Research Commons

The Effects of Ocean Acidification on Microbial Nutrient Cycling and Productivity in Coastal Marine Sediments

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Doctor of Philosophy in Biological Sciences

at

The University of Waikato

by

SHELLY MIA MARIE BRANDT

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2022

Statement of Authorship

I hereby declare that this is a submission of my own work, and to the best of my knowledge does not contain material that has been previously published by another individual, nor has it been used for the awarding of another degree.

Acknowledgements

List of Figures

Chapter 1: The Effects of Ocean Acidification on Microbial Communities in Benthic Coastal Sediments -A Review

Chapter 2: *The Effects of Ocean Acidification on Benthic Microbial Communities from a Coastal Intertidal Sand Flat on the North Island of New Zealand*

Chapter 3: *The Effects of Ocean Acidification on Benthic Subtidal Microbial Communities from The Hauraki Gulf, New Zealand*

Chapter 4: *Benthic Subtidal Sediment Communities Structurally Respond to the Addition of Organic Carbon and CaCO₃ Enrichments under Differing Acidified Ocean Conditions*

List of Tables

Chapter 4: *Benthic Subtidal Sediment Communities Structurally Respond to the Addition of Organic Carbon and CaCO3 Enrichments under Differing Acidified Ocean Conditions*

Table of Contents

Chapter 1: *The Effects of Ocean Acidification on Microbial Communities in Benthic Coastal Sediments -A Review*

Chapter 2: *The Effects of Ocean Acidification on Benthic Microbial Communities from a Coastal Intertidal Sand Flat on the North Island of New Zealand*

Chapter 3: *The Effects of Ocean Acidification on Benthic Subtidal Microbial Communities from The Hauraki Gulf, New Zealand*

Chapter 4: *Benthic Subtidal Sediment Communities Structurally Respond to the Addition of Organic Carbon and CaCO3 Enrichments Under Differing Acidified Ocean Conditions*

Chapter 5: *The Effects of Ocean Acidification on Microbial Nutrient Cycling and Productivity in Coastal Marine Sediments: A Summary*

Chapter 1: The Effects of Ocean Acidification on Microbial Communities in Benthic Coastal Sediments -A Review

Abstract

Ocean Acidification (OA), commonly referred to as the "other CO_2 problem," illustrates the current rise in atmospheric carbon dioxide (CO_2) levels, precipitated in large by human-related activity (e.g., fossil fuel combustion and mass deforestation). The dissolution of atmospheric CO_2 into the surface of the ocean over time has reduced oceanic pH levels by 0.1 units since the start of the pre-industrial era and has resulted in wholesale shifts in seawater carbonate chemistry on a planetary scale. The chemical processes of ocean acidification are increasingly well documented, demonstrating clear rates of increase for global CO_2 emissions predicted by the IPCC (Intergovernmental Panel on Climate Change) under the business-as-usual CO_2 emissions scenario. The ecological impact of ocean acidification alters seawater chemical speciation and disrupts vital biogeochemical cycling processes for various chemicals and compounds. Whereby the unidentified potential fallout of this is the cascading effects on the microbial communities within the benthic sediments. These microorganisms drive the marine ecosystem through a network of vast biogeochemical cycling processes aiding in the moderation of ecosystem-wide primary productivity and fundamentally regulating the global climate. The benthic sediments are determinably one of the largest and most diverse ecosystems on the planet. Marine sediments are also conceivably one of the most productive in terms of microbial activity and nutrient flux between the water-sediment interface (i.e., boundary layer). The absorption and sequestering of CO_2 from the atmosphere have demonstrated significant impacts on various marine taxa and their associated ecological processes. This is commonly observed in the reduction in calcium carbonate saturation states in most shell-forming organisms (i.e., plankton, benthic mollusks, echinoderms, and Scleractinia corals). However, the response of benthic sediment microbial communities to a reduction in global ocean pH remains considerably less well characterized. As these microorganisms operate as the lifeblood of the marine ecosystem, understanding their response and physiological plasticity to increased levels of CO_2 is of critical importance when it comes to investigating regional and global implications for the effects of ocean acidification.

Chapter 1: A Review of The Literature

Introduction

This chapter is designed with the intention of familiarizing the reader with the global problem that is Ocean Acidification, from a microbial perspective, followed by the extreme variability of nearshore and coastal habitats that represent a unique and potentially sensitive ecosystem given their proximity to land and therefore increased human-related disturbances. From there, the chapter begins by introducing the different physical and chemical processes of these environments as it relates to changes in pH (i.e., alkalinity, metabolism, photosynthetic processes). We then follow this with examining buffering capacity, mitigation strategies, and blue carbon ecosystems. We then discuss differences in coastal versus open ocean ecosystems, ad pelagic versus benthic environments in terms of their response to ocean acidification. From here, we spend time addressing the complexity and immense heterogeneity of the benthic sedimentary environment and its variable response to ocean acidification based on sediment composition, type, and present benthic macrofaunal species. We assess the current knowledge of coastal benthic sediment microbiological community response to the effects of low pH, in addition to the projected impacts of ocean acidification on microbial composition, structure, and physiology. Briefly, we evaluate the modern genomic methodologies and their standard applications before discussing, in short, each of the experimental chapters. This includes a quick summary of our aims and hypotheses, as well as the intended philosophical explanation for each experimental design.

1.1 Ocean Acidification: An Overview

The ocean acts as a natural carbon sink, aiding in the regulation and mitigation of climatic shifts through the absorption of atmospheric CO_2 (Sabine et al., 2004; Land et al., 2015). On average, an estimated 36 billion metric tons of CO_2 is emitted into the atmosphere annually, where one-third of that total volume is then absorbed directly into the surface of the ocean (P. Land et al., 2015). In the past two centuries, an estimated 25-30% of all CO_2 emissions have been absorbed directly into the global ocean, a process more frequently termed *ocean acidification* (OA) (Zeebe & Wolf-Galdrow, 2001; Sabine & Tanhua, 2010; Boyd, 2011; Burrell et al., 2015). The chemical process of ocean acidification involves the absorption and dissolution of atmospheric CO_2 into seawater (**1**.)(Upton & Folger, 2015); typically, a rapid chemical exchange operating on a time scale of a few months, relative to the atmospheric-

oceanic equilibrium where the increased concentration of atmospheric CO_2 is proportionate to the increased concentration of CO_2 in the ocean. The secondary process (**2.**) shows the formation of H_2CO_3 (Carbonic acid) from the dissolution of CO_2 and hydration of H_2O; which then quickly dissociates in an acid-base reaction, resulting in the release of free hydrogen ions and the subsequent reduction in logarithmic pH (A. Dickson, Sabine, & Christian, 2007; Zeebe & Wolf-Gladrow, 2001).

$$1.)\quad CO_2\,(atm) \rightleftharpoons CO_2\,(aq)$$

$$2.)\quad CO_2\,(aq) + H_2O \;\rightleftharpoons\; H_2CO_3\,H^+ + HCO_3^- \rightleftharpoons\; 2H^+ + CO_3^{\,2-}$$
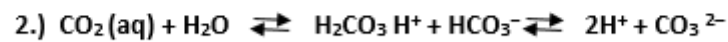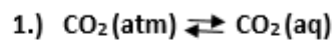
Since the start of the preindustrial era, the concentration of atmospheric CO_2 had increased by an estimated 40% from 280 ppm (parts per million volume) to 384 ppm by 2007 (Solomon et al., 2007; Doney, 2010), and easily surpassing 416 ppm by 2021 (NOAA (The National Oceanic and Atmospheric Administration)). The IPCC (Intergovernmental Panel on Climate Change) has determined 800 ppm to be the projected end-of-the-century concentration of CO_2, operating under the business-as-usual CO_2 emissions scenario (Doney et al., 2009; Stocker et al., 2013). This has effectively reduced the global ocean pH by 0.1 units from 8.2 to the current 8.1 over the past 250 years (Stocker et al., 2013). The IGBP (International Geosphere-Biosphere Program) anticipates an additional drop in oceanic pH by 0.3-0.4 by the end of the century (IGBP, 2013). This is further corroborated by current predictive models, which demonstrate that surface seawater involved in a gas exchange equilibrium with the atmosphere will exhibit a steady increase in pCO_2 (partial pressure CO_2) from the present 390 µatm concentration to 700-1000 µatm by 2100 (Solomon, 2007; Melzner et al., 2013). Previous studies examining higher trophic levels have demonstrated that 550 ppm atmospheric CO_2 is the threshold at which coral begins to erode (Doney, 2010). Naturally, this is anticipated to have significant collateral effects on various reef-dependent taxa on both macro and micro scales given the dramatic change in seawater carbonate chemistry (26% increase in acidity) and reduction (16%) in carbonate ion concentration within the last 250 years (P. Land et al., 2015). The rate of global CO_2 emission is an order of

magnitude faster than any other carbon-related event that has occurred within the last few million years. Additionally, the concentration of atmospheric CO_2 is also higher than anything the planet has experienced in the past 800,000 years (Doney & Schimel, 2007; Lüthi et al., 2008). The continued dissolution of atmospheric CO_2 into the surface of the ocean is likely to reduce the efficiency of carbon storage over time as the ocean reaches a CO_2 saturation point (Sabine et al., 2004; Land et al., 2015). This is predicted to have a significant impact on coastal and estuarine ecosystems across both spatial and temporal scales, with further implications for their co-associated benthic microbial communities (Lidbury et al., 2012).

1.2   Coastal Marine Ecosystems

Coastal or nearshore zones are one of the most dynamic and energetic ecosystems on the planet, operating as the interface between the terrestrial and oceanic biomes (McLean et al., 2001) (**Figure 1**.). These regions are responsible for dictating the regulation and transfer of genes, energy, and matter (Monaco & Prouzet, 2015). Collectively, they make up one of the most complex and important environments of the natural biosphere (Monaco & Prouzet, 2015). The coastal ecosystem is also critical for supporting primary productivity through the recycling of major biological elements, the metabolization of contaminants, and the regulation of hydrological processes (Bolin & Cook, 1983). These systems represent the coalescing of the physical, chemical, and biological interactions between land-dominated global processes and ocean-dominated global processes; across multiple biogeochemical, geomorphological, and environmental gradients (Crossland et al., 2005). This variability can be seen in the complexity and diversity of the distinct ecosystem gradients along the world's coastline (350,000 km), which consists of rivers, estuaries, tidal wetlands, and the proximal continental shelf (Bauer et al., 2013; J. P. Gattuso, Frankignoulle, & Wollast, 1998). Collectively, this further trademarks them as one of the most intricate and unique environmental domains on the planet (Bauer et al., 2013). In terms of global significance, the coastal ecosystem plays a considerable role in oceanic biogeochemical nutrient cycling processes, given that these systems receive massive amounts of organic matter and nutrient input via terrestrial run-off and ground-water discharge (J. P. Gattuso, Frankignoulle, & Wollast, 1998). Coastal regions also perform large-scale energetic matter exchange with the open ocean, which again collectively constitutes them as one of the most biologically active and geochemically active zones within the global biosphere (J. P. Gattuso, Frankignoulle, &

Chapter 1: A Review of The Literature

Wollast, 1998). The coastal zone accounts for an estimated 30% of all oceanic primary productivity, 80% of organic matter burial, 90% of sediment mineralization, 75% of suspended particle sink from river input, and 50% of calcium carbonate deposition (Mantoura, 1991; Pernetta & Milliman, 1995). Economically, the global coastline represents 43% of the value of the total planetary ecosystem services and natural capital (Costanza et al., 1997) and accounts for an estimated 90% of world fish catch (Pauly & Christensen, 1995). Additionally, approximately 37% of the global human population lives within 70-100km of coastline and relies on its goods and services (Cohen, 1997). However, despite its global significance, the coastal region remains a challenging and intrinsically complex environment to study and thus is often underrepresented in its potential and importance (J. P. Gattuso, Frankignoulle, & Wollast, 1998), especially in ocean acidification research.
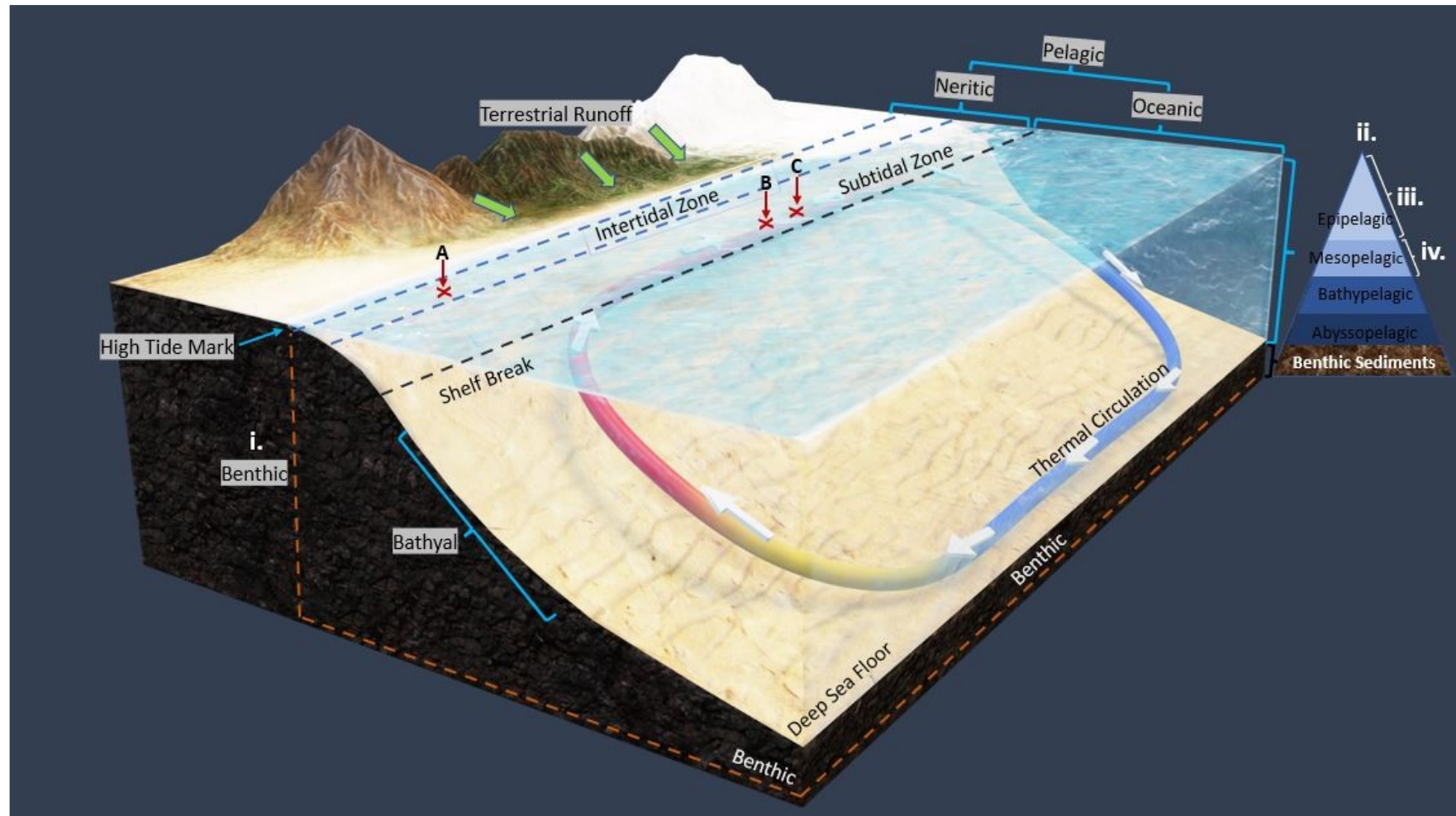
Figure 1. A model of the coastal marine ecosystem displaying the geological structure of the intertidal and subtidal zones (with surrounding environmental inputs: i.e., upwelling, terrestrial loading) as areas of interest throughout this study where "A" represents the proximal region of experiment one, "B" experiment two, and "C" experiment three. **i.** identifies the benthic sedimentary environment, **ii.** describes the coupling of the neighboring pelagic system to the benthos below. **iii.** Shows the water column profile for an *intertidal* ecosystem, where **iv.** Shows a similar profile for a *subtidal* ecosystem. A modified Microsoft© Open Access Model used with permission.

Chapter 1: A Review of The Literature

1.2.1   Seawater pH

Coastal systems have demonstrated complex and heterogeneous pH patterns over time (Borges & Gypens, 2010; Duarte, Hendriks, et al., 2013; Johnson et al., 2013; Provoost et al., 2010; Wootton, Pfister, & Forester, 2008). This is a characteristic not representative of the more monotonic open ocean environment in response to increased levels of CO_2 (Bates et al., 2014; Doney, Fabry, Feely, & Kleypas, 2009b; Ríos et al., 2015). The dynamic and almost extreme oscillations witnessed in coastal ocean pH concentrations appear idiosyncratic in terms of their diverse patterning (Baumann & Smith, 2018; Duarte, Hendriks, et al., 2013). However, there is difficulty in assessing and understanding the regulation of pH in response to anthropogenic impacts, as coastal waters are confounded by a complex interconnected web of interacting processes that drive pH variability (Cai et al., 2017; Carstensen & Duarte, 2019; Duarte, Hendriks, et al., 2013; Johnson et al., 2013; Mongin et al., 2016; Wootton et al., 2008). For example, coastal ecosystem variabilty can be primarily driven by terrestrial input, including freshwater influx which can dilute seawater alkalinity and alter buffering capacity (Cai et al., 2017; Duarte, Hendriks, et al., 2013; Johnson et al., 2013; Mongin et al., 2016; Wootton et al., 2008). Additionally, coastal regions exposed to regular anthropogenic nutrient inputs exhibit eutrophication acidification dynamics, hypoxia, anoxia and low pH, which can enhance productivity and where changes in coastal respiratory processes as a result of nutrient loading, only further exacerbate the effects of OA (Hagens et al., 2015; Sunda & Cai, 2012; Ulfsbo, Hulth, & Anderson, 2011; Wallace, Baumann, Grear, Aller, & Gobler, 2014a). The combination of the effects of both anthropogenic and biological CO_2 inputs can contribute to even greater acidification rate in coastal environments compared to open ocean systems (Cai 2017). "

The initial methodology used to investigate this variability in coastal pH was based on concepts and models initially developed for open ocean, ocean acidification scenarios. These studies examined data trends for anthropogenic CO_2 emissions and mixing between marine and freshwater systems that controlled and regulated carbonate chemistry in the coastal marine ecosystem (Feely et al., 2010). However, further analysis identified that the control of coastal oceanic pH involved an assortment of additional drivers (e.g., watershed and biological processes) which then drive various metabolically active communities (Borges & Gypens, 2010; Cai et al., 2017; Carstensen & Duarte, 2019; Duarte, Hendriks, et al., 2013;

Mongin et al., 2016). One example would be the localized effects of coastal eutrophication, sometimes termed eutrophication-induced acidification which occurs when an algal bloom produced by excess nutrient input dies off, resulting in an influx in organic material to the sediments (Borges & Gypens, 2010; Melzner et al., 2013b). Rapid remineralization of the organic matter by the residential heterotrophic bacteria at the surface of the sediment and sediment-water interface draws down bioavailable O_2 and subsequently releases additional CO_2, where the process of eutrophication exerts a strong hold on regional pH patterning (Provoost et al., 2010; Cai, 2012). In heavily populated coastal regions, the combination of anthropogenic ocean acidification and eutrophication-induced acidification compounds to influence reductions in seawater pH (Borges & Gypens, 2010; Melzner et al., 2013b; Provoost et al., 2010; Cai, 2012). More recent studies have also shown that reduced seawater pH in the overlying water column can have significant effects on nutrient fluxes across the sediment-water interface (Kroeker et al., 2011; Widdicombe et al., 2009; Widdicombe & Needham, 2007; Wood, Spicer, & Widdicombe, 2008). It is, therefore, of critical importance to investigate the linkage between the effects of ocean acidification on microbial communities across spatially dynamic and heterogeneously diverse benthic sediment systems.

1.2.2   Alkalinity

It is essential to distinguish the difference between pH and alkalinity in relation to an acidifying ocean environment. pH provides a measure of the acidity or alkalinity of the water on a logarithmic scale (0-14 pH) where the dissolution and concentration of free hydrogen ions (H^+) in the water column equates to how acidic or basic the environment is. This is fundamentally described in the chemical equation ($H^+ + OH^- = H_2O$), where the equalization of the H^+ ion concentration to the OH^- ion concentrations imply neutral pH. However, when the concentration of H^+ ions is greater than the OH^- ion concentration, the water becomes measurably acidic (Butler, 1991; Stumm & Morgan, 2012). Alkalinity essentially describes the buffering capacity of either the overlying water column or the benthic sediments, where the presence of electron donors and acceptors dictates oceanic chemistry buffering potential (Middelburg et al., 2020). It also plays a role in calcium carbonate mineral precipitation and dissolution, which directly or indirectly affects various marine organisms (Middelburg et al., 2020). Alkalinity plays an important part in both the sequestering and storage of

anthropogenic carbon dioxide (CO_2) but also in minimizing the potential impacts of ocean acidification on marine organisms (Kroeker et al., 2013a). The ability to measure and quantify the potential for buffering within the marine environment is crucial for understanding the impacts of ocean acidification as it pertains to the modern world (Anthropocene) state (Orr et al., 2018). Total alkalinity (TA) is a standard and frequently measured parameter of the marine environment and is often used to determine the neutralizing potential and capacity of seawater (Pytkowicz & Atlas, 1975). Measuring TA alongside pH or DIC (Dissolved Inorganic Carbon) is used to provide estimations of pCO_2 (partial pressure of carbon dioxide) (Lueker et al., 2000; Wanninkhof et al., 1999). TA, in its simplest form, refers to the number of H^+ ions (millimoles) required to neutralize weak bases in a 1 kg volume of seawater (Grasshoff et al., 2009). The higher the alkalinity levels in seawater, the greater the capacity to resist changes in overlying water column pH (Lukawska-Matuszewska, 2016).

When discussing global ocean surface waters, alkalinity remains tightly coupled with salinity and temperature (Lee et al., 2006; Millero, Lee, et al., 1998). Alkalinity is also influenced by regional and local drivers, as well as various chemical and physical processes (i.e., precipitation or dissolution of $CaCO_3$) or other hydrological dynamics (i.e., deep-water upwelling) (Millero et al., 1998). Open ocean (seawater) TA is predominantly determined by bicarbonate, carbonate, and borate ions, whereas in coastal (estuarine) systems, carboxylic acid groups and phenolic groups from dissolved organic matter (DOM) can be additional sources of alkalinity (Cai et al., 1998; Kuliński et al., 2014; Hernández-Ayon et al., 2007; Muller & Bleie, 2008). However, marine sediments are an exception, where the difference between carbonate alkalinity (derived from HCO_3^- and CO_3^{2-}) and TA is considerable. This is attributed to the various abiotic and microbiological reactions (i.e., dissolution and precipitation) during early diagenesis, which results in differences between the chemical composition of sediment pore water and overlying seawater (Carman & Rahm, 1997; Chatterjee et al., 2011). Some studies have shown that the differences between porewater and seawater alkalinity can be as much as 20x higher in overlying seawater than in sediments (Berner et al., 1970; Carman & Rahm, 1997; Chatterjee et al., 2011; Lukawska-Matuszewska & Kielczewska, 2016). Benthic exchange, or the transition of substances across the sediment-water interface, identifies pore water as a significant source of alkalinity for the overlying water column (Carman & Rahm, 1997; Cyronak & Eyre, 2016; Cyronak et al., 2013; Drupp et al., 2016). The generation of

alkalinity in fine-grained organically rich sediments is a result of the (anoxic) mineralization of organic matter (OM) typically via denitrification or sulfate reduction, in addition to the reduction of manganese (Mn IV) and iron (Fe III) by the residential microbial taxa (Berner et al., 1970). In the context of ocean acidification, high alkalinity in the benthic sediments translates to increased buffering capacity under high CO_2 exposure, where the residential microbial communities may maintain *near* normal environmental conditions due to the concentration of pore water alkalinity (Andreas J Andersson, Bates, & Mackenzie, 2007; Morse, Andersson, & Mackenzie, 2006a).

1.2.3   Coastal Processes and Metabolism

Research investigating how concurrent acidification and hypoxia impact marine life remains underrepresented, and data regarding this effect on benthic sediments and the microbial diversity contained within them remains all but unexplored. Though some recent studies have shown that low oxygen in coastal systems plays a far more dominant role than the effects of pH (T. W. Kim, Barry, & Micheli, 2013). However, other studies demonstrated that the combined effects of both hypoxia and acidification proved to be far more consequential than either of the two individual stressors independently (Gobler, Clark, Griffith, & Lusty, 2017; Steckbauer et al., 2015). Recently, seawater pH measurements have become a vital parameter for understanding the impacts of ocean acidification on coastal ecosystems and are now essential for predicting their vulnerability to biogeochemical and ecological degradation (Doney et al., 2009b; Howarth et al., 2011). As previously identified, hypoxic regimes caused by nutrient over-enrichment and algal blooms are highly problematic to coastal systems (McLaughlin et al., 2017). However, this is in addition to other external factors within these environments that act as compounding variables to pH fluctuation, such as freshwater inputs, tidal forcing, water column stratification, and the upwelling of low pH waters (Borges & Gypens, 2010; Doney et al., 2009b; Guinotte & Fabry, 2008; Howarth et al., 2011). It is important to note that these perturbations may occur in the background to ocean acidification, further confounding the ability to decouple the direct effects of ocean acidification on the coastal environments from that of the secondary effects of pH change (McLaughlin et al., 2017).

Coastal systems will exhibit signs of acidification or basification, depending on the relationship between the input of anthropogenic CO_2 and organic matter and watershed

export of alkalinity (Duarte et al., 2013). The regulation of pH within coastal surface waters is unique as pH variation is dependent on the input of open ocean chemistry as well as watershed inputs of Ca^{2+}, carbonate alkalinity, organic/inorganic carbon, nutrients, ecosystem metabolic activity, and the hydrological processes that govern the mixing of both coastal and open ocean waters (Aufdenkampe et al., 2011; Duarte et al., 2013). The initial uptake of CO_2 is principally governed by the rate of oceanic circulation, or the exchange between surface and subsurface waters infused with CO_2 (Doney, 2010). The acid-base chemistry of seawater is buffered largely impart by inorganic carbon, where CO_2 functions as a weak acid (Doney, 2010). Other processes such as air-to-sea flux, or the bacterial respiration of organic matter, influence the concentration of hydrogen ions (H^+), subsequently decreasing pH ($pH = log_{10}\{H^+\}$) (Doney, 2010). The extent of the impact of anthropogenically derived ocean acidification, and co-associated climate change, is dependent on whether organic matter is recycled in the form of CO_2 or remains permanently buried in benthic sediments (Snelgrove, 1997). This is further influenced and perhaps confounded by the synergy between microbial breakdown, sedimentation, and depositional feeders responsible for mixing and burying particles vertically within the sediment (Snelgrove, 1997). The coastal ecosystem, in all its complexity, is biogeochemically governed by the interactions between terrestrial input and the relationship between the atmosphere and open ocean (Doney, 2010; Aufedenkampe et al., 2011). The connectivity that exists between the pelagic and benthic ecosystems are also influenced by these contributing land-based processes such as the transport of dissolved organic matter (DOM), sedimentation, and erosion, as well as other abiotic factors such as the transport of gaseous compounds (O_2 and CO_2) (Asmus, 2012).

1.2.4   Photosynthetic Activity

Phytoplankton within the photosynthetic surficial layer of the ocean is a major driver of ocean biogeochemical cycling through the exportation-based flux of organic matter and supply of calcareous/siliceous bio-minerals derived from planktonic shells to the benthic sediments (Doney, 2010). The biologically produced particulate matter that sinks to the ocean floor is consumed by the resident microbial consortia and macrofauna within the sediment (Hedges & Keil, 1995; Meysman et al., 2006; Doney, 2010). The degradation of organic carbon results in a final by-product of CO_2 with the corresponding consumption of bioavailable

subsurface O_2 (Hedges & Keil, 1995; Meysman et al., 2006; Doney, 2010). The newly processed organic matter is then either assimilated via heterotrophic microorganisms by means of secondary production or respired and remineralized back into the system (Middelburg & Levin, 2009). This indicates that export production maintains strong vertical gradients in biogeochemical tracers between the active microbial community within the sediment and the overlying water column (Doney, 2010). Additionally, over 80% of the nitrogen within the system that is utilized by photosynthetic organisms in shallow coastal regions comes from the regeneration of organic material by the microbial communities in the oxygenated layer of the sediment (Dale & Prego, 2002). This highlights the importance of the microbial communities within coastal benthic sediments in driving the marine ecosystem processes through the vast network of complex biogeochemical cycling pathways (Dale & Prego, 2002).

1.2.5   Buffering Capacity and Organic Loading

Alkalinity is driven predominantly by the cycling of organic matter (OM) within the coastal benthic sediments, driven by a series of reduction-oxidation (redox) reactions (Burdige, 2012; Krumins, Gehlen, et al., 2013; Sippo, Maher, et al., 2016). For instance, sulfate is one of the more abundant oxidants within the water column and subsequently drives the dominant anaerobic reduction pathway within the sediments (Burdige, 2012). In most marine sediments, the mineralization of organic matter is directed by a diagenetic reaction sequence, where aerobic processes at the surface of the sediment are followed by anaerobic processes within the deeper stratified sediment layers (Burdige, 2012; Hu & Cai, 2011). This is a more simplified two-dimensional explanation of the organic matter mineralization process, which does not effectively account for the true complexity and variability of other sediment processes such as bioturbation, where the presence of macrofaunal burrows exerts a strong influence over hydrology (Tait et al., 2016) and OM mineralization rates (Alongi, 2009). Mangrove sediments, for instance, best illustrate the degradation of organic matter through multiple diagenetic pathways, where sulfate reduction becomes the dominant mineralization pathway for organic carbon within the system (Borges et al., 2003; Bouillon et al., 2007). The process of sulfate reduction results in the generation of alkalinity for any sulfide produced that is not immediately re-oxidized (Alongi et al., 1998; Burdige, 2012; Ovalle et al., 1990). Tidal activity within the coastal zone drives the enriched (pCO_2, alkalinity, DIC) pore water

solutes out to the coastal ocean and off-gases CO_2 to the atmosphere (Alongi et al., 2012; Borges et al., 2003; Bouillon et al., 2007; Bouillon et al., 2007; Call et al., 2015; Koné & Borges, 2008; Maher et al., 2013), where collectively these processes describe the metabolic activity of coastal marine sediments in relation to organic carbon consumption.

1.2.6 Blue Carbon

Given the current global climate situation, efforts have been made to transition to a low-carbon economy by means of CO_2 biosequestration in order to aid in the mitigation of global warming and maintain a planetary temperature below $2^{\circ}C$ (Macreadie et al., 2017). Biosequestering, also known as "blue carbon," employs the use of vegetated coastal habitats (i.e., seagrasses, tidal marshes, and mangrove forests), often used as natural blue carbon ecosystems (McLeod et al., 2011). These blue carbon ecosystems only occupy an estimated 0.2% of the global ocean surface but collectively account for 50% of the total amount of carbon buried in marine sediments (Duarte et al., 2013). The most important aspect of these blue carbon ecosystems is that they have the ability to accumulate carbon without becoming supersaturated and can therefore store legacy carbon in the sediments over a millennium (McLeod et al., 2011). Microbial communities within vegetated terrestrial environments play a critical role in mediating the global carbon sink (i.e., Amazonian forests, permafrost regions) a process that also occurs less obviously within the marine ecosystem (Chen et al., 2021). However, unlike terrestrial ecosystems, which store organic carbon primarily in vegetation, living biomass, and soil organic matter, the vegetated coastal ecosystems store the majority of organic matter within the benthic sediments (Crusius et al., 2005; Fourqurean et al., 2012; Greiner et al., 2013; McLeod et al., 2011). This is because marine sediments are characteristically anoxic at depth and accumulate continual sediment deposition on a regular basis (Greiner et al., 2013; Kennedy et al., 2010). Therefore, organic carbon can remain unaltered within the sediment on long time scales (i.e., decadal to millennial) (Mateo et al., 1997; Mckee, Cahoon, & Feller, 2007; Orem et al., 1999)**.**

Carbon burial in coastal sediments is facilitated by fast accumulation rates, low oxygen regimes, low sediment hydraulic conductivity, and slow microbial turnover and decomposition (Duarte, Kennedy, Marbà, & Hendriks, 2013; Duarte et al., 2010; Mateo, 2006). It is also important to note that differences exist between vegetated and non-

vegetated coastal sediments in relation to carbon burial efficiency. As the presence of vegetation (i.e., seagrasses) compared to non-vegetated sediments can result in as much as a threefold reduction in resuspension rates within fine-grained sediment (Gacia & Duarte, 2001; Gacia, Granata, & Duarte, 1999; Hansen & Reidenbach, 2012). The particulate matter that is captured via the seagrass stripping particles from the water column and deposited to the sediments is typically organically rich (~4.1%) (Greiner et al., 2013; Kennedy et al., 2010). This process results in a net transfer of allochthonous carbon to the benthic sediments below coastal seagrass beds and increases the long-term storing capacity of sequestered carbon within the sediments (Kennedy et al., 2010)**.** The loss of seagrass coverage and density within these coastal environments, often as a result of human activity or eutrophication, shows a clear reduction in this capturing effect highlighting the importance of vegetative ecosystems (Gacia & Duarte, 2001; Hemminga, 2000). However, as seen with terrestrial carbon sinks, ecosystem degradation, whether by natural or anthropogenic means, can transition a blue carbon ecosystem from being a carbon sink to being a carbon source (Pendleton et al., 2012).

1.3 Comparing Coastal to Open Ocean Systems

The coastal environment is dominated by various types of engineering species within the benthic ecosystem (e.g., corals, seagrass, mangroves, macroalgae, salt marshes, oyster reefs) who demonstrate the ability to modulate the chemical and physical properties of their surrounding environment (Duarte, Hendriks, et al., 2013; Gutiérrez et al., 2012). The interaction of these different factors in the coastal zone highlights a carbon system that is vastly more complex than what is observed in the open ocean (Borges & Gypens, 2010; Cai, 2011). This also implies that the impact of atmospheric CO_2 on the coastal ocean system may play a much smaller role (Borges & Gypens, 2010; Cai, 2011; Hofmann et al., 2011; Provoost et al., 2010). In contrast, the effect of anthropogenic atmospheric CO_2 in the open ocean is more likely to be the primary dominating factor for pH change (Borges & Gypens, 2010; Cai, 2011; Hofmann et al., 2011; Provoost et al., 2010). This paradigm addresses the concept that there are multiple mechanisms that are involved in driving shifts in coastal ocean pH (e.g., land usage, nutrient influx, ecosystem structure and metabolism, and atmospheric gas emission to the carbon system); whereas a change in open ocean pH is almost singularly driven by anthropogenic atmospheric CO_2 (Duarte et al., 2013). Additionally, the regulation of coastal surface pH is much more complex as it is influenced both by open ocean processes

as well as watershed inputs (e.g., Ca^{2+}, carbonate, alkalinity, inorganic carbon, organic carbon, nutrient input, ecosystem metabolism, hydrological processes) which govern the mixing between coastal and open ocean waters (Duarte et al., 2013). Net community production and calcification rates are typically an order of magnitude greater in coastal ocean systems when compared to open ocean systems (Duarte & Cebrián, 1996; J. P. Gattuso, Frankignoulle, & Wollast, 1998), and therefore play a more important role in ecosystem functionality (Anthony, Kleypas, & Gattuso, 2011).

A survey of various coastal marine habitats demonstrates that these systems all have characteristic site-specific diel, diurnal (semi), and stochastic patterning with varying levels of amplitude (Hofmann et al., 2011). For example, seagrass meadows, mangrove forests, salt marshes, coral reefs, and macroalgae are metabolically intense ecosystems and can tolerate diel changes in oceanic pH up to as much as 1.0 pH unit (Hofmann et al., 2011). Other coastal ecological events such as phytoplankton blooms can spike the pH from 8.6-9.0 during the peak of the bloom, with a subsequent drop in pH by 0.3-0.4 units at the point of breakdown and remineralization after seasonal decline (Brussaard et al., 1996; Dai et al., 2008; Spilling, 2007; Taguchi & Fujiwara, 2010; Zhai et al., 2012). In terms of bottom-up effects, benthic microbial processes can also modulate pH and alkalinity through changes in sediment-water interface flux rates within the coastal ecosystem (Cyronak et al., 2013). Seawater pH in the open ocean which is considered to be relatively stable with a very narrow range in pH variability (<0.1 pH units inter-annually) (Doney et al., 2009b). Whereas coastal ocean pH can oscillate by as much as 1.0 pH unit within a 24-hour (i.e., diel) cycle (Hofmann et al., 2011) and by an amplitude of much as >0.3 pH units on a seasonal to decadal basis (Barton et al., 2012; Borges & Gypens, 2010; Cai et al., 2011; Hofmann et al., 2011; Melzner et al., 2013a; Mercado & Gordillo, 2011; Provoost et al., 2010; Waldbusser et al., 2011).

1.4 Pelagic Versus Benthic Processes

In coastal ocean waters, the effects of acidification on marine organisms may also be determined by the frequency, amplitude, and duration of carbonate chemistry events as they influence various species thresholds (Waldbusser & Salisbury, 2014). In the coastal region, the combination of local hydrological processes with chemical acid-base reactions can result in

localized "carbonate weather" patterning events, whose variability can affect many marine taxa (e.g., bivalves) (Firth & Hawkins, 2011; Hofmann & Todgham, 2009; Somero, 2012). The biogeochemical conditions and processes in benthic coastal sediments vary from those of water column processes based on the differences in transport mechanisms such as molecular versus eddy diffusion and particle settling versus bioturbational mixing (Middelburg & Levin, 2009). The deposition of labile and reactive materials via the water column is also responsible for providing nutrients and energy for the heterotrophic consumers living within the sediment (Hedges & Keil, 1995; Meysman et al., 2006). When it comes to understanding the impact of ocean acidification on pelagic versus benthic ecosystems, it is also important to examine differences in how these two environments are equipped to respond to increased CO_2. For instance, CO_2 levels in the benthic sediments are higher than that of the overlying water column, with subsequently lower oxygen levels (Widdicombe & Spicer, 2008). This would suggest that benthic organisms will have enhanced tolerance to acidification and hypoxia (Widdicombe & Spicer, 2008).

1.4.1   Pelagic Carbonate Chemistry

The effects of increased atmospheric CO_2 uptake in seawater helps to facilitate increased organic production in coastal and particularly estuarine systems (Cai, 2011), which results in a subsequent increase in the capture, burial, and subsequent respiration of CO_2 (Cai, 2011). Coastal zones are already subjected to the greatest range of net community production across multiple time scales compared to other oceanic systems, and therefore it is unclear how these regions will continue to respond or potentially adapt to increased CO_2 (J. P. Gattuso, Frankignoulle, & Wollast, 1998). Continual chemical fluctuations will directly alter carbonate chemistry through changes in alkalinity, resulting in indirect or secondary effects on nutrients involved in primary production (Fekete et al., 2001). Some of the most influential drivers of coastal carbonate chemistry variability are the effects of biological production and respiration (Sunda & Cai, 2012). The introduction of deep nutrient and carbon-rich water into the photic zone through upwelling systems can be advected into the coastal region, which will then drive net community production and subsequently alters oceanic chemistry (Barton et al., 2012; Fassbender et al., 2011; Feely et al., 2010; Feely et al. l., 2008; Hales et al., 2005; Mathis et al., 2012). As previously described, terrestrial input into the coastal zone can have a significant impact on system carbonate chemistry (Duarte et al., 2013). Land-based sources

are directly or indirectly affecting carbonate chemistry through the deposition of bioactive constituents (i.e., dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) into the water column (Doney et al., 2007; Paerl, 1985; Turk et al., 2010). This also relates to the anthropogenic impacts of freshwater input into the coastal system due to alterations in landscape and agriculture (Diaz & Rosenberg, 2008; Moser et al., 2012; Seitzinger et al., 2005) that can introduce weathered rock products which include alkalinity, nutrients, and particulate and dissolved organic carbon, effectively altering coastal ocean carbonate chemistry (Keulet et al., 2010; Salisbury et al., 2008). This process also perturbs the global nitrogen cycle, as terrestrial input often contains fertilizers or other agricultural by-products, which contribute to increased fluxes of labile nitrogen (Canfield et al., 2010). This also occurs along with increased DIC flux within the same coastal regions (Caraco & Cole, 1999; Green et al., 2004; Seitzinger et al., 2005). Therefore, understanding how these different drivers interact together to alter carbonate chemistry when confounded by regional variation in watershed processes becomes a crucial element for understanding the localized effects of ocean acidification in the coastal environment (Waldbusser & Salisbury, 2014).

1.4.2   Benthic Carbonate Chemistry

Benthic sediments are dynamic and highly heterogenous systems where significant amounts of organic matter are delivered from the water column where they are either recycled back into the system or remain buried in the sediments (Hansen & Blackburn, 1992). The cycling of organic matter is attributed to shallow water column profiles, high production rates in the overlying seawater, rapid organic matter deposition, and nutrient availability (Giorgio & Williams, 2005). In estuarine systems, an estimated 24% of total organic input is respired by the coastal benthic sediments (Smith & Hopkinson, 2005). The percentage of benthic respiration, compared to pelagic respiration, seems small relative to surface area (Smith & Hopkinson, 2005). However, the amount of productivity that is generated from benthic sediments is derived from only a few millimeters or centimeters of the upper oxygenated sediment layers (Glud, 2008). The suboxic layer of the sediment is where aerobic respiration is still taking place but at a reduced level (Soetaert et al., 2007). In this region is where microbial metabolic processes (e.g., denitrification, sulfate reduction) oxidize organic matter through available alternate terminal electron acceptors (Soetaert et al., 2007). Suboxic metabolism is also known to produce CO_2 while simultaneously increasing alkalinity, which

somewhat counteracts the effects of excess CO_2 production (Soetaert et al., 2007). These suboxic metabolic processes can also occur at the sediment-water interface, effectively altering system chemistry; for example, by changing calcium carbonate saturation states across seasonal timescales, where these compounds are more or less thermodynamically available in summer or winter months (Green & Aller, 1998).

Respiration in coastal sediments is largely driven by organic matter enrichment, electron acceptor transport dynamics, and additional environmental factors such as temperature and light (Green & Aller, 1998). However, the effects of anaerobic metabolism can also produce toxic metabolites (e.g., sulfides); therefore, regions high in organic matter concentrations often exclude megafauna, driving even greater benthic habitat heterogeneity (Green & Aller, 1998; Waldbusser & Salisbury, 2014). The presence of oxygen combined with organic substrate has a strong influence on sediment pore water saturation states (Macintyre et al., 1996). The primary producers within the photic sediments are also capable of generating large amounts of O_2 alongside excess CO_2 production (Macintyre et al., 1996). This is further confounded by diurnal-scale variability of the carbonate chemistry within the surface sediments, which can drive diurnal sediment-pore water oxygen fluctuations from 20-180% saturation due to primary production in the upper oxygenated layer of the sediments (Wenzhöfer & Glud, 2004).

When it comes to the cycling of calcium carbonate in marine sediments, it is also closely linked to the respiration of organic matter. Krumins et al., 2013, used a mono-dimensional reaction transport model to assess the flux of alkalinity in coastal sediments, effectively demonstrating that an estimated 50% of the alkalinity flux was attributed to calcium carbonate dissolution out of shallow, non-photic sediments. The residual 50% is likely linked to the generation of alkalinity by means of suboxic metabolism (Krumins et al., 2013). However, the dominant driving force on variability in carbonate chemistry remains the continued uptake and release of CO_2, which directly affects speciation within the carbonate system (Waldbusser & Salisbury, 2014). There is also increasing evidence that suggests that oceanic nitrogen (N_2) fixation can be enhanced by diazotrophic processes can be enhanced under these conditions, and should this phenomenon occurs on a larger scale, it will mean that this is an essential nitrogen source that can play an important part in regulating net carbon fixation (Bombar et al., 2011; Subramaniam et al., 2008). Finally, the advective inputs

of water from tidal flats, marshes, or groundwater sources are also thought to be strong contributors of carbonate chemistry variation in shallow coastal waters through impacts on sediment pore water exchange (Waldbusser & Salisbury, 2014). The carbonate chemistry, and therefore the pH of the coastal and nearshore ecosystem is not static, where spatiotemporal variations governed by these processes (both natural and anthropogenic in their disturbance), result in a dynamic range of coastal carbonate system parameters often outside the end-of-century projections (Waldbusser & Salisbury, 2014).

1.5 Coastal Benthic Sediment Heterogeneity

1.5.1 Benthic Sediment Heterogeneity

Benthic heterogeneity is closely linked to variability in sediment composition and type, where for instance, cohesive and non-cohesive sediments demonstrate notably different nutrient, carbon, and oxygen dynamics (Hicks et al., 2017). Coastal non-cohesive sediments exhibit incredibly high rates of carbon oxidation when compared to deep ocean sediment systems and are important biological carbon-sequestering sites (Burdige, 2006; Ståhl et al., 2004). More cohesive sediments such as estuarine muds characteristically have greater organic content, mainly comprised of silt or clay (Black et al., 2002). These sediments are highly chemically and biologically active, with the diffusive processes being the most dominant aspect of their biogeochemistry (Hicks et al., 2017). The less cohesive or sandy sediments are much more permeable and therefore driven more by advective flow transport than diffusion (Huettel et al., 2003). These two contrasting sediment types display unique biological, physical, and chemical activity, a level of variation that is also likely to translate to differences in microbial community response to ocean acidification based on the composition and potential buffering capacity of the surrounding sedimentary environment (Currie et al., 2017).

Benthic heterogeneity may also be influenced by other physical or chemical factors (i.e., DIC, DOC, salinity, alkalinity), in addition to sediment composition, such as gradients in salinity, tidal action, or wind-generated wave fetch organic content and particle distribution, and hydrodynamic or hydrological processes (Norkko, Cummings, Thrush, Hewitt, & Hume, 2001; T. Ysebaert et al., 2003)(Norkko, Cummings, Thrush, Hewitt, & Hume, 2001; T. Ysebaert et al., 2003)(Norkko, Cummings, Thrush, Hewitt, & Hume, 2001; T. Ysebaert et al.,

2003)(Norkko, Cummings, Thrush, Hewitt, & Hume, 2001; T. Ysebaert et al., 2003)(Norkko, Cummings, Thrush, Hewitt, & Hume, 2001; T. Ysebaert et al., 2003)(Norkko, Cummings, Thrush, Hewitt, & Hume, 2001; T. Ysebaert et al., 2003)(Norkko et al., 2001; Snelgrove & Butman, 1995; Turner et al., 1997; Warwick et al., 1991; Warwick & Uncles, 1980; Ysebaert et al., 2003). Benthic variability in soft coastal region sediments is linked to the spatial and temporal variability of macrobenthic communities in terms of sediment type (spatially) and seasonal fluctuation (temporally) (Braeckman et al., 2014). The biogeochemical cycling processes of these communities are likely to be influenced by seasonal fluctuations (e.g., environmental variation) or the variability in community abundance and distribution within the stratified sediment layers (i.e., temperature change) (Ouellette et al., 2004; Maire et al., 2007; Braeckman et al., 2010).

1.5.2 Bioturbation

Coastal sediments are highly stratified systems that are closely interlinked with the microbial communities within; and where the vertical zonation of the bacteria in the sediments can be influenced by macrofaunal activity. The direct influence is dictated by the availability of O_2, changes in pH, pCO_2, organic matter concertation, and the bioavailability of essential nutrients (Torsvik et al., 2002; Horner-Devine, Lage, Hughes, & Bohannan, 2004; Lozupone & Knight, 2007). Bioturbation is described as the physicochemical disturbance of a sediment body by macrofaunal or meiofaunal organisms. This disturbance is a known driver of microbial dynamics by affecting community composition, structure, and diversity within the sediment as a result of large, complex burrow systems made by various benthic taxa (Griffis & Suchanek, 1991; Laverock, Tait, Gilbert, Osborn, & Widdicombe, 2014; Laverock et al., 2010; Papaspyrou et al., 2005). Complex burrows formed by bioturbating macrofaunal organisms create a distinct habitat structure, which changes the topography of the benthic sediments and creates pockets of nutrient deposition and oxygen availability to the stratified and potentially previously anoxic regions of the sediment (Reise, 2001).

The direct effects of bioturbation include the addition of resuspended sediment in the water column and surrounding surface sediments, the fractionation and agglutination of sediment particle composition, the distribution and diffusive mixing of sediment particles, and the fluidizing and loosening of the surrounding sediment (Reise, 2001). Indirect effect includes the changing of the microbial metabolic process and surrounding sediment

chemistry, the drainage of the sediment during tidal cycling, the flux of dissolved nutrients, metabolites and substances in the overlying water column, and the alteration of the sediment topography, ventilation, and nearshore hydrodynamics (Reise, 2002). Collectively, bioturbation modifies the sediment profile and regularly alters the bioavailability of O_2 and nutrients to the surrounding sediments, where the irrigational mixing process of bioturbation on the sediment shows clear effects on microbial community structure, diversity, distribution, and relative abundance (Laverock et al., 2011; Bonnie Laverock et al., 2010b; Vopel et al., 2007). The infaunal burrow walls alone induce a 10-fold higher number of bacteria than compared to the surrounding surface sediment (Papaspyrou et al., 2005). This selectivity is thought to be a result of increased organic matter availability, the abundance of biopolymers, and the extension of the oxic-anoxic interface within the burrow compared to the external sediment, all of which influences solute transport and redox potential (Kristensen, 2000). For example, burrowing structures formed by *thalassinidean* shrimp have been shown to enhance the nitrification potential in the walls of the burrow, and increase the rate of denitrification in the proximal sediment, as well as increasing the efflux of dissolved inorganic nitrogen out of the sediment (Andrea et al., 2004; Howe et al., 2004; Webb & Eyre, 2004). Temporal variability in the abundance and activity of nitrifying microbial communities caused by bioturbation could have magnifying effects on the seasonal dynamics and fluxes of nitrogen between the sediment-water interface, effectively altering biogeochemical processes in the benthic ecosystem (Laverock et al., 2014). Given that bioturbating macrofauna are known to enhance the flux of dissolved inorganic nitrogen across the sediment-water interface, in addition to increasing the rates of denitrification and coupled denitrification, understanding the response of bioturbating species to the effects of ocean acidification is important for interpreting the associated effects for bacterial communities in the benthic sediment (Blackford & Gilbert, 2007).

1.6 Coastal Benthic Sediment Microbial Response to Ocean Acidification

Microorganisms are among the most abundant biological species on the planet, accounting for the greatest (unseen) fraction of biomass and biodiversity in the world (Whitman et a., 1998; Tseng & Tang, 2014). All life in the ocean is dominated by these microorganisms, which drive biogeochemical cycles, recycle nutrients, organic carbon, regulate gaseous atmospheric composition, and breakdown chemical pollutants in the

environment (Azam & Malfatti, 2007; Karl, 2007; Kerfahi et al., 2014; Nunes-Alves, 2015). The benthic sediments are a vast reservoir for marine biodiversity, accounting for an estimated 98% of all marine species (Widdicombe & Spicer, 2008). These bacteria drive the transformation of nitrogen within the nitrification process, thereby providing an essential link between the degradation of organic matter and the regeneration of nutrients, which aids in support of ecosystem-wide primary productivity (Nixon, 1981; Graf, 1992; Valiela, 1995; Raffaelli et al., 2003; Bianchi, 2007; Doney et al., 2009; Torsvik et al., 2002; Horner-Devine et al., 2004; Lozupone & Knight, 2007). Anthropogenic disturbances in the coastal environment are known to alter microbially-mediated biogeochemical transformations of nutrients (i.e., Nitrogen (N) cycle) (Hutchins & Fu, 2017; Kitidis et al., 2017), where the effects of OA on specific N-cycle pathways suggests that nitrification (i.e., oxidation of NH_{4+} to NO_{3-}) is one of the more sensitive pathways to changes in pH (Suzuki, Dular, & Kwok, 1974). This is a result of the decline in the availability of ammonia (NH_{3-}) for nitrifying microbial communities (Suzuki, Dular, & Kwok, 1974).

Current studies indicate that an acidified ocean will result in increased levels of N_2, with simultaneous decreases in nitrification-based processes (Hutchins et al., 2009). This would result in a reduced supply of oxidized nitrogen substrate for the denitrifiers by decreasing the levels of nitrate-supported primary production (Hutchins et al., 2009). Ultimately this could initiate a dramatic shift in plankton composition (Hutchins et al., 2009). Micro-scale redox gradients within coastal sediments influence the interaction between aerobic and anaerobic nitrogen (N) cycling processes (Smith et al., 2015). Within the surficial layers of the sediments, the availability of oxygen and ammonium are responsible for the promotion of nitrification in terms of supporting anaerobic microbial community activity (Smith et al., 2015). Nitrification (oxidation of ammonia to nitrate from nitrite) is performed by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Watanabe et al., 2015). The primary step in the nitrification process (ammonia oxidation) requires the use of NH_3 rather than NH^{4+} (Suzuki et al., 1974; Ward, 1987). Under lower pH conditions, the pH buffering capacity of seawater shifts the NH_3/NH^{4+} ratio in favor of the NH^{4+} ion (Braeckman et al., 2014). Given that nitrification is the microbial oxidation of ammonia to nitrate from nitrite, it is responsible for playing a major role in the coupling of organic nitrogen mineralization to denitrification, where the impact of this shift, therefore, has the ability to

weaken the nitrate sink within the sediment and disrupt the functional role of nitrifying AOB and AOA on a microbial level (An & Joye, 2001; Crowe et al., 2012).

A study by Martens-Habbena et al. (2009) suggests that AOA have a greater substrate affinity for ammonia than AOB. This suggests that AOA have a competitive advantage over AOB in reduced or low pH environments (Martens-Habbena et al., 2009; Watanabe et al., 2015). In extension, elevated CO_2 levels and reduced pH may be an important factor influencing AOA and AOB ratios in sediments under future ocean acidification regimes (Bouskill et al., 2012; Bowen et al., 2013; Tait et al., 2014). A study conducted by Braeckman et al. (2014) indicated that the effects of ocean acidification in coastal marine sediments would likely reduce community O_2 consumption and nitrification by 60%. Additional research has also demonstrated that benthic microbial communities which participate in the nitrification process within fine-grained sandy sediment vs. permeable sediment material were more likely to be adapted to low pH conditions as $CaCO_3$ mineral dissolution will act as a buffer for pH fluctuations within the water column (Wenzhöfer et al., 2001; Morse et al., 2006; Widdicombe et al., 2011; Braeckman et al., 2014). As stated earlier, a reduction in seawater pH has been shown to have a significant impact on both benthic bioturbation and bio-irrigational processes, effectively altering redox gradients and the subsequent composition of the benthic microbial community (Godbold & Solan, 2013; Laverock et al., 2013; Tait et al., 2013). The increased level of CO_2 is thought to affect bioirrigation processes and bioturbating macrofaunal species by reducing the NH_3 and O_2 concentration available for nitrification and altering the balance of microbial oxidizers (Laverock et al., 2013; Tait et al., 2014). This can be particularly important as bioturbation and irrigation bring O_2 into direct contact with solid sulfides changing the microbial remineralization processes within the sediment (Aller, 1982; Howarth, 1984; McNichol et al., 1988).

Benthic nitrification in response to elevated CO_2 and reduced pH is highly complex in nature (Laverock et al., 2013; Tait et al., 2014). An analysis by Lidbury et al., 2012 concluded that the community composition of microorganisms along a marine CO_2 gradient would be compositionally or structurally altered as a result of the effects of ocean acidification. A meta-analysis by Liu et al., (2010) indicated the potential for microbial communities to adapt to decreased pH by genetic modification occurring at the species level and through the replacement of the more sensitive species with the less sensitive species at the community

level (Liu et al., 2010; Kerfahi et al., 2014). With the potential for ocean acidification to alter bacterial community composition, it is highly likely that the biogeochemical functionality of marine sediments will be affected (Johnson et al., 2013). Changes in microbial community composition within the sediments are also likely to affect the turnover of organic material and the supply of nutrients, eventuating in a decrease in overall primary productivity (Tait et al., 2013). However, a mesocosm study by Kitidis et al., 2011, demonstrated that the benthic ammonia oxidation process across various different sediment types was not affected by elevated CO_2 concentrations. The lack of response to the increased levels of CO_2 and reduced pH may be a result of high buffering capacity in marine sediments or the potential adaptation of ammonia-oxidizing microorganisms within the sediment (Watanabe et al., 2015). This also suggests that a change in community composition and structure of the present oxidizing microorganisms allows for the nitrification process to be maintained under low pH conditions, possibly by means of functional redundancy (Tait et al., 2014). However, the impact of ocean acidification on microbial processes in marine sediments is still quite novel in relation to significant environmental changes (Laverock et al., 2013).

1.7 Suspected Impacts of Ocean Acidification on Microbial Processes and Dynamics

The continued enrichment of anthropogenic CO_2 into the ocean is anticipated to have far-reaching consequences for marine biogeochemistry and associated marine microbial communities. Shifts in heterotrophic bacteria in response to the effects of ocean acidification are thought to have residual impacts on the rest of the microbial loop (Weinbauer et al., 2011). Deviations on a cellular level could translate to a whole community-level response in terms of composition, diversity, and structure that could result in the subsequent alteration of major associated biogeochemical processes (Joint et al., 2009). Higher CO_2 and lower pH environments have been shown to disrupt natural enzymatic activity where the degradation and recycling processes are directly impacted (Yamada & Suzumura, 2010). In the marine microbial loop and biogeochemical cycling, hydrolytic enzymes are known to play an important role, where bacterial enzymatic hydrolysis promotes the regeneration of DOM (Dissolved Organic Matter) nutrients and trace metals from the breakdown of particulate matter and corresponding organic aggregates (Azam & Long, 2001; Smith et al., 1992). When it comes to the regeneration of organic nutrients, the process of bacterial enzymatic hydrolysis is critical for sustaining total ocean primary productivity (Hoppe et al., 2002).

Decreases in seawater pH have been shown to disrupt this natural enzymatic activity by decreasing protease, glucosidase, and even lipase activity which are important promoters of the degradation of marine organic matter (Yamada & Suzumura, 2010). In addition, decreasing oceanic pH levels have also been shown to directly affect marine polysaccharide degradation (Piontek et al., 2010). Other processes are also susceptible to the effects of OA, such as microbial nitrogen fixation, which is a major source of nitrogen input into the ecosystem(Sohm et al., 2011). Nitrogen fixation generates *new* bioavailable nitrogen for the rest of the marine ecosystem, aiding in the support of biological carbon export and subsequent sequestration (Sohm et al., 2011). Reductions in seawater pH have been shown to have both positive (increasing) and negative (decreasing) N_2 fixation effects on the microbial community depending on the microorganism and the availability of trace metals (i.e., iron) (Shetye et al., 2013).

Ocean acidification has also had a measurable effect on microbial community composition and structure, likely to result in other substantial ecological effects (Weinbauer et al., 2011). The knowledge of pelagic and benthic microbial diversity is limited, as much of the microbial consortia fall under the category of viable yet uncultivatable, where only a small subset of taxa have been successfully isolated and cultured (Dash et al., 2013). This remains one of the many reasons why microorganisms are less than ideal model organisms for predicting the effects of ocean acidification (Das & Mangwani, 2015a). The response of marine microbial communities to ocean acidification has resulted in varying taxonomic responses where high CO_2 environments have both positively and negatively affected composition and structure (Ibrahim et al., 2014; Krause et al., 2012; Liu et al., 2010; Raulf et al., 2015). This could suggest that species specificity in response to ocean acidification differs amongst organisms as a result of natural selective processes (Collins et al., 2014). However, other studies such as Maas et al., 2013 demonstrated that exposure time (i.e., acidified incubation period) varied bacterial diversity, leading them to speculate that *prolonged* exposure to low pH environments will substantially modify the composition and structure of the modern ocean. They summarized that the diversity of marine microbial communities is not solely dependent on pH changes at the individual level but that there is greater ecological connectivity than previously explored (Maas et al., 2013).

1.8 Current Methods and Previous Limitations

1.8.1   Previous Limitations

Microbial communities are recognized for their role in the biogeochemical cycling of major elements and essential ecosystem-based functions, but in the past, it was difficult to assess the extent of microbial diversity and subsequent functionality within the ocean (Zinger et al., 2011), where >99% of marine microorganisms have not been cultured under standard laboratory conditions (Hofer, 2018; Jiao et al., 2021). Neither the patterns and drivers of this diversity nor the corresponding metabolic and genetic inventory associated with these microorganisms could be thoroughly explored in full detail (Zinger et al., 2011). This invoked ecological questions that were previously hindered by the limitation in sampling efforts and methodologies for assessing marine microbial communities (e.g., cultivation resistance) (Stingl et al., 2007). The earlier advancements in molecular (cultivation-independent) techniques led to the exploration of marine microorganisms in unprecedented detail, which provided a description of their microbial diversity in new and comprehensive ways (Sogin et al., 2006; Caporaso et al., 2011). Since cultivation-independent methods were first pioneered by the early cloning and sequencing studies, there have been tremendous advancements in high throughput DNA sequencing technologies (Sogin et al., 2006; Caporaso et al., 2011).

Over the past 20 years, sequencing technologies have grown and evolved (Krehenwinkel et al., 2019; Metzker, 2010; Reuter et al., 2015) This is evidenced by the application of massive high-throughput sequencing for ribosomal genes where the 16S rRNA genes are routinely amplified with the use of PCR (Polymerase Chain Reaction) from DNA that has been extracted from just about every niche in marine systems, including sediments (Ryan et al., 2006; Wasmund et al., 2021). The progression of high-throughput sequencing technologies has opened new doors when it comes to investigating the microbial taxa in a given environmental sample (Cordier et al., 2021; Reuter et al., 2015). The utilization of the 16S rRNA gene for phylogenetic analyses is essential for providing insights into the microbial biodiversity and dynamics of entire communities (Cordier et al., 2021; Fadeev et al., 2021; Ghate et al., 2021). In the past two decades, 16S rRNA gene-based molecular surveys have been thoroughly standardized (i.e., The Earth Microbiome Project) (Gilbert, Jansson, & Knight,

2014)) for the investigation of microbial diversity and ecology, ranging from more coastal and estuarine systems to that of deep-sea hydrothermal vents (Geobel & Stackebrandt, 1994; Baker & Banfield, 2003; Johnson & Hallberg, 2003; Zinger et al., 2011; Kuang et al., 2013; Chen et al., 2015).

1.8.2   Current Methodologies

The application of 16S rRNA gene-based analyses (16S rDNA and 16S rRNA) for the assessment of microbial community composition, structure, and diversity has been a method of standard practice for microbial ecologists for over twenty years and remains a useful tool for assessing environmental samples (Cordier et al., 2021; Reuter et al., 2015; Shokralla et al.,, 2012). Some of the benefits of quantifying microbial community compositional changes with 16S rRNA amplicon sequencing include: the effective detection of genetic variation embedded in complex genetic backgrounds, a more cost effective technology than untargeted sequencing particularly when there are large quantities of undesired genetic material present (i.e., host associated microbial population, genes in large genomes), and it is a more precise, sensitive, and fiscal sequencing tool that has been applied by thousands of published scientific studies each year (Callahan et al., 2019). However, the application of the 16S rRNA gene for DNA analysis is limited in its ability to detect the metabolically active members of the community or describe which bacterial genes are being up regulated or down regulated in response to low pH regimes, though 16S rDNA is highly informative for identifying potential shifts in community structure or composition in response to these reductions in pH (Webster et al., 2013). This is critical to note, as structural or compositional deviations within the community at low pH may be indicative of a possible destabilization in the microbial majority, with even greater potential consequences for the functionality and productivity of the coastal marine ecosystem (Maas et al., 2013).

Despite overcoming large challenges in sequencing costs and computational requirements, 16S still remains the most appropriate method for studying microbial taxonomy where ribosomal genes are not well assembled with MAGS (metagenome assembled genomes), and nanopore is still highly error prone, and the high nucleotide diversity within sediments makes it exceptionally changeling to address compositional changes (Branton et al., 2008; Setubal, 2021). 16S cDNA does provide information regarding

which members of the community are metabolically active at low pH, without elucidating their functional role (Tait et al., 2013). The active cDNA community is also used to inform microbial plasticity and resistance to shifts in pH and can be used to assess the ecological sensitivity or physiological robustness of certain taxonomic individuals to the effects of global anthropogenic ocean acidification and regional eutrophication-induced acidification. Both 16S rDNA and 16S rRNA are useful for discerning the response of marine microbial communities to changes in the oceanic climate (i.e., ocean acidification) (Coelho et al., 2016; K. Tait et al., 2013, 2014).

Following the use of compositional analyses, the natural "omic" progression would be the use of metagenomic (metabolic functional inference) or metatranscriptomic approaches (gene expression and functional activity), to evaluate the genetic (upregulation or down regulation of genes) response of sensitive taxa to low, and to determine the functional role of certain bacteria within the community under acidified conditions (Chen et al., 2015; Cordier et al., 2021; Rinta-Kanto et al.,2012) The pace and progression of the use of environmental genomics technologies enabled High Throughput Sequencing (HTS) methodologies which illustrates a transformation in our ability to document natural microbial biodiversity within any ecosystem (Cristescu & Hebert, 2018; Escalas et al., 2019; Quince et al., 2017; Ruppert, Kline, & Rahman, 2019; Singer et al.,2017).

The direction of HTS techniques shows that the application of metagenomic and metatranscriptomic methodologies for environmental assessment are becoming the more informative and descriptive of available technologies to date, shifting ahead of amplicon sequencing and metabarcoding in their ability to explain system wide response and activity on a microbial scale (Aguiar-Pulido et al., 2016; Chen et al., 2022; Nancy, Boparai, & Sharma, 2021; Song et al., 2021). The use of 16S rRNA amplicon sequencing analysis comes with its own set of inherent limitations for understanding how the microbial community responds to an imposed disturbance (Callahan et al., 2019; Poretsky, Rodriguez-R, Luo, Tsementzi, & Konstantinidis, 2014). Therefore, given this limitation, we recommended that when conducting environmental analyses, both general surveying techniques (amplicon sequencing/metabarcoding) be coupled with metagenomic or metatranscriptomic technologies for optimal genetic coverage of community wide activity and response.

Chapter 1: A Review of The Literature

1.9 Summary

Microorganisms within the sediment are vital players when it comes to the cycling of nutrients throughout the marine system and the overall support of oceanic primary productivity (Bolin & Cook, 1983). The sustainability and reliability of ecosystem-based primary productivity are also paramount for providing insights into future coastal-ocean responses to impending anthropogenic impacts. It is well established that pH plays an important environmental and ecological role for microbial communities in terms of influencing enzymatic activities such as energy respiration, physiology, and growth, and as highlighted earlier, pH is *a,* if not *the* critical environmental parameter governing the composition, structure, and function of microbial communities (Hutchins et al., 2009; Fabry & Balch, 2010; Liu et al., 2010; Beman et al., 2011; Joint et al., 2011). This is, however, further influenced by the complexity and high degree of heterogeneity within the coastal benthic environment. This variability also creates habitat heterogeneity for the microbial communities within marine sediments and adds an additional level of spatial variation that makes predicting bacterial response to ocean acidification that much more challenging. This is in addition to the entropic contribution of seasonal dynamics, diel and diurnal cycling, the turnover of organic matter, and natural nutrient bioavailability. Analyses of microbial communities along spatial and temporal gradients that address this inherent heterogeneity within the system are of critical importance for elucidating questions about which factors drive microbial community composition and structure (Scala & Kerkhof, 2000). The coastal zone remains one of the most complex and dynamic ecosystems on the planet, where the benthic microbes are exposed to a higher degree of stress than that of open ocean systems based on terrestrial nutrient input, tidal cycling, upwelling, and seasonal variation (Harley et al., 2006; Doney, 2010; Aufdenkampe et al., 2011; Duarte et al., 2013). Current pH IPCC predictions for the year 2100 are already exceeded on a daily basis in coastal marine systems, indicating the potential for these environments to be more readily adapted to low pH regimes than their more stable open ocean counterparts; simply owed to the daily disturbances that occur in these systems naturally (Borges & Gypens, 2010; Provoost et al., 2010; Hofmann et al., 2011; Cai et al., 2011; Duarte et al., 2013).

Changes in oceanic pH can cause significant ecological community restructuring within the coastal ecosystem, manifested in the form of reductions in ecosystem function via the

removal of the less tolerant species (Fischlin et al., 2007). This restructuring could also mean the change of compensatory roles among functionally similar organisms or species through the process of functional redundancy, where the function is maintained but is now performed by a different organism (Ives, Gross, & Klug, 1999; McNaughton, 1977; Tilman, 1996). However, it is important to note that the replacing of numerous species by other species to maintain an ecosystem function is not likely to compensate the system enough to stabilize it, particularly when numerous species respond in the same way to environmental disturbance (Fischer, Frost, & Ives, 2001). Various ocean acidification studies have used mortality as a metric for understanding an organism's sensitivity or resistance to low pH environments. However, if the exposure to low pH does not determine the death of an individual, that is not a true measure of the robustness or intolerance of that organism's population or community (Widdicombe & Spicer, 2008). However, even if the community or population was not fatally disrupted by reductions in pH, it cannot be assumed that there is no residual physiological impact on the community to the disturbance, as in trade-offs between running maintenance processes such as respiration, growth, or reproduction which could imply that the community is still vulnerable to large reductions in pH (Widdicombe & Spicer, 2008). Additionally, it is important to examine whether the community members who are responding are on the fringes of that community and if they are displaying signs of intolerance, given that they are already operating on the edges of their tolerance range.

2.0 This Study

The previously discussed information in terms of current research and methodologies became the collective knowledge base that was used as the foundation for the question driven hypotheses and experimental design. Microbial communities are the unseen majority of the marine ecosystem that drive all major biogeochemical cycles within the ocean which maintain the functionality and regulation of the global climate (Azam & Malfatti, 2007; Karl, 2007; Kerfahi et al., 2014; Nunes-Alves, 2015). As the oceans absorb more and more CO_2 over time, resulting in the effects of ocean acidification, structural or compositional changes on the microbial level could equate to severe destabilization of the entire ecosystem and the subsequent services it provides (i.e., primary productivity). Each of the following experiments seeks to investigate the response of sediment microbial communities from coastal and nearshore habitats to high CO_2 and low pH regimes, where each study introduces another

level of environmental complexity. The gradient of experiments outlined in this chapter share a commonality, where each experiment tries to identify the resistance of the microbial communities to both natural and anthropogenically driven changes in seawater pH ranging from a heavily controlled and simplified intertidal environment to a increasingly complex subtidal system that examines both OA and eutrophication induced acidification.

2.0.1 Chapter 2: Experiment 1

Given the extreme pH oscillations that are observed on a seasonal or even daily basis within the intertidal zone permits us to hypothesize that benthic sediment bacterial communities endemic to this dynamic intertidal region will display increased levels of compositional plasticity and structural robustness on a community level in response to reductions in seawater pH. To assess the supposed inherent durability of benthic microbial communities to the effects of ocean acidification, we spatially sampled sediments along an intertidal sand flat with low incidents of human-related impact during tidal exchange, in Tauranga Harbour, in New Zealand. Where sediment was collected with in a small 1 x 2 m quadrat by depth intervals. Intertidal sediment cores that were first homogenized and sieved at different depth intervals prior to restructuring were incubated in a flow-through pH-controlled mesocosm used to simulate 3 different environmental pH treatments based on IPCC (Intergovernmental Panel on Climate Change) projections for 2100 (Figure 2.). Here we apply two highly contrasting pH treatments used to measure the proverbial breaking point of the community, where the aim of this study is not terribly dissimilar from a toxicological survey of the saturation point of communities under increasing levels of CO_2. If the community experiences a sudden unexpected shock to the system how capable are the bacteria of reacting in a way that allows them to sustain function? Conversely, if the community is carefully walked through subtle changes in surrounding pH are we still able to measure a response to even slight variations in the environment? Could this indicate that the responding bacteria are biologically sentient to these micro disturbances and may provide insights in their role as potential sentinels (i.e., bioindicators) to anthropogenic impact within the environment and further reinforcing their significance within the coastal ecosystem? The first pH treatment captures the extreme pH dynamics observed in the intertidal zone, with pH values that sit just outside the lowest predicted pH levels for futuristic 2100 oceanic

conditions. With the justification that under current predicted global atmospheric CO_2 emissions scenarios, planetary seawater pH will continue to decline further after the year 2100. Additionally, as these communities are regularly exposed to fluctuations in pH, in order to elicit a measurable response from the community, using pH values that extend beyond their respective tolerance ranges was imperative for identifying just how truly resistant they are too high CO_2 environments.

The second pH treatment served a dual purpose, acting as an additional pseudo control to the first experimental pH treatment, where incrementally changing the pH slowly over time allowed us to capture the subtle responses of the bacterial community at each interval. By using a rapid shift approach as shown in the first pH treatment subtle responses from the bacteria may otherwise be overlooked as background noise or misinterpreted as a stress-like response. Therefore, the stepdown approach allows us to systematically shift the pH along a gradient over an extended period of time to observe even slight variations in the community and at which point the greatest level of sensitivity to high CO_2 was detected. This stepwise treatment was also designed to mirror a more stable marine ecosystem (i.e., subtidal) providing a rudimentary side-by-side comparison of these two contrasting oceanic zones. Setting the stage for the subtidal work that was conducted in both the second and third research experiments.
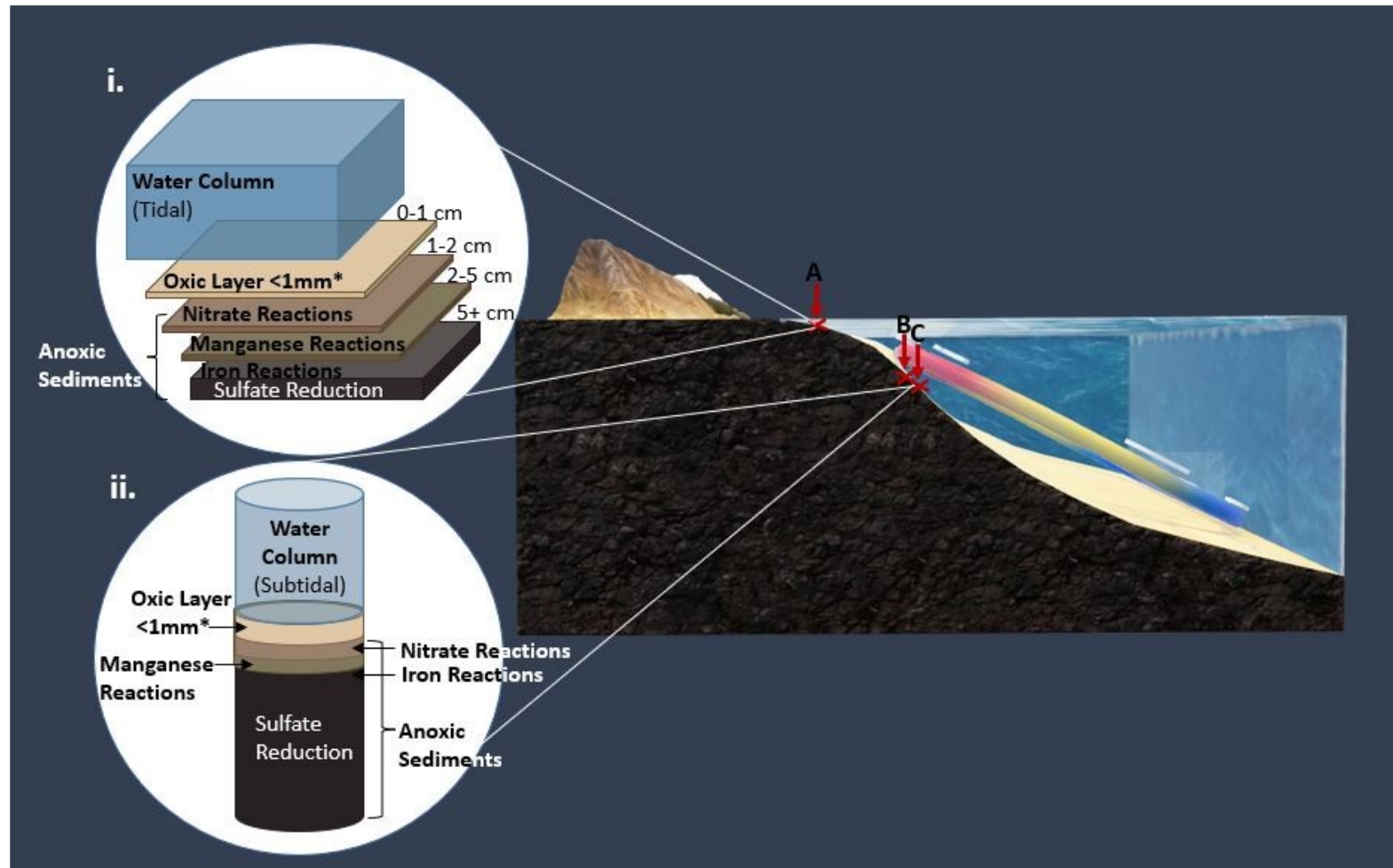
Figure 2. A side profile of the coastal marine ecosystem model shown in Figure 1. Providing a different look at the areas of interest used in this study (A: Experiment 1. (Intertidal); B: Experiment 2. (Subtidal); C: Experiment 3. (Subtidal)). **i.** is an elaboration of how the intertidal benthic sediment for the first experiment was manually sectioned and sampled by depth intervals, **ii.** also elucidates the sampling process applied in the second and third subtidal experiments using a modified gravity core approach. In all experiments, molecular analysis was targeted at examining the uppermost oxygenated portion of the sediment (~1 mm), directly interfaced with the overlying water column. A modified Microsoft© Open Access Model used with permission.

2.0.2 **Chapter 3: Experiment 2**

Following on from the previous work done in chapter 2, a second experiment was implemented that branched off from the knowledge gained from that first study Here we addressed a more stable and consistent environment (i.e., subtidal zone)(Figure 2.) based loosely on what we observed in the stepwise treatment utilized in chapter 2, here we hypothesized that bacterial communities collected from the subtidal region would select for inherently less compositional plasticity compared to the intertidal consortia. However, though we anticipate reduced levels of plasticity in the subtidal communities, we also predict that the majority of the bacterial core community will remain relatively unaffected by the newly acidified environment. We applied a very similar experimental design approach as seen in the previous experiment (Chapter 2: Experiment 1), but now using whole intact sediment cores from the sublittoral zone. This sublittoral (i.e., subtidal) region operates as the intermediary between the active intertidal zone and the deep open ocean and is considered a much less physically dynamic environment compared to the intertidal ecosystem. The step-down approach applied in Chapter 2. where the pH was incrementally reduced over time to mimic a more natural pH gradient characteristic of the subtidal zone in what was determined to be the more conservative of the two treatments, was again reapplied in this experiment across 4 independent mesocosm chambers, each with its own environmental pH condition. Our main aim in this study was to take what we learned from the previous experiment, to strengthen the experimental design, to reinforce our statistical power in finding the most appropriate way to test different pH conditions across a very consistent microbial community. As previously mentioned, the stepwise treatment was identified as being the more controlled, conservative, and environmentally realistic treatment method. By repurposing for this study we are able to test our question driven hypotheses but also to go back and reinforce our experimental design approach in the previous chapter (chapter 2.) by comparing the results from both studies. The reoccurrence of certain taxa between both studies could indicate a pattern of microbiological activity where particular responding individuals could be reflective of changes in the environment (i.e., bioindicators).

Microelectrode oxygen profiling was used to inform the starting point for the experiment based on an established redox profile. The system was maintained in total

darkness to limit the impact of photosynthetic organisms. With advanced bioinformatic and statistical approaches, 16S rRNA and rDNA sequencing data were used to resolve the response of the subtidal benthic bacterial community to a lower pH exposure and to identify members of the community who demonstrate sensitivity to these new conditions.

2.0.3 **Chapter 4: Experiment 3**

In this final mesocosm experiment, we sought to understand how subtidal sediment augmented with increased organics and/or calcium carbonate would respond to ocean acidification (Figure 2.). We hypothesized that subtidal sediment microbial communities that were amended with $CaCO_3$ addition would be more structurally and compositionally resolute than sediment communities that did not receive $CaCO_3$ as a result of the buffering capacity provided by the calcite amendment. We postulated that buffering the sediments with $CaCO_3$ will effectively resolve whether the microbial community is inherently resilient under low pH or if the geochemical properties of the sediment and porewater are providing transient protection. This study also looks at both the global anthropogenic impacts of ocean acidification in conjunction with the regional and local disturbances of coastal eutrophication-induced acidification, which maintains a compounding effect on seawater pH characteristic of nearshore environments, where the regional driver may be the more pronounced of the two effects. This study combines the collective sum of knowledge gained between the first (Chapter 2.) and second (Chapter 3.) experiments. The data and results of which were used to construct the experimental design used in this study. This final research chapter brings together all levels of controllable abiotic and biotic variable complexity to reenact an environmental system that would be a as reflective of a natural environment as possible. Using four independent mesocosm chambers, we ran two pH treatments (7.7) and two pH controls (8.1) for each of the treatments. From there, we exposed the sediment cores to two different types of amendments, organic matter (e.g., brown algae) and calcium carbonate (e.g., finely crushed oyster shell hash). Another notable difference between this experiment and the previous experiments is that this system was run on a 24-hour dark-light cycle to mimic a more natural diel day-night cycling pattern. This coordinated approach allowed us to examine the bacterial response to different environmental factors and to different nutrient treatments. The use of 16S rRNA and rDNA was used to look at the total [DNA] and active

[cDNA] community structure and composition under organically enriched treatments and CaCO$_3$ additions under ambient and low pH conditions.

2.0.4 Chapter 5: A Summary

Here we discuss the intended and unintended outcomes of each of the experimental studies, in addition to the limitations and constraints of each of the experiments, and the future directions for this type of manipulation work.

3.0 References

Adams, R. I., Bateman, A. C., Bik, H. M., & Meadow, J. F. (2015). Microbiota of the indoor environment: a meta-analysis. *Microbiome*, *3*(1), 1–18.

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., & Narasimhan, G. (2016). Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis: Supplementary Issue: Bioinformatics Methods and Applications for Big Metagenomics Data. *Evolutionary Bioinformatics*, *12s1*, EBO.S36436. https://doi.org/10.4137/EBO.S36436

Aller, R. C. (1982). The effects of macrobenthos on chemical properties of marine sediment and overlying water. In *Animal-sediment relations* (pp. 53–102). Springer.

Allison, S. D., & Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, *105*(Supplement 1), 11512–11519.

Allison, S. D., & Martiny, J. B. H. (2009). Resistance, resilience, and redundancy in microbial communities. *In the Light of Evolution*, *2*, 149–166. https://doi.org/10.17226/12501

Alongi, D. M. (1985). Effect of physical disturbance on population dynamics and trophic interactions among microbes and meiofauna. *Journal of Marine Research*, *43*(2), 351–364. https://doi.org/10.1357/002224085788438694

Alongi, D. M., Sasekumar, A., Tirendi, F., & Dixon, P. (1998). The influence of stand age on benthic decomposition and recycling of organic matter in managed mangrove forests of Malaysia. *Journal of Experimental Marine Biology and Ecology*, *225*(2), 197–218. https://doi.org/10.1016/S0022-0981(97)00223-2

Alongi, D M, Pfitzner, J., Trott, L. A., Tirendi, F., Dixon, P., & Klumpp, D. W. (2005). Rapid sediment accumulation and microbial mineralization in forests of the mangrove Kandelia candel in the Jiulongjiang Estuary, China. *Estuarine, Coastal and Shelf Science*, *63*(4), 605–618.

Alongi, Daniel M., de Carvalho, N. A., Amaral, A. L., da Costa, A., Trott, L., & Tirendi, F. (2012). Uncoupled surface and below-ground soil respiration in mangroves: Implications for estimates of dissolved inorganic carbon export. *Biogeochemistry*, *109*(1–3), 151–162. https://doi.org/10.1007/s10533-011-9616-9

Alpert, P. A., Kilthau, W. P., Bothe, D. W., Radway, J. C., Aller, J. Y., & Knopf, D. A. (2015). The influence of marine microbial activities on aerosol production: A laboratory mesocosm study. *Journal of Geophysical Research: Atmospheres*, *120*(17), 8841–8860. https://doi.org/https://doi.org/10.1002/2015JD023469

An, S., & Joye, S. B. (2001). Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnology and Oceanography*, *46*(1), 62–74.

Andersen, K. S., Kirkegaard, R. H., Karst, S. M., & Albertsen, M. (2018). ampvis2: An R package to analyse and visualise 16S rRNA amplicon data. *BioRxiv*, 10–11. https://doi.org/10.1101/299537

Chapter 1: A Review of The Literature

Anderson, M. J., Ford, R. B., Feary, D. A., & Honeywill, C. (2004). Quantitative measures of sedimentation in an estuarine system and its relationship with intertidal soft-sediment infauna. *Marine Ecology Progress Series*, *272*, 33–48. https://doi.org/10.3354/meps272033

Andersson, A. J., & MacKenzie, F. T. (2012). Revisiting four scientific debates in ocean acidification research. *Biogeosciences*, *9*(3), 893–905. https://doi.org/10.5194/bg-9-893-2012

Andersson, Andreas J., Bates, N. R., & Mackenzie, F. T. (2007). Dissolution of carbonate sediments under rising pCO2 and ocean acidification: Observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, *13*(3), 237–264. https://doi.org/10.1007/s10498-007-9018-8

Andersson, Andreas J. (2015). A fundamental paradigm for coral reef carbonate sediment dissolution. *Frontiers in Marine Science*, *2*, 52.

Andersson, Andreas J, Bates, N. R., & Mackenzie, F. T. (2007). Dissolution of carbonate sediments under rising p CO 2 and ocean acidification: observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, *13*(3), 237–264.

Andersson, Andreas J, Mackenzie, F. T., & Lerman, A. (2005). Coastal ocean and carbonate systems in the high CO2 world of the Anthropocene. *American Journal of Science*, *305*(9), 875–918.

Andrea, A. F. D., Brown, C. A., Griffen, B. D., Eldridge, P. M., Dewitt, T. H., Andrea, A. F. D., … Eldridge, P. M. (2004). Impact of burrowing shrimp populations on nitrogen cycling and water quality in western North American temperate estuaries . In : A . Tamaki (ed .), Proceedings of the Symposium on Ecology of Large Bioturbators in Tidal Flats and Shallow Sublittoral Sedi. *Quality*, (June 2014), 107–118.

Anthony, K. R. N., A. Kleypas, J., & Gattuso, J. P. (2011). Coral reefs modify their seawater carbon chemistry - implications for impacts of ocean acidification. *Global Change Biology*, *17*(12), 3655–3666. https://doi.org/10.1111/j.1365-2486.2011.02510.x

Aparicio, F. L., Nieto-Cid, M., Borrull, E., Calvo, E., Pelejero, C., Sala, M. M., … Marrasé, C. (2016). Eutrophication and acidification: Do they induce changes in the dissolved organic matter dynamics in the coastal Mediterranean Sea? *Science of the Total Environment*, *563*, 179–189.

Atkinson, B. L., Grace, M. R., Hart, B. T., & Vanderkruk, K. E. N. (2008). Sediment instability affects the rate and location of primary production and respiration in a sand-bed stream. *Journal of the North American Benthological Society*, *27*(3), 581–592. https://doi.org/10.1899/07-143.1

Aufdenkampe, A. K., Mayorga, E., Raymond, P. A., Melack, J. M., Doney, S. C., Alin, S. R., … Yoo, K. (2011). Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, *9*(1), 53–60.

Auguie, B., & Antonov, A. (2017). *gridExtra*. 1–9. Retrieved from https://cran.r-project.org/web/packages/gridExtra/gridExtra.pdf

Azam, F., & Long, R. A. (2001). Sea snow microcosms. *Nature*, *414*(6863), 495–498.

Azam, F., & Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nature Reviews Microbiology*, *5*, 966. https://doi.org/10.1038/nrmicro1798

Badger, M. R., Price, G. D., Long, B. M., & Woodger, F. J. (2006). The environmental plasticity and ecological genomics of the cyanobacterial CO2 concentrating mechanism. *Journal of Experimental Botany*, *57*(2), 249–265.

Baker, B. J., & Banfield, J. F. (2003). Microbial communities in acid mine drainage. *FEMS Microbiology Ecology*, *44*(2), 139–152. https://doi.org/10.1016/S0168-6496(03)00028-X

Banks, S. C., Cary, G. J., Smith, A. L., Davies, I. D., Driscoll, D. A., Gill, A. M., … Peakall, R. (2013). How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, *28*(11), 670–679.

Barcelos e Ramos, J., Biswas, H., Schulz, K. G., LaRoche, J., & Riebesell, U. (2007). Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer Trichodesmium. *Global Biogeochemical Cycles*, *21*(2).

Barnes, R. S. K., & Hughes, R. N. (1999). *An introduction to marine ecology*. John Wiley & Sons.

Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., & Feelyd, R. A. (2012). The Pacific oyster, Crassostrea gigas, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. *Limnology and Oceanography*, *57*(3), 698–710. https://doi.org/10.4319/lo.2012.57.3.0698

Basso, D. (2012). Carbonate production by calcareous red algae and global change. *Geodiversitas*, *34*(1), 13–33.

Bates, N. R., Astor, Y. M., Church, M. J., Currie, K., Dore, J. E., González-Dávila, M., … Santana-Casiano, J. M. (2014). A time-series view of changing surface ocean chemistry due to ocean uptake of anthropogenic CO2 and ocean acidification. *Oceanography*, *27*(1), 126–141. https://doi.org/10.5670/oceanog.2014.16

Battaglia, G., Steinacher, M., & Joos, F. (2016). A probabilistic assessment of calcium carbonate export and dissolution in the modern ocean. *Biogeosciences*, *13*(9), 2823–2848.

Bauer, J. E., Cai, W.-J., Raymond, P. A., Bianchi, T. S., Hopkinson, C. S., & Regnier, P. A. G. (2013). The changing carbon cycle of the coastal ocean. *Nature*, *504*(7478), 61.

Baumann, H., & Smith, E. M. (2018). Quantifying Metabolically Driven pH and Oxygen Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries and Coasts*, *41*(4), 1102–1117. https://doi.org/10.1007/s12237-017-0321-3

Beman, J. M., Chow, C.-E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., … Hutchins, D. A. (2011a). Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proceedings of the National Academy of Sciences*, *108*(1), 208–213. https://doi.org/10.1073/pnas.1011053108

Beman, J. M., Chow, C. E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., … Hutchins, D. A. (2011b). Global declines in oceanic nitrification rates as a consequence of ocean

acidification. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(1), 208–213. https://doi.org/10.1073/pnas.1011053108

Ben-Yaakov, S. (1973). pH BUFFERING OF PORE WATER OF RECENT ANOXIC MARINE SEDIMENTS. *Limnology and Oceanography*, *18*(1), 86–94. https://doi.org/10.4319/lo.1973.18.1.0086

Bender, E. A., Case, T. J., & Gilpin, M. E. (1984). Perturbation Experiments in Community Ecology: Theory and Practice. *Ecology*, *65*(1), 1–13. https://doi.org/https://doi.org/10.2307/1939452

Berelson, W. M., Balch, W. M., Najjar, R., Feely, R. A., Sabine, C., & Lee, K. (2007). Relating estimates of CaCO3 production, export, and dissolution in the water column to measurements of CaCO3 rain into sediment traps and dissolution on the sea floor: A revised global carbonate budget. *Global Biogeochemical Cycles*, *21*(1).

Berga, M., Székely, A. J., & Langenheder, S. (2012). Effects of disturbance intensity and frequency on bacterial community composition and function. *PLoS One*, *7*(5), e36959.

Berner, R. A., Scott, M. R., & Thomlinson, C. (1970). CARBONATE ALKALINITY IN THE PORE WATERS OF ANOXIC MARINE SEDIMENTS 1. *Limnology and Oceanography*, *15*(4), 544–549.

Bernhard, A. E., Donn, T., Giblin, A. E., & Stahl, D. A. (2005). Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. *Environmental Microbiology*, *7*(9), 1289–1297. https://doi.org/10.1111/j.1462-2920.2005.00808.x

Bianchi, C. N. (2007). Biodiversity issues for the forthcoming tropical Mediterranean Sea. *Hydrobiologia*, *580*(1 LB-Bianchi2007), 7. https://doi.org/10.1007/s10750-006-0469-5

Black, K. S., Tolhurst, T. J., Paterson, D. M., & Hagerthey, S. E. (2002). Working with Natural Cohesive Sediments. *Journal of Hydraulic Engineering*, *128*(1), 2–8. https://doi.org/10.1061/(asce)0733-9429(2002)128:1(2)

Blackford, J. C., & Gilbert, F. J. (2007). pH variability and CO2 induced acidification in the North Sea. *Journal of Marine Systems*, *64*(1–4), 229–241. https://doi.org/10.1016/j.jmarsys.2006.03.016

Blanchet, H., Lavesque, N., Ruellet, T., Dauvin, J. C., Sauriau, P. G., Desroy, N., … de Montaudouin, X. (2008). Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats-Implications for the implementation of the European Water Framework Directive. *Ecological Indicators*, *8*(4), 360–372. https://doi.org/10.1016/j.ecolind.2007.04.003

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., … Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods*, *10*(1), 57–59. https://doi.org/10.1038/nmeth.2276

Bolin, B. (1959). *atmosphere and the sea in motion: scientific contributions to the Rossby memorial volume*.

Bolin, B., & Cook, R. B. (1983). *The major biogeochemical cycles and their interactions (Scope*

Report 21).

Bombar, D., Moisander, P. H., Dippner, J. W., Foster, R. A., Voss, M., Karfeld, B., & Zehr, J. P. (2011). Distribution of diazotrophic microorganisms and nifH gene expression in the Mekong River plume during intermonsoon. *Marine Ecology Progress Series*, *424*, 39–52. https://doi.org/10.3354/meps08976

Bonin, P., Ranaivoson, E. R., Raymond, N., Chalamet, A., & Bertrand, J. C. (1994). Evidence for denitrification in marine sediment highly contaminated by petroleum products. *Marine Pollution Bulletin*, *28*(2), 89–95.

Borges, A. V., Djenidi, S., Lacroix, G., Théate, J., Delille, B., & Frankignoulle, M. (2003). Atmospheric CO2 flux from mangrove surrounding waters. *Geophysical Research Letters*, *30*(11), 12–15. https://doi.org/10.1029/2003GL017143

Borges, Alberto V., & Gypens, N. (2010). Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. *Limnology and Oceanography*, *55*(1), 346–353. https://doi.org/10.4319/lo.2010.55.1.0346

Borgesa, A. V, & Gypensb, N. (2010). Carbonate chemistry in the coastal zone responds more strongly to eutrophication than ocean acidification. *Limnology and Oceanography*, *55*(1), 346–353.

Borrero-Santiago, A. R., Bautista-Chamizo, E., DelValls, T., & Riba, I. (2017). A possible CO2 leakage event: Can the marine microbial community be recovered? *Marine Pollution Bulletin*, *117*(1–2), 380–385. https://doi.org/10.1016/j.marpolbul.2017.02.027

Bosak, T., & Newman, D. K. (2003). Microbial nucleation of calcium carbonate in the Precambrian. *Geology*, *31*(7), 577–580. https://doi.org/10.1130/0091-7613(2003)031<0577:MNOCCI>2.0.CO;2

Bosselmann, K. (2007). *Sulfate reduction and iron-manganese cycling in intertidal surface sediments of the southern North Sea*. Universität Oldenburg.

Boudreau, B. P., Canfeld, D. E., & Mucci, A. (1992). Early diagenesis in a marine sapropel, Mangrove Lake, Bermuda. *Limnology and Oceanography*, *37*(8), 1738–1753.

Boudreau, B. P., Huettel, M., Forster, S., Jahnke, R. A., McLachlan, A., Middelburg, J. J., … Sundby, B. (2001). Permeable marine sediments: Overturning an old paradigm. *Eos*, *82*(11), 133–136. https://doi.org/10.1029/EO082i011p00133-01

Bouillon, S., Middelburg, J. J., Dehairs, F., Borges, A. V., Abril, G., Flindt, M. R., … Kristensen, E. (2007). Importance of intertidal intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania). *Biogeosciences*, *4*(3), 311–322. https://doi.org/10.5194/bg-4-311-2007

Bouillon, Steven, Borges, A. V., Castañeda-Moya, E., Diele, K., Dittmar, T., Duke, N. C., … Twilley, R. R. (2008). Mangrove production and carbon sinks: A revision of global budget estimates. *Global Biogeochemical Cycles*, *22*(2), 1–12. https://doi.org/10.1029/2007GB003052

Bouillon, Steven, Dehairs, F., Velimirov, B., Abril, G., & Borges, A. V. (2007). Dynamics of

organic and inorganic carbon across contiguous mangrove and seagrass systems (Gazi Bay, Kenya). *Journal of Geophysical Research: Biogeosciences*, *112*(2), 1–14. https://doi.org/10.1029/2006JG000325

Bouskill, N., Tang, J., Riley, W., & Brodie, E. (2012). Trait-Based Representation of Biological Nitrification: Model Development, Testing, and Predicted Community Composition. *Frontiers in Microbiology*, *3*(364). https://doi.org/10.3389/fmicb.2012.00364

Bowen, J. L., Kearns, P. J., Holcomb, M., & Ward, B. B. (2013). Acidification alters the composition of ammonia-oxidizing microbial assemblages in marine mesocosms. *Marine Ecology Progress Series*, *492*, 1–8.

Bowman, J. P., & McCuaig, R. D. (2003). Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Applied and Environmental Microbiology*, *69*(5), 2463–2483.

Boyd, P. W. (2011). Beyond ocean acidification. *Nature Geoscience*, *4*, 273. https://doi.org/10.1038/ngeo1150

Braeckman, U., Provoost, P., Gribsholt, B., Van Gansbeke, D., Middelburg, J. J., Soetaert, K., … Vanaverbeke, J. (2010). Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. *Marine Ecology Progress Series*, *399*, 173–186.

Braeckman, U., Van Colen, C., Guilini, K., Van Gansbeke, D., Soetaert, K., Vincx, M., & Vanaverbeke, J. (2014). Empirical Evidence Reveals Seasonally Dependent Reduction in Nitrification in Coastal Sediments Subjected to Near Future Ocean Acidification. *PLoS ONE*, *9*(10), e108153. https://doi.org/10.1371/journal.pone.0108153

Branton, D., Deamer, D. W., Marziali, A., Bayley, H., Benner, S. A., Butler, T., … Schloss, J. A. (2008). The potential and challenges of nanopore sequencing. *Nature Biotechnology*, *26*(10), 1146–1153. https://doi.org/10.1038/nbt.1495

Breiman, L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32.

Brewer, P. G. (1997). Ocean chemistry of the fossil fuel CO_2 signal: The haline signal of "business as usual." *Geophysical Research Letters*, *24*(11), 1367–1369.

Brose, U., & D. Martinez, N. (2004). Estimating the richness of species with variable mobility. *Oikos*, *105*(2), 292–300.

Brussaard, C. P. D., Gast, G. J., Van Duyl, F. C., & Riegman, R. (1996). Impact of phytoplankton bloom magnitude on a pelagic microbial food web. *Marine Ecology Progress Series*, *144*(1–3), 211–221. https://doi.org/10.3354/meps144211

Burdige, D. J. (2012). Estuarine and Coastal Sediments - Coupled Biogeochemical Cycling. In *Treatise on Estuarine and Coastal Science* (Vol. 5). https://doi.org/10.1016/B978-0-12-374711-2.00511-8

Burdige, David J. (2006). *Geochemistry of marine sediments*. Princeton University Press.

Burns, R. A., MacDonald, C. D., McGinn, P. J., & Campbell, D. A. (2005). INORGANIC CARBON REPLETION DISRUPTS PHOTOSYNTHETIC ACCLIMATION TO LOW TEMPERATURE IN THE CYANOBACTERIUM SYNECHOCOCCUS ELONGATUS 1. *Journal of Phycology*, *41*(2), 322–

334.

Burrell, R. B., Keppel, A. G., Clark, V. M., & Breitburg, D. L. (2016). An automated monitoring and control system for flow-through co-cycling hypoxia and pH experiments. *Limnology and Oceanography: Methods*, *14*(3), 168–185.

Burrell, T. J., Maas, E. W., Hulston, D. A., & Law, C. S. (2015). Bacterial abundance, processes and diversity responses to acidification at a coastal CO2vent. *FEMS Microbiology Letters*. https://doi.org/10.1093/femsle/fnv154

Butler, J. N. (1991). *Carbon dioxide equilibria and their applications*. CRC Press.

Byoung-Joon, P., Soo-Je, P., Dae-No, Y., Stefan, S., S., S. D. J., & Sung-Keun, R. (2010). Cultivation of Autotrophic Ammonia-Oxidizing Archaea from Marine Sediments in Coculture with Sulfur-Oxidizing Bacteria. *Applied and Environmental Microbiology*, *76*(22), 7575–7587. https://doi.org/10.1128/AEM.01478-10

Cai, W.-J. (2011). Estuarine and Coastal Ocean Carbon Paradox: CO_2 Sinks or Sites of Terrestrial Carbon Incineration? *Annual Review of Marine Science*, *3*(1), 123–145. https://doi.org/10.1146/annurev-marine-120709-142723

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., … Gong, G.-C. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, *4*, 766. https://doi.org/10.1038/ngeo1297 https://www.nature.com/articles/ngeo1297#supplementary-information

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., … Wang, Y. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, *4*(11), 766–770.

Cai, W.-J., Reimers, C. E., & Shaw, T. (1995). Microelectrode studies of organic carbon degradation and calcite dissolution at a California continental rise site. *Geochimica et Cosmochimica Acta*, *59*(3), 497–511.

Cai, W.-J., Wang, Y., & Hodson, R. E. (1998). Acid-base properties of dissolved organic matter in the estuarine waters of Georgia, USA. *Geochimica et Cosmochimica Acta*, *62*(3), 473–483.

Cai, W. J., Hu, X., Huang, W. J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., … Gong, G. C. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, *4*(11), 766–770. https://doi.org/10.1038/ngeo1297

Cai, W. J., Huang, W. J., Luther, G. W., Pierrot, D., Li, M., Testa, J., … Michael Kemp, W. (2017). Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications*, *8*(1), 1–12. https://doi.org/10.1038/s41467-017-00417-7

Cai, W., & Reimers, C. E. (1993). The development of pH and pCO2 microelectrodes for studying the carbonate chemistry of pore waters near the sediment-water interface. *Limnology and Oceanography*, *38*(8), 1762–1773.

Caldeira, K., & Wickett, M. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical*

Research C: Oceans, *110*(9), 1–12. https://doi.org/10.1029/2004JC002671

Call, M., Maher, D. T., Santos, I. R., Ruiz-Halpern, S., Mangion, P., Sanders, C. J., … Eyre, B. D. (2015). Spatial and temporal variability of carbon dioxide and methane fluxes over semi-diurnal and spring-neap-spring timescales in a mangrove creek. *Geochimica et Cosmochimica Acta*, *150*, 211–225. https://doi.org/10.1016/j.gca.2014.11.023

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., Wong, J., Heiner, C., Oh, S., Theriot, C. M., Gulati, A. S., … Dougherty, M. K. (2019). High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. *Nucleic Acids Research*, *47*(18), e103–e103. https://doi.org/10.1093/nar/gkz569

Canfield, Donald E., Glazer, A. N., & Falkowski, P. G. (2010). The evolution and future of earth's nitrogen cycle. *Science*, *330*(6001), 192–196. https://doi.org/10.1126/science.1186120

Canfield, Donald Eugene, Jørgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., … Hall, P. O. J. (1993). Pathways of organic carbon oxidation in three continental margin sediments. *Marine Geology*, *113*(1–2), 27–40.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Gordon, J. I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, *108*(Supplement 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Caraco, N. F., & Cole, J. J. (1999). Human Impact on Nitrate Export: An Analysis Using Major World Rivers. *Ambio*, *28*(2), 167–170.

Carman, R., & Rahm, L. (1997). Early diagenesis and chemical characteristics of interstitial water and sediments in the deep deposition bottoms of the Baltic proper. *Journal of Sea Research*, *37*(1–2), 25–47.

Carstensen, J., & Duarte, C. M. (2019). Drivers of pH Variability in Coastal Ecosystems. *Environmental Science and Technology*, *53*(8), 4020–4029. https://doi.org/10.1021/acs.est.8b03655

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., … Webster, N. S. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, *17*(9), 569–586. https://doi.org/10.1038/s41579-019-0222-5

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 265–270.

Chatterjee, S., Dickens, G. R., Bhatnagar, G., Chapman, W. G., Dugan, B., Snyder, G. T., & Hirasaki, G. J. (2011). Pore water sulfate, alkalinity, and carbon isotope profiles in

shallow sediment above marine gas hydrate systems: A numerical modeling perspective. *Journal of Geophysical Research: Solid Earth*, *116*(B9).

Chen, G., Bai, R., Zhang, Y., Zhao, B., & Xiao, Y. (2022). Application of metagenomics to biological wastewater treatment. *Science of The Total Environment*, *807*, 150737. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.150737

Chen, H., Wang, F., Kong, W., Jia, H., Zhou, T., Xu, R., ... Wu, J. (2021). Soil microbial CO2 fixation plays a significant role in terrestrial carbon sink in a dryland ecosystem: A four-year small-scale field-plot observation on the Tibetan Plateau. *Science of the Total Environment*, *761*. https://doi.org/10.1016/j.scitotenv.2020.143282

Chen, L. X., Hu, M., Huang, L. N., Hua, Z. S., Kuang, J. L., Li, S. J., & Shu, W. S. (2015). Comparative metagenomic and metatranscriptomic analyses of microbial communities in acid mine drainage. *ISME Journal*. https://doi.org/10.1038/ismej.2014.245

Christiansen, B., Denda, A., & Christiansen, S. (2020). Potential effects of deep seabed mining on pelagic and benthopelagic biota. *Marine Policy*, *114*(September 2018), 103442. https://doi.org/10.1016/j.marpol.2019.02.014

Christiansen, T., Wiberg, P. L., & Milligan, T. G. (2000). Flow and sediment transport on a tidal salt marsh surface. *Estuarine, Coastal and Shelf Science*, *50*(3), 315–331. https://doi.org/10.1006/ecss.2000.0548

Cloern, J. E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, *210*, 223–253.

Coelho, F. J. R. C., Cleary, D. F. R., Costa, R., Ferreira, M., Polónia, A. R. M., Silva, A. M. S., ... Gomes, N. C. M. (2016). Multitaxon activity profiling reveals differential microbial response to reduced seawater pH and oil pollution. *Molecular Ecology*, *25*(18), 4645–4659.

Cohen, J. E. (1997). Estimates of Coastal Populations. *Science.*, Vol. 278, pp. 1209c – 1213. https://doi.org/10.1126/science.278.5341.1209c

Collins, S., Rost, B., & Rynearson, T. A. (2014). Evolutionary potential of marine phytoplankton under ocean acidification. *Evolutionary Applications*, *7*(1), 140–155.

Cordier, T. (2020). Bacterial communities' taxonomic and functional turnovers both accurately predict marine benthic ecological quality status. *Environmental DNA*, *2*(2), 175–183. https://doi.org/10.1002/edn3.55

Cordier, T., Alonso-Sáez, L., Apothéloz-Perret-Gentil, L., Aylagas, E., Bohan, D. A., Bouchez, A., ... Lanzén, A. (2021). Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap. *Molecular Ecology*, *30*(13), 2937–2958. https://doi.org/https://doi.org/10.1111/mec.15472

Costanza, R., D'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., ... van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. LK - https://royalroads.on.worldcat.org/oclc/4592801201. *Nature TA - TT -*, *387*(6630), 253–260. Retrieved from https://www-nature-com.ezproxy.royalroads.ca/articles/387253a0.pdf

Cristescu, M. E., & Hebert, P. D. N. (2018). Uses and Misuses of Environmental DNA in Biodiversity Science and Conservation. *Annual Review of Ecology, Evolution, and Systematics*, *49*(1), 209–230. https://doi.org/10.1146/annurev-ecolsys-110617-062306

Crossland, C. J., Baird, D., Ducrotoy, J.-P., Lindeboom, H., Buddemeier, R. W., Dennison, W. C., … Swaney, D. P. (2005). *The Coastal Zone — a Domain of Global Interactions*. (Oecd 2001), 1–37. https://doi.org/10.1007/3-540-27851-6_1

Crowe, S. A., Canfield, D. E., Mucci, A., Sundby, B., & Maranger, R. (2012). Anammox, denitrification and fixed-nitrogen removal in sediments from the Lower St. Lawrence Estuary. *Biogeosciences*, *9*(11), 4309–4321.

Crummett, L. T. (2020). Acidification decreases microbial community diversity in the Salish Sea, a region with naturally high pCO2. *PLoS ONE*, *15*(10 October), 1–17. https://doi.org/10.1371/journal.pone.0241183

Crusius, J., Koopmans, D., Bratton, J. F., Charette, M. A., Kroeger, K., Henderson, P., … Colman, J. A. (2005). Submarine groundwater discharge to a small estuary estimated from radon and salinity measurements and a box model. *Biogeosciences*, *2*(2), 141–157. https://doi.org/10.5194/bg-2-141-2005

Currie, A. R., Tait, K., Parry, H., de Francisco-Mora, B., Hicks, N., Mark Osborn, A., … Stahl, H. (2017). Marine microbial gene abundance and community composition in response to ocean acidification and elevated temperature in two contrasting coastal marine sediments. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.01599

Cyronak, T., & Eyre, B. D. (2016). The synergistic effects of ocean acidification and organic metabolism on calcium carbonate (CaCO3) dissolution in coral reef sediments. *Marine Chemistry*, *183*, 1–12.

Cyronak, T., Santos, I. R., Erler, D. V, & Eyre, B. D. (2013). Groundwater and porewater as major sources of alkalinity to a fringing coral reef lagoon (Muri Lagoon, Cook Islands). *Biogeosciences*, *10*(4), 2467–2480.

Cyronak, T., Santos, I. R., McMahon, A., & Eyre, B. D. (2013). Carbon cycling hysteresis in permeable carbonate sands over a diel cycle: Implications for ocean acidificationn. *Limnology and Oceanography*, *58*(1), 131–143. https://doi.org/10.4319/lo.2013.58.1.0131

Dai, M., Zhai, W., Cai, W. J., Callahan, J., Huang, B., Shang, S., … Chen, Z. (2008). Effects of an estuarine plume-associated bloom on the carbonate system in the lower reaches of the Pearl River estuary and the coastal zone of the northern South China Sea. *Continental Shelf Research*, *28*(12), 1416–1423. https://doi.org/10.1016/j.csr.2007.04.018

Dale, A. W., & Prego, R. (2002). Physico-biogeochemical controls on benthic pelagic coupling of nutrient fluxes and recycling in a coastal upwelling system. *Marine Ecology Progress Series*, *235*, 15–28.

Das, S., & Mangwani, N. (2015a). Ocean acidification and marine microorganisms: responses and consequences. *Oceanologia*, *57*(4), 349–361. https://doi.org/10.1016/j.oceano.2015.07.003

Das, S., & Mangwani, N. (2015b). Ocean acidification and marine microorganisms: responses

and consequences. *Oceanologia*, *57*(4), 349–361. https://doi.org/10.1016/J.OCEANO.2015.07.003

Dash, H. R., Mangwani, N., Chakraborty, J., Kumari, S., & Das, S. (2013). Marine bacteria: potential candidates for enhanced bioremediation. *Applied Microbiology and Biotechnology*, *97*(2), 561–571.

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 1–14.

De Beer, D., Wenzhöfer, F., Ferdelman, T. G., Boehme, S. E., Huettel, M., Van Beusekom, J. E. E., … Dubilier, N. (2005). Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. *Limnology and Oceanography*, *50*(1), 113–127. https://doi.org/10.4319/lo.2005.50.1.0113

De Vrieze, J., Pinto, A. J., Sloan, W. T., & Ijaz, U. Z. (2018). The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. *Microbiome*, *6*(1), 63. https://doi.org/10.1186/s40168-018-0449-9

Desjardins, P. R., Buatois, L. A., & Mángano, M. G. (2012). Tidal Flats and Subtidal Sand Bodies. In *Developments in Sedimentology* (Vol. 64). https://doi.org/10.1016/B978-0-444-53813-0.00018-6

Diaz, R. J., & Rosenberg, R. (2008). Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, *321*(5891), 926–929. Retrieved from http://www.jstor.org.ezproxy.waikato.ac.nz/stable/20144596

Dickson, A., Sabine, C., & Christian, J. (2007). Guide to Best Practices for Ocean C02 Measurments. In *North Pacific Marine Science Organization* (Vol. 3). Retrieved from www.pices.int

Dickson, A G, & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(10), 1733–1743.

Dickson, Andrew G. (1990). Standard potential of the reaction: AgCl (s)+ 12H2 (g)= Ag (s)+ HCl (aq), and and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, *22*(2), 113–127.

Doney, S. C. (2010). The Growing Human Footprint on Coastal and Open-Ocean Biogeochemistry. *Science*, *328*(5985), 1512–1516. Retrieved from http://www.jstor.org.ezproxy.waikato.ac.nz/stable/40656418

Doney, S. C., Busch, D. S., Cooley, S. R., & Kroeker, K. J. (2020). The Impacts of Ocean Acidification on Marine Ecosystems and Reliant Human Communities. *Annual Review of Environment and Resources*, *45*(1), 1–30. https://doi.org/10.1146/annurev-environ-012320-083019

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009a). Ocean Acidification: The Other CO $_2$ Problem. *Annual Review of Marine Science*. https://doi.org/10.1146/annurev.marine.010908.163834

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009b). Ocean acidification: The other CO2 problem. *Annual Review of Marine Science*, *1*, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834

Doney, S. C., Mahowald, N., Lima, I., Feely, R. A., Mackenzie, F. T., Lamarque, J. F., & Rasch, P. J. (2007). Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(37), 14580–14585. https://doi.org/10.1073/pnas.0702218104

Doney, S. C., & Schimel, D. S. (2007). *Carbon and Climate System Coupling on Timescales from the Precambrian to the Anthropocene **. *32*(1), 31–66. https://doi.org/10.1146/annurev.energy.32.041706.124700

Douglas, E. J., Lohrer, A. M., & Pilditch, C. A. (2019). Biodiversity breakpoints along stress gradients in estuaries and associated shifts in ecosystem interactions. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-54192-0

Drupp, P. S., De Carlo, E. H., & Mackenzie, F. T. (2016). Porewater CO2–carbonic acid system chemistry in permeable carbonate reef sands. *Marine Chemistry*, *185*, 48–64.

Drylie, T. P., Needham, H. R., Lohrer, A. M., Hartland, A., & Pilditch, C. A. (2019). Calcium carbonate alters the functional response of coastal sediments to eutrophication-induced acidification. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-48549-8

Duarte, C. M., & Cebrián, J. (1996). The fate of marine autotrophic production. *Limnology and Oceanography*, *41*(8), 1758–1766. https://doi.org/10.4319/lo.1996.41.8.1758

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., … McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts*, *36*(2), 221–236.

Duarte, C. M., Kennedy, H., Marbà, N., & Hendriks, I. (2013). Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean and Coastal Management*, *83*, 32–38. https://doi.org/10.1016/j.ocecoaman.2011.09.001

Duarte, C. M., Marbà, N., Gacia, E., Fourqurean, J. W., Beggins, J., Barrón, C., & Apostolaki, E. T. (2010). Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles*, *24*(4), 1–8. https://doi.org/10.1029/2010GB003793

Dunn, R. R., Fierer, N., Henley, J. B., Leff, J. W., & Menninger, H. L. (2013). Home life: factors structuring the bacterial diversity found within and between homes. *PloS One*, *8*(5), e64133.

Dyer, K. R., Christie, M. C., Feates, N., Fennessy, M. J., Pejrup, M., & van der Lee, W. (2000). An Investigation into Processes Influencing the Morphodynamics of an Intertidal Mudflat, the Dollard Estuary, The Netherlands: I. Hydrodynamics and Suspended Sediment. *Estuarine, Coastal and Shelf Science*, *50*(5), 607–625. https://doi.org/https://doi.org/10.1006/ecss.1999.0596

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edmonds, J. W., Weston, N. B., Joye, S. B., Mou, X., & Moran, M. A. (2009). Microbial community response to seawater amendment in low-salinity tidal sediments. *Microbial Ecology*, *58*(3), 558–568. https://doi.org/10.1007/s00248-009-9556-2

Eggers, S. L., Lewandowska, A. M., Barcelos e Ramos, J., Blanco-Ameijeiras, S., Gallo, F., & Matthiessen, B. (2014). Community composition has greater impact on the functioning of marine phytoplankton communities than ocean acidification. *Global Change Biology*, *20*(3), 713–723.

Egleston, E. S., Sabine, C. L., & Morel, F. M. M. (2010). Revelle revisited: Buffer factors that quantify the response of ocean chemistry to changes in DIC and alkalinity. *Global Biogeochemical Cycles*, *24*(1), 1–9. https://doi.org/10.1029/2008GB003407

Escalas, A., Hale, L., Voordeckers, J. W., Yang, Y., Firestone, M. K., Alvarez-Cohen, L., & Zhou, J. (2019). Microbial functional diversity: From concepts to applications. *Ecology and Evolution*, *9*(20), 12000–12016. https://doi.org/https://doi.org/10.1002/ece3.5670

Fabry, V. J., & Balch, W. M. (2010). *Direct measurements of calcification rates in planktonic organisms 12*.

Fadeev, E., Cardozo-Mino, M. G., Rapp, J. Z., Bienhold, C., Salter, I., Salman-Carvalho, V., … Boetius, A. (2021). Comparison of Two 16S rRNA Primers (V3–V4 and V4–V5) for Studies of Arctic Microbial Communities . *Frontiers in Microbiology* , Vol. 12. Retrieved from https://www.frontiersin.org/article/10.3389/fmicb.2021.637526

Fassbender, A. J., Sabine, C. L., Feely, R. A., Langdon, C., & Mordy, C. W. (2011). Inorganic carbon dynamics during northern California coastal upwelling. *Continental Shelf Research*, *31*(11), 1180–1192. https://doi.org/10.1016/j.csr.2011.04.006

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., … Maloy, C. (2010). The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science*, *88*(4), 442–449. https://doi.org/10.1016/j.ecss.2010.05.004

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., & Hales, B. (2008). Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science*, *320*(5882), 1490–1492. https://doi.org/10.1126/science.1155676

FEELY, R. A., SABINE, C. L., LEE, K., BERELSON, W., KLEYPAS, J., FABRY, V. J., & MILLERO, F. J. (2004). Impact of anthropogenic CO2 on the CaCO3 system in the oceans. *Science*, *305*(5682), 362–366. https://doi.org/10.1126/science.1097329

Fekete, B. M., Vörösmarty, C. J., & Lammers, R. B. (2001). Scaling gridded river networks for macroscale hydrology: Development, analysis, and control of error. *Water Resources Research*, *37*(7), 1955–1967. https://doi.org/10.1029/2001WR900024

Fenchel, T. M., & Riedl, R. J. (1970). The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *Marine Biology*, *7*(3), 255–268. https://doi.org/10.1007/BF00367496

Fenchel, T., & Riedl, R. (1970). The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *International Journal on Life in Oceans and Coastal Waters*, *7*(3), 255–268. https://doi.org/10.1007/BF00367496

Fernandes, A. D., Vu, M. T. H. Q., Edward, L. M., Macklaim, J. M., & Gloor, G. B. (2018). A reproducible effect size is more useful than an irreproducible hypothesis test to analyze high throughput sequencing datasets. *ArXiv*, 1–15.

Fernández-Gó Mez, B., Richter, M., Schü Ler, M., Pinhassi, J., Acinas, S. G., González, J. M., & Pedró S-Alió, C. (2013). Ecology of marine Bacteroidetes: a comparative genomics approach. *The ISME Journal*, *7*, 1026–1037. https://doi.org/10.1038/ismej.2012.169

Ferrández-Gómez, B., Sánchez, A., Jordá, J. D., Fonfría, E. S., Bordehore, C., & Cerdán, M. (2021). Effectiveness of Oxygen-Saturated Seawater Injections and Air Sparging Technologies in Remediation of Coastal Marine Sediments from Sludge. *Environmental Geochemistry and Health*, *43*(12), 4975–4986. https://doi.org/10.1007/s10653-021-00952-9

Findlay, R. H., Pollard, P. C., Moriarty, D. J. W., & White, D. C. (1985). Quantitative determination of microbial activity and community nutritional status in estuarine sediments: evidence for a disturbance artifact. *Canadian Journal of Microbiology*, *31*(5), 493–498. https://doi.org/10.1139/m85-091

Findlay, R., Trexler, M., Guckert, J., & White, D. (1990). Laboratory study of disturbance in marine sediments: response of a microbial community. *Marine Ecology Progress Series*, *62*(Aller 1982), 121–133. https://doi.org/10.3354/meps062121

Fink, A., den Haan, J., Chennu, A., Uthicke, S., & de Beer, D. (2017). Ocean Acidification Changes Abiotic Processes but Not Biotic Processes in Coral Reef Sediments. *Frontiers in Marine Science*. https://doi.org/10.3389/fmars.2017.00073

Firth, L. B., & Hawkins, S. J. (2011). Introductory comments - Global change in marine ecosystems: Patterns, processes and interactions with regional and local scale impacts. *Journal of Experimental Marine Biology and Ecology*, *400*(1–2), 1–6. https://doi.org/10.1016/j.jembe.2011.02.001

Fischer, J. M., Frost, T. M., & Ives, A. R. (2001). Compensatory dynamics in zooplankton community responses to acidification: Measurement and mechanisms. *Ecological Applications*, *11*(4), 1060–1072. https://doi.org/10.1890/1051-0761(2001)011[1060:CDIZCR]2.0.CO;2

Fischlin, A., Midgley, G. F., Price, J. T., Leemans, R., Gopal, B., Turley, C., … Velichko, A. A. (2007). Ecosystems , their properties , goods and services Coordinating Lead Authors : Lead Authors : Contributing Authors : Review Editors. *Change*, *48*(3), 211–272. Retrieved from http://www.treesearch.fs.fed.us/pubs/33102

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., … Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, *5*(7), 505–509. https://doi.org/10.1038/ngeo1477

Gacia, E., & Duarte, C. M. (2001). Sediment retention by a Mediterranean Posidonia oceanica meadow: The balance between deposition and resuspension. *Estuarine,*

Coastal and Shelf Science, *52*(4), 505–514. https://doi.org/10.1006/ecss.2000.0753

Gacia, E., Granata, T. C., & Duarte, C. M. (1999). *Gacia et al. 1999 sedimentation.pdf*. *65*, 255–268.

Galand, P. E., Lucas, S., Fagervold, S. K., Peru, E., Pruski, A. M., Vétion, G., … Guizien, K. (2016). Disturbance increases microbial community diversity and production in marine sediments. *Frontiers in Microbiology*, *7*(DEC), 1–11. https://doi.org/10.3389/fmicb.2016.01950

Garnier, S. (2017). viridis: Default Color Maps from "matplotlib." *R Package Version 0.5.1*, 1–6. Retrieved from https://cran.r-project.org/package=viridis

Gattuso, J-P, Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., … Eakin, C. M. (2015). Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. *Science*, *349*(6243).

Gattuso, J. P., Frankignoulle, M., Bourge, I., Romaine, S., & Buddemeier, R. W. (1998). Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change*, *18*(1–2), 37–46. https://doi.org/10.1016/S0921-8181(98)00035-6

Gattuso, J. P., Frankignoulle, M., & Wollast, R. (1998). Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics*, *29*, 405–434. https://doi.org/10.1146/annurev.ecolsys.29.1.405

Gattuso, Jean-Pierre, Allemand, D., & Frankignoulle, M. (1999). Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. *American Zoologist*, *39*(1), 160–183.

Gattuso, Jean Pierre, Mach, K. J., & Morgan, G. (2013). Ocean acidification and its impacts: An expert survey. *Climatic Change*, *117*(4), 725–738. https://doi.org/10.1007/s10584-012-0591-5

Gazeau, F., Van Rijswijk, P., Pozzato, L., & Middelburg, J. J. (2014). Impacts of ocean acidification on sediment processes in shallow waters of the Arctic Ocean. *PLoS One*, *9*(4), e94068.

Ghate, S. D., Shastry, R. P., Arun, A. B., & Rekha, P. D. (2021). Unraveling the bacterial community composition across aquatic sediments in the Southwestern coast of India by employing high-throughput 16S rRNA gene sequencing. *Regional Studies in Marine Science*, *46*, 101890. https://doi.org/https://doi.org/10.1016/j.rsma.2021.101890

Gilbert, J. A., Jansson, J. K., & Knight, R. (2014). The Earth Microbiome project: successes and aspirations. *BMC Biology*, *12*(1), 1–4.

Gill, A. B. (2005). Offshore renewable energy: Ecological implications of generating electricity in the coastal zone. *Journal of Applied Ecology*, *42*(4), 605–615. https://doi.org/10.1111/j.1365-2664.2005.01060.x

Giorgio, P. del, & Williams, P. (2005). *Respiration in Aquatic Ecosystems*. https://doi.org/10.1093/acprof:oso/9780198527084.001.0001

Giraud, X., Le Quéré, C., & da Cunha, L. C. (2008). Importance of coastal nutrient supply for

global ocean biogeochemistry. *Global Biogeochemical Cycles*, *22*(2), 1–15. https://doi.org/10.1029/2006GB002717

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: and this is not optional. *Frontiers in Microbiology*, *8*, 2224.

Glud, R. N. (2008). Oxygen dynamics of marine sediments. *Marine Biology Research*, *4*(4), 243–289.

Gobler, C. J., & Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. *Biology Letters*, *12*(5), 20150976.

Gobler, C. J., Clark, H. R., Griffith, A. W., & Lusty, M. W. (2017). Diurnal fluctuations in acidification and hypoxia reduce growth and survival of larval and juvenile bay scallops (Argopecten irradians) and hard clams (Mercenaria mercenaria). *Frontiers in Marine Science*, *3*(JAN), 1–12. https://doi.org/10.3389/FMARS.2016.00282

Godbold, J. A., & Solan, M. (2013). Long-term effects of warming and ocean acidification are modified by seasonal variation in species responses and environmental conditions. *Phil. Trans. R. Soc. B*, *368*(1627), 20130186.

Goebel, B. M., & Stackebrandt, E. (1994). Cultural and phylogenetic analysis of mixed microbial populations found in natural and commercial bioleaching environments. *Applied and Environmental Microbiology*, *60*(5), 1614.

Goldberg, S. J., Nelson, C. E., Viviani, D. A., Shulse, C. N., & Church, M. J. (2017). Cascading influence of inorganic nitrogen sources on DOM production, composition, lability and microbial community structure in the open ocean. *Environmental Microbiology*, *19*(9), 3450–3464. https://doi.org/10.1111/1462-2920.13825

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. In *Journal of Statistical Software* (Vol. 22). https://doi.org/10.18637/jss.v022.i07

Graf, G. (1992). Benthic-pelagic coupling: a benthic view. *Oceanography and Marine Biology Annual Review*, *30*, 149–190.

Grasshoff, K., Kremling, K., & Ehrhardt, M. (2009). *Methods of seawater analysis*. John Wiley & Sons.

Gray, J. S. (1974). Animal-sediment relationships. Oceanogr. *Mar. Biol. Ann. Rev.*, *12*, 223–261.

Green, M. A., & Aller, R. C. (1998). Seasonal patterns of carbonate diagenesis in nearshore terrigenous muds: Relation to spring phytoplankton bloom and temperature. *Journal of Marine Research*, *56*(5), 1097–1123. https://doi.org/10.1357/002224098765173473

Green, P. A., Vörösmarty, C. J., Meybeck, M., Galloway, J. N., Peterson, B. J., & Boyer, E. W. (2004). Pre-industrial and contemporary fluxes of nitrogen through rivers: A global assessment based on typology. *Biogeochemistry*, *68*(1), 71–105. https://doi.org/10.1023/B:BIOG.0000025742.82155.92

Greiner, J. T., McGlathery, K. J., Gunnell, J., & McKee, B. A. (2013). Seagrass Restoration

Enhances "Blue Carbon" Sequestration in Coastal Waters. *PLoS ONE*, *8*(8), 1–8. https://doi.org/10.1371/journal.pone.0072469

Griffis, R. B., & Suchanek, T. H. (1991). A model of burrow architecture and trophic modes in thalassinidean shrimp (Decapoda: Thalassinidea). *Marine Ecology Progress Series*, Vol. 79, pp. 171–183. https://doi.org/10.3354/meps079171

Guan, Y., Hohn, S., Wild, C., & Merico, A. (2020). Vulnerability of global coral reef habitat suitability to ocean warming, acidification and eutrophication. *Global Change Biology*, *26*(10), 5646–5660.

Guinotte, J. M., & Fabry, V. J. (2008). Ocean acidification and its potential effects on marine ecosystems. *Annals of the New York Academy of Sciences*, *1134*(1), 320–342.

Gutiérrez, J. L., Jones, C. G., Byers, J. E., Arkema, K. K., Berkenbusch, K., Commito, A., … Wild, C. (2012). Physical Ecosystem Engineers and the Functioning of Estuaries and Coasts. In *Treatise on Estuarine and Coastal Science* (Vol. 7). https://doi.org/10.1016/B978-0-12-374711-2.00705-1

Gutowska, M. A., Pörtner, H. O., & Melzner, F. (2008). Growth and calcification in the cephalopod Sepia officinalis under elevated seawater pCO2. *Marine Ecology Progress Series*, *373*, 303–309.

Hagens, M., Slomp, C. P., Meysman, F. J. R., Seitaj, D., Harlay, J., Borges, A. V, & Middelburg, J. J. (2015). Biogeochemical processes and buffering capacity concurrently affect acidification in a seasonally hypoxic coastal marine basin. *Biogeosciences*, *12*(5), 1561–1583.

Hagopian, D. S., & Riley, J. G. (1998). *A closer look at the bacteriology of nitrification*. *18*(May), 223–244.

Hale, R., Calosi, P., Mcneill, L., Mieszkowska, N., & Widdicombe, S. (2011). Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos*, *120*(5), 661–674. https://doi.org/10.1111/j.1600-0706.2010.19469.x

Hales, B., Takahashi, T., & Bandstra, L. (2005). Atmospheric CO2 uptake by a coastal upwelling system. *Global Biogeochemical Cycles*, *19*(1), 1–11. https://doi.org/10.1029/2004GB002295

Hansen, J. C. R., & Reidenbach, M. A. (2012). Wave and tidally driven flows in eelgrass beds and their effect on sediment suspension. *Marine Ecology Progress Series*, *448*, 271–287. https://doi.org/10.3354/meps09225

Hansen, L. S., & Blackburn, T. H. (1992). Mineralization budgets in sediment microcosms: Effect of the infauna and anoxic conditions. *FEMS Microbiology Letters*, *102*(1), 33–43. https://doi.org/10.1111/j.1574-6968.1992.tb05793.x

Harley, C. D. G., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., … Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228–241.

Harvey, L. D. D. (2008). Mitigating the atmospheric CO2 increase and ocean acidification by

adding limestone powder to upwelling regions. *Journal of Geophysical Research: Oceans*, *113*(C4).

Hassenruck, C., Fink, A., Lichtschlag, A., Tegetmeyer, H. E., de Beer, D., & Ramette, A. (2016). Quantification of the effects of ocean acidification on sediment microbial communities in the environment: the importance of ecosystem approaches. *Fems Microbiology Ecology*, *92*(5). https://doi.org/ARTN fiw027 10.1093/femsec/fiw027

Haugan, P. M., & Drange, H. (1996). Effects of CO2 on the ocean environment. *Energy Conversion and Management*, *37*(6–8), 1019–1022.

Hedges, J. I., & Keil, R. G. (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. *Marine Chemistry*, *49*(2), 81–115. https://doi.org/10.1016/0304-4203(95)00008-F

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., … Humphries, E. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae*, *8*(1), 3–13.

Hendriks, I. E., Duarte, C. M., Olsen, Y. S., Steckbauer, A., Ramajo, L., Moore, T. S., … McCulloch, M. (2015). Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems. *Estuarine, Coastal and Shelf Science*, *152*, A1–A8. https://doi.org/10.1016/j.ecss.2014.07.019

Hicks, N., Ubbara, G. R., Silburn, B., Smith, H. E. K., Kröger, S., Parker, E. R., … Stahl, H. (2017). Oxygen dynamics in shelf seas sediments incorporating seasonal variability. *Biogeochemistry*, *135*(1–2), 35–47. https://doi.org/10.1007/s10533-017-0326-9

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., … Hatziolos, M. E. (2007). Coral reefs under rapid climate change and ocean acidification. *Science (New York, N.Y.)*, *318*(5857), 1737–1742. https://doi.org/10.1126/science.1152509

Hoehler, T. M., Alperin, M. J., Albert, D. B., & Martens, C. S. (2001). Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. *FEMS Microbiology Ecology*, *38*(1), 33–41.

Hofer, U. (2018). The majority is uncultured. *Nature Reviews Microbiology*, *16*(12), 716–717.

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., … Takeshita, Y. (2011). High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PloS One*, *6*(12), e28983.

Hofmann, G. E., & Todgham, A. E. (2009). Living in the now: Physiological mechanisms to tolerate a rapidly changing environment. *Annual Review of Physiology*, *72*, 127–145. https://doi.org/10.1146/annurev-physiol-021909-135900

Hondt, S. D., Jørgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., … Acosta, S. (2004). *Esearch rticle*. (December), 2216–2222.

Hoppe-Jones, C., Oldham, G., & Drewes, J. E. (2010). Attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems. *Water Research*, *44*(15), 4643–4659. https://doi.org/10.1016/j.watres.2010.06.022

Hoppe, H.-G., Arnosti, C., & Herndl, G. J. (2002). *Ecological significance of bacterial enzymes in the marine environment*. New York, USA: Marcel Dekker.

Horner-Devine, M. C., Lage, M., Hughes, J. B., & Bohannan, B. J. M. (2004). A taxa–area relationship for bacteria. *Nature*, *432*(7018), 750.

Hou, J., Song, C., Cao, X., & Zhou, Y. (2013). Shifts between ammonia-oxidizing bacteria and archaea in relation to nitrification potential across trophic gradients in two large Chinese lakes (Lake Taihu and Lake Chaohu). *Water Research*, *47*(7), 2285–2296. https://doi.org/https://doi.org/10.1016/j.watres.2013.01.042

Hou, M., Xiong, J., Wang, K., Ye, X., Ye, R., Wang, Q., … Zhang, D. (2014). Communities of sediment ammonia-oxidizing bacteria along a coastal pollution gradient in the East China Sea. *Marine Pollution Bulletin*, *86*(1–2), 147–153. https://doi.org/10.1016/j.marpolbul.2014.07.031

Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., & Billen, G. (2011). Coupled biogeochemical cycles: Eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Frontiers in Ecology and the Environment*, *9*(1), 18–26. https://doi.org/10.1890/100008

Howe, R. L., Rees, A. P., & Widdicombe, S. (2004). The impact of two species of bioturbating shrimp (Callianassa subterranea and Upogebia deltaura) on sediment denitrification. *Journal of the Marine Biological Association of the United Kingdom*, *84*(3), 629–632. https://doi.org/10.1017/S002531540400966Xh

Hu, X., & Cai, W. J. (2011). An assessment of ocean margin anaerobic processes on oceanic alkalinity budget. *Global Biogeochemical Cycles*, *25*(3), 1–11. https://doi.org/10.1029/2010GB003859

Huettel, M., Ziebis, W., & Forster, S. (1996). Flow-induced uptake of particulate matter in permeable sediments. *Limnology and Oceanography*, *41*(2), 309–322. https://doi.org/10.4319/lo.1996.41.2.0309

Huettel, Markus, Røy, H., Precht, E., & Ehrenhauss, S. (2003). Hydrodynamical impact on biogeochemical processes in aquatic sediments. *Hydrobiologia*, *494*(May), 231–236. https://doi.org/10.1023/A:1025426601773

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609–623.

Hurd, C. L., Beardall, J., Comeau, S., Cornwall, C. E., Havenhand, J. N., Munday, P. L., … McGraw, C. M. (2020). Ocean acidification as a multiple driver: how interactions between changing seawater carbonate parameters affect marine life. *Marine and Freshwater Research*, *71*(3), 263–274.

Hutchins, D. A., & Fu, F. (2017). Microorganisms and ocean global change. *Nature Microbiology*. https://doi.org/10.1038/nmicrobiol.2017.58

Hutchins, D. A., Mulholland, M. R., & Fu, F. (2009). Nutrient cycles and marine microbes in a CO_2-enriched ocean. *Oceanography*, *22*(4), 128–145.

Ibrahim, H. A. H., El-Sayed, W. M. M., Shaltout, N. A., & El-Shorbagi, E. K. (2014). Effects of

different pCO2 concentrations on marine bacterial community structure, Eastern Harbor, Alexandria, Egypt. *Life Sci J*, *11*, 781–789.

Igbp, I. O. C. (n.d.). SCOR (2013) Ocean Acidification Summary for Policymakers–Third Symposium on the Ocean in a High-CO2 World. *International Geosphere-Biosphere Programme. Stockolm, Sweden*.

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., … von Dassow, P. (2008). Phytoplankton calcification in a high-CO2 world. *Science*, *320*(5874), 336–340.

Inoue, S., Yoshizawa, S., Nakajima, Y., Kojima, K., Tsukamoto, T., Kikukawa, T., & Sudo, Y. (2018). Spectroscopic characteristics of: Rubricoccus marinus xenorhodopsin (Rm XeR) and a putative model for its inward H+ transport mechanism. *Physical Chemistry Chemical Physics*, *20*(5), 3172–3183. https://doi.org/10.1039/c7cp05033j

Ives, A. R., Gross, K., & Klug, J. L. (1999). Stability and variability in competitive communities. *Science*, *286*(5439), 542–544. https://doi.org/10.1126/science.286.5439.542

Jaekel, U., Zedelius, J., Wilkes, H., & Musat, F. (2015). Anaerobic degradation of cyclohexane by sulfate-reducing bacteria from hydrocarbon-contaminated marine sediments. *Frontiers in Microbiology*, *6*, 116.

Jansen, S., Walpersdorf, E., Werner, U., Billerbeck, M., Böttcher, M. E., & De Beer, D. (2009). Functioning of intertidal flats inferred from temporal and spatial dynamics of O2, H2S and pH in their surface sediment. *Ocean Dynamics*, *59*(2), 317–332. https://doi.org/10.1007/s10236-009-0179-4

Jessup, C. M., Forde, S. E., & Bohannan, B. J. M. (2005). Microbial Experimental Systems in Ecology. *Advances in Ecological Research*, *37*(04), 273–307. https://doi.org/10.1016/S0065-2504(04)37009-1

Jessup, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., & Bohannan, B. J. M. (2004). Big questions, small worlds: Microbial model systems in ecology. *Trends in Ecology and Evolution*, *19*(4), 189–197. https://doi.org/10.1016/j.tree.2004.01.008

Jiao, J.-Y., Liu, L., Hua, Z.-S., Fang, B.-Z., Zhou, E.-M., Salam, N., … Li, W.-J. (2021). Microbial dark matter coming to light: challenges and opportunities. *National Science Review*, *8*(3), nwaa280.

Johnson, D. B., & Hallberg, K. B. (2003). The microbiology of acidic mine waters. *Research in Microbiology*, *154*(7), 466–473. https://doi.org/10.1016/S0923-2508(03)00114-1

Johnson, V., Brownlee, C., Rickaby, R., Graziano, M., Milazzo, M., & Hall-Spencer, J. (2013). Responses of marine benthic microalgae to elevated CO 2. *International Journal on Life in Oceans and Coastal Waters*, *160*(8), 1813–1824. https://doi.org/10.1007/s00227-011-1840-2

Johnson, Z. I., Wheeler, B. J., Blinebry, S. K., Carlson, C. M., Ward, C. S., & Hunt, D. E. (2013). Dramatic variability of the carbonate system at a temperate coastal ocean site (Beaufort, North Carolina, USA) is regulated by physical and biogeochemical processes on multiple timescales. *PLoS ONE*, *8*(12), 1–8. https://doi.org/10.1371/journal.pone.0085117

Joint, I., Doney, S. C., & Karl, D. M. (2011a). Will ocean acidification affect marine microbes. *ISME Journal*. https://doi.org/10.1038/ismej.2010.79

Joint, I., Doney, S. C., & Karl, D. M. (2011b). Will ocean acidification affect marine microbes. *ISME Journal*, *5*(1), 1–7. https://doi.org/10.1038/ismej.2010.79

Joint, I., Karl, D. M., Doney, S. C., Armbrust, E., Balch, W., Beman, M., … Heidelberg, J. (2009). *Consequences of high CO2 and ocean acidification for microbes in the global ocean*.

Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2020). *Package ' adespatial .'* https://doi.org/10.1890/11-1183.1>.Maintainer

Jorgensen, B. B., Revsbech, N. P., & Cohen, Y. (1983). Photosynthesis and structure of benthic microbial mats: Microelectrode and SEM studies of four cyanobacterial communities1. *Limnology and Oceanography*, *28*(6), 1075–1093.

Jourabchi, P., Van Cappellen, P., & Regnier, P. (2005). Quantitative interpretation of pH distributions in aquatic sediments: A reaction-transport modeling approach. *American Journal of Science*, *305*(9), 919–956.

K, A. A., Emilio, M., A, R. P., M, M. J., C, D. S., R, A. S., … Kyungsoo, Y. (2011). Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, *9*(1), 53–60. https://doi.org/doi:10.1890/100014

Karen, T., Bonnie, L., Jennifer, S., J., S. P., & Steve, W. (2013). Minor impact of ocean acidification to the composition of the active microbial community in an Arctic sediment. *Environmental Microbiology Reports*, *5*(6), 851–860. https://doi.org/doi:10.1111/1758-2229.12087

Karl, D. M. (2007). Microbial oceanography: paradigms, processes and promise. *Nature Reviews Microbiology*, *5*(10), 759.

Kassambara, A. (2016). Practical guide to principal component methods in R: PCA, M (CA), FAMD, MFA, HCPC, factoextra. Vol. 2. *Sthda*. Retrieved from http://www.sthda.com/english/rpkgs/factoextra BugReports

Kassambara, Alboukadel, & Kassambara, M. A. (2020). *Package 'ggpubr.'*

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., … Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kennedy, H., Beggins, J., Duarte, C. M., Fourqurean, J. W., Holmer, M., Marbá, N., & Middelburg, J. J. (2010). Seagrass sediments as a global carbon sink: Isotopic constraints. *Global Biogeochemical Cycles*, *24*(4), 1–8. https://doi.org/10.1029/2010GB003848

Kerfahi, D., Hall-Spencer, J., Tripathi, B., Milazzo, M., Lee, J., & Adams, J. (2014). Shallow Water Marine Sediment Bacterial Community Shifts Along a Natural CO 2 Gradient in the Mediterranean Sea Off Vulcano, Italy. *Microbial Ecology*, *67*(4), 819–828. https://doi.org/10.1007/s00248-014-0368-7

Kerfahi, D., Harvey, B. P., Agostini, S., Kon, K., Huang, R., Adams, J. M., & Hall-Spencer, J. M. (2020). Responses of Intertidal Bacterial Biofilm Communities to Increasing p CO 2. *Marine Biotechnology*, 1–12.

Kessler, A. J., Chen, Y. J., Waite, D. W., Hutchinson, T., Koh, S., Popa, M. E., … Greening, C. (2019). Bacterial fermentation and respiration processes are uncoupled in anoxic permeable sediments. *Nature Microbiology*. https://doi.org/10.1038/s41564-019-0391-z

Kessouri, F., McWilliams, J. C., Bianchi, D., Sutula, M., Renault, L., Deutsch, C., … Howard, E. M. (2021). Coastal eutrophication drives acidification, oxygen loss, and ecosystem change in a major oceanic upwelling system. *Proceedings of the National Academy of Sciences*, *118*(21).

Keul, N., Morse, J. W., Wanninkhof, R., Gledhill, D. K., & Bianchi, T. S. (2010). Carbonate chemistry dynamics of surface waters in the Northern Gulf of Mexico. *Aquatic Geochemistry*, *16*(3), 337–351. https://doi.org/10.1007/s10498-010-9091-2

Kim, K. H., & Kim, D. (2007). *Seasonal and spatial variability of sediment oxygen fluxes in the Beobsan*. *11*(4), 323–329.

Kim, T. W., Barry, J. P., & Micheli, F. (2013). The effects of intermittent exposure to low pH and oxygen conditions on survival and growth of juvenile red abalone. *Biogeosciences Discussions*, *10*(2), 3559–3576. https://doi.org/10.5194/bgd-10-3559-2013

Kitidis, V, Tait, K., Nunes, J., Brown, I., Woodward, E. M. S., Harris, C., … Kröger, S. (2017). Seasonal benthic nitrogen cycling in a temperate shelf sea: the Celtic Sea. *Biogeochemistry*, *135*(1), 103–119.

Kitidis, Vassilis, Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., … Widdicombe, S. (2011a). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters*. https://doi.org/10.1029/2011GL049095

Kitidis, Vassilis, Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., … Widdicombe, S. (2011b). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters*, *38*(21), n/a-n/a. https://doi.org/10.1029/2011GL049095

Kolda, A., Petrić, I., Mucko, M., Gottstein, S., Žutinić, P., Goreta, G., … Udovič, M. G. (2019). How environment selects: Resilience and survival of microbial mat community within intermittent karst spring Krčić (Croatia). *Ecohydrology*, *12*(2), e2063.

Koné, Y. J. M., & Borges, A. V. (2008). Dissolved inorganic carbon dynamics in the waters surrounding forested mangroves of the Ca Mau Province (Vietnam). *Estuarine, Coastal and Shelf Science*, *77*(3), 409–421. https://doi.org/10.1016/j.ecss.2007.10.001

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., & Sass, H. (2005). Microbial diversity in coastal subsurface sediments: A cultivation approach using various electron acceptors and substrate gradients. *Applied and Environmental Microbiology*, *71*(12), 7819–7830. https://doi.org/10.1128/AEM.71.12.7819-7830.2005

Körtzinger, A., Hedges, J. I., & Quay, P. D. (2001). Redfield ratios revisited: Removing the

biasing effect of anthropogenic CO2. *Limnology and Oceanography*, *46*(4), 964–970.

Köster, M., Dahlke, S., & Meyer-Reil, L. A. (2005). Microbial colonization and activity in relation to organic carbon in sediments of hypertrophic coastal waters (Nordrügensche Bodden, Southern Baltic Sea). *Aquatic Microbial Ecology*, *39*(1), 69–83. https://doi.org/10.3354/ame039069

Köster, M., Wardenga, R., & Blume, M. (2008). Microscale investigations of microbial communities in coastal surficial sediments. *Marine Ecology*, *29*(1), 89–105. https://doi.org/10.1111/j.1439-0485.2007.00219.x

Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M. B., & Gerdts, G. (2012). Small Changes in pH Have Direct Effects on Marine Bacterial Community Composition: A Microcosm Approach. *PLoS ONE*, *7*(10), e47035. https://doi.org/10.1371/journal.pone.0047035

Krehenwinkel, H., Pomerantz, A., & Prost, S. (2019). Genetic Biomonitoring and Biodiversity Assessment Using Portable Sequencing Technologies: Current Uses and Future Directions. *Genes* , Vol. 10. https://doi.org/10.3390/genes10110858

Kristensen, E. (2000). Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. *Hydrobiologia*, *426*, Life.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., … Gattuso, J. P. (2013a). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*. https://doi.org/10.1111/gcb.12179

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., … Gattuso, J. P. (2013b). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*(6), 1884–1896. https://doi.org/10.1111/gcb.12179

Kroeker, K. J., Micheli, F., Gambi, M. C., & Martz, T. R. (2011). Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(35), 14515–14520. https://doi.org/10.1073/pnas.1107789108

Krumins, V., Gehlen, M., Arndt, S., Van Cappellen, P., & Regnier, P. (2013). Dissolved inorganic carbon and alkalinity fluxes from coastal marine sediments: Model estimates for different shelf environments and sensitivity to global change. *Biogeosciences*, *10*(1), 371–398. https://doi.org/10.5194/bg-10-371-2013

Kuang, J.-L., Huang, L.-N., Chen, L.-X., Hua, Z.-S., Li, S.-J., Hu, M., … Shu, W.-S. (2013). Contemporary environmental variation determines microbial diversity patterns in acid mine drainage. *The ISME Journal*, *7*(5), 1038.

Kuhn, M. (2011). The caret Package. *A Unified Interface for Predictive Models*, 1–27. Retrieved from http://cran.r-project.org/web/packages/caret/vignettes/caretTrain.pdf%0Apapers2://publication/uuid/D8CA271E-F548-44F3-B081-B129A8765F04

Kuliński, K., Schneider, B., Hammer, K., Machulik, U., & Schulz-Bull, D. (2014). The influence of dissolved organic matter on the acid–base system of the Baltic Sea. *Journal of Marine Systems*, *132*, 106–115.

L.Pimm, S. (1985). The complexity and stability of ecosystems. *Nature*, *315*(6021), 635–636.

Lamim, V. B., & Procópio, L. (2021). Influence of Acidification and Warming of Seawater on Biofouling by Bacteria Grown over API 5L Steel. *Indian Journal of Microbiology*, *61*(2), 151–159. https://doi.org/10.1007/s12088-021-00925-7

Land, P. E., Shutler, J. D., Findlay, H. S., Girard-Ardhuin, F., Sabia, R., Reul, N., … Bhadury, P. (2015). Salinity from space unlocks satellite-based assessment of ocean acidification. *Environmental Science and Technology*. https://doi.org/10.1021/es504849s

Land, P., Shutler, J., Findlay, H., Girard-Ardhuin, F., Sabia, R., Reul, N., … Bhadury, P. (2015). Salinity from Space Unlocks Satellite-Based Assessment of Ocean Acidification. *Environmental Science & Technology*, *49*(4), 1987. https://doi.org/10.1021/es504849s

Laurent, A., Fennel, K., Cai, W. J., Huang, W. J., Barbero, L., & Wanninkhof, R. (2017). Eutrophication-induced acidification of coastal waters in the northern Gulf of Mexico: Insights into origin and processes from a coupled physical-biogeochemical model. *Geophysical Research Letters*, *44*(2), 946–956. https://doi.org/10.1002/2016GL071881

Laverock, B., Tait, K., Gilbert, J. A., Osborn, A. M., & Widdicombe, S. (2014). Impacts of bioturbation on temporal variation in bacterial and archaeal nitrogen-cycling gene abundance in coastal sediments. *Environmental Microbiology Reports*, *6*(1), 113–121. https://doi.org/10.1111/1758-2229.12115

Laverock, B, Kitidis, V., Tait, K., Gilbert, J. A., Osborn, A. M., & Widdicombe, S. (2013). Bioturbation determines the response of benthic ammonia-oxidizing microorganisms to ocean acidification. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1627), <xocs:firstpage xmlns:xocs=""/>. https://doi.org/10.1098/rstb.2012.0441

Laverock, Bonnie, Gilbert, J. A., Tait, K., Osborn, A. M., & Widdicombe, S. (2011). Bioturbation: impact on the marine nitrogen cycle. *Biochemical Society Transactions*. https://doi.org/10.1042/BST0390315

Laverock, Bonnie, Smith, C. J., Tait, K., Osborn, A. M., Widdicombe, S., & Gilbert, J. A. (2010a). Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. *The ISME Journal*. https://doi.org/10.1038/ismej.2010.86

Laverock, Bonnie, Smith, C. J., Tait, K., Osborn, A. M., Widdicombe, S., & Gilbert, J. A. (2010b). Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. *ISME Journal*, *4*(12), 1531–1544. https://doi.org/10.1038/ismej.2010.86

Lee, K., Tong, L. T., Millero, F. J., Sabine, C. L., Dickson, A. G., Goyet, C., … Key, R. M. (2006). Global relationships of total alkalinity with salinity and temperature in surface waters of the world's oceans. *Geophysical Research Letters*, *33*(19).

Levin, S. A., & Lubchenco, J. (2008). Resilience, robustness, and marine ecosystem-based management. *Bioscience*, *58*(1), 27–32.

Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., … BERMAN-FRANK, I. (2007). Elevated CO2 enhances nitrogen fixation and growth in the marine cyanobacterium Trichodesmium. *Global Change Biology*, *13*(2), 531–538.

Lewis, E. R., & Wallace, D. W. R. (1998). *Program developed for CO2 system calculations*. Environmental System Science Data Infrastructure for a Virtual Ecosystem.

Liao, H., Lin, X., Li, Y., Qu, M., & Tian, Y. (2020). Reclassification of the taxonomic framework of orders cellvibrionales, oceanospirillales, pseudomonadales, and alteromonadales in class gammaproteobacteria through phylogenomic tree analysis. *Msystems*, *5*(5), e00543-20.

Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R News*, *2*(3), 18–22.

Lidbury, I., Johnson, V., Hall-Spencer, J. M., Munn, C. B., & Cunliffe, M. (2012). Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. *Marine Pollution Bulletin*, *64*(5), 1063–1066. https://doi.org/https://doi.org/10.1016/j.marpolbul.2012.02.011

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J.-P. (2010a). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*, *61*(3), 291–305.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J. P. (2010b). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*. https://doi.org/10.3354/ame01446

Llirós, M., Inceoₓlu, Ö., García-Armisen, T., Anzil, A., Leporcq, B., Pigneur, L. M., … Servais, P. (2014). Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. *PLoS ONE*, *9*(12), 1–27. https://doi.org/10.1371/journal.pone.0116145

Lomas, M. W., Hopkinson, B. M., Ryan, J. L. L. D. E., Shi, D. L., Xu, Y., & Morel, F. M. M. (2012). Effect of ocean acidification on cyanobacteria in the subtropical North Atlantic. *Aquatic Microbial Ecology*, *66*(3), 211–222.

Long, M. H., Charette, M. A., Martin, W. R., & McCorkle, D. C. (2015). Oxygen metabolism and pH in coastal ecosystems: Eddy Covariance Hydrogen ion and Oxygen Exchange System (ECHOES). *Limnology and Oceanography: Methods*, *13*(8), 438–450.

Lozupone, C. A., & Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences*, *104*(27), 11436–11440.

Lueker, T. J., Dickson, A. G., & Keeling, C. D. (2000). Ocean pCO2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of CO2 in gas and seawater at equilibrium. *Marine Chemistry*, *70*(1–3), 105–119.

Lukawska-Matuszewska, K. (2016). Contribution of non-carbonate inorganic and organic alkalinity to total measured alkalinity in pore waters in marine sediments (Gulf of Gdansk, SE Baltic Sea). *Marine Chemistry*, *186*, 211–220.

Lukawska-Matuszewska, K., & Kielczewska, J. (2016). Effects of near-bottom water oxygen concentration on biogeochemical cycling of C, N and S in sediments of the Gulf of Gdansk (southern Baltic). *Continental Shelf Research*, *117*, 30–42.

Lusk, R. W. (2014). Diverse and widespread contamination evident in the unmapped depths of high throughput sequencing data. *PloS One*, *9*(10), e110808.

Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.-M., Siegenthaler, U., … Kawamura, K. (2008). High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature*, *453*(7193), 379.

Maas, E. W., Law, C. S., Hall, J. A., Pickmere, S., Currie, K. I., Chang, F. H., … Caird, D. (2013). Effect of ocean acidification on bacterial abundance, activity and diversity in the Ross Sea, Antarctica. *Aquatic Microbial Ecology*, *70*(1), 1–15.

Macintyre, H. L., Geider, R. J., & Miller, D. C. (1996). Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries*, *19*(2), 186–201. https://doi.org/10.2307/1352224

Mackay, I. M. (2004). Real-time PCR in the microbiology laboratory. *Clinical Microbiology and Infection*, *10*(3), 190–212.

Mackenzie, F. T., Andersson, A. J., Arvidson, R. S., Guidry, M. W., & Lerman, A. (2011). Land–sea carbon and nutrient fluxes and coastal ocean CO2 exchange and acidification: Past, present, and future. *Applied Geochemistry*, *26*, S298–S302.

Mackey, R. L., & Currie, D. J. (2001). The diversity–disturbance relationship: is it generally strong and peaked? *Ecology*, *82*(12), 3479–3492.

Macreadie, P. I., Nielsen, D. A., Kelleway, J. J., Atwood, T. B., Seymour, J. R., Petrou, K., … Ralph, P. J. (2017). Can we manage coastal ecosystems to sequester more blue carbon? *Frontiers in Ecology and the Environment*, *15*(4), 206–213. https://doi.org/10.1002/fee.1484

Maher, D. T., Santos, I. R., Golsby-Smith, L., Gleeson, J., & Eyre, B. D. (2013). Groundwater-derived dissolved inorganic and organic carbon exports from a mangrove tidal creek: The missing mangrove carbon sink? *Limnology and Oceanography*, *58*(2), 475–488. https://doi.org/10.4319/lo.2013.58.2.0475

Maire, O., DuchГ€ne, J. C., GrГ©mare, A., Malyuga, V. S., & Meysman, F. J. R. (2007). A comparison of sediment reworking rates by the surface deposit-feeding bivalve Abra ovata during summertime and wintertime, with a comparison between two models of sediment reworking. *Journal of Experimental Marine Biology and Ecology*, *343*(1), 21–36. https://doi.org/10.1016/j.jembe.2006.10.052

Malits, A., Boras, J. A., Balagué, V., Calvo, E., Gasol, J. M., Marrasé, C., … Vaqué, D. (2021). Viral-mediated microbe mortality modulated by ocean acidification and eutrophication: Consequences for the carbon fluxes through the microbial food web. *Frontiers in Microbiology*, *12*.

Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial

composition. *Microbial Ecology in Health & Disease*, *26*(0). https://doi.org/10.3402/mehd.v26.27663

Mandic, M., Ramon, M. L., Gracey, A. Y., & Richards, J. G. (2014). Divergent transcriptional patterns are related to differences in hypoxia tolerance between the intertidal and the subtidal sculpins. *Molecular Ecology*, *23*(24), 6091–6103. https://doi.org/10.1111/mec.12991

Mantoura, R. F. C. (1991). *Ocean margin processes in global change*.

Marinelli, R. L., & Boudreau, B. P. (1996). An experimental and modeling study of pH and related solutes in an irrigated anoxic coastal sediment. *Journal of Marine Research*, *54*(5), 939–966.

Marinelli, R. L., Lovell, C. R., Wakeham, S. G., Ringelberg, D. B., & White, D. C. (2002). Experimental investigation of the control of bacterial community composition in macrofaunal burrows. *Marine Ecology Progress Series*, *235*, 1–13. https://doi.org/10.3354/meps235001

Martens-Habbena, W., Berube, P. M., Urakawa, H., José, R., & Stahl, D. A. (2009). Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature*, *461*(7266), 976.

Martín Hernández-Ayon, J., Zirino, A., Dickson, A. G., Camiro-Vargas, T., & Valenzuela-Espinoza, E. (2007). Estimating the contribution of organic bases from microalgae to the titration alkalinity in coastal seawaters. *Limnology and Oceanography: Methods*, *5*(7), 225–232.

Mason, G. (2010). *Poisoning and Acidification of the Earth's Oceans*. Nova Science Publishers.

Mateo 2006 Carbon Flux in Seagrass Ecosystems.pdf. (2006).

Mateo, M. A., Romero, J., Pérez, M., Littler, M. M., & Littler, D. S. (1997). Dynamics of millenary organic deposits resulting from the growth of the Mediterranean seagrass Posidonia oceanica. *Estuarine, Coastal and Shelf Science*, *44*(1), 103–110. https://doi.org/10.1006/ecss.1996.0116

Mathis, J. T., Pickart, R. S., Byrne, R. H., McNeil, C. L., Moore, G. W. K., Juranek, L. W., … Feely, R. A. (2012). Storm-induced upwelling of high pCO2 waters onto the continental shelf of the western Arctic Ocean and implications for carbonate mineral saturation states. *Geophysical Research Letters*, *39*(7), 4–9. https://doi.org/10.1029/2012GL051574

Maung-Saw-Htoo-Thaw, Ohara, S., Matsuoka, K., Yurimoto, T., Higo, S., Khin-Ko-Lay, … Koike, K. (2017). Seasonal dynamics influencing coastal primary production and phytoplankton communities along the southern Myanmar coast. *Journal of Oceanography*, *73*(3), 345–364. https://doi.org/10.1007/s10872-016-0408-7

McArley, T. J., Hickey, A. J. R., Wallace, L., Kunzmann, A., & Herbert, N. A. (2019). Intertidal triplefin fishes have a lower critical oxygen tension (P crit), higher maximal aerobic capacity, and higher tissue glycogen stores than their subtidal counterparts. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*,

189(3–4), 399–411. https://doi.org/10.1007/s00360-019-01216-w

McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R., … DeLong, E. F. (2010). Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proceedings of the National Academy of Sciences*, *107*(38), 16420–16427.

McIlroy, S. J., & Nielsen, P. H. (2014). The family saprospiraceae. In *The prokaryotes: Other major lineages of bacteria and the Archaea* (pp. 863–889). Springer Science+ Business Media.

Mckee, K. L., Cahoon, D. R., & Feller, I. C. (2007). Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Global Ecology and Biogeography*, *16*(5), 545–556. https://doi.org/10.1111/j.1466-8238.2007.00317.x

McLaughlin, K., Dickson, A., Weisberg, S. B., Coale, K., Elrod, V., Hunter, C., … Weis, K. R. (2017). An evaluation of ISFET sensors for coastal pH monitoring applications. *Regional Studies in Marine Science*, *12*(March), 11–18. https://doi.org/10.1016/j.rsma.2017.02.008

McLeod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., … Silliman, B. R. (2011). A blueprint for blue carbon: Toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2. *Frontiers in Ecology and the Environment*, *9*(10), 552–560. https://doi.org/10.1890/110004

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

McMurdie, P. J., & Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLOS Computational Biology*, *10*(4), e1003531. Retrieved from https://doi.org/10.1371/journal.pcbi.1003531

McNaughton, S. J. (1977). Diversity and Stability of Ecological Communities: A Comment on the Role of Empiricism in Ecology. *The American Naturalist*, *111*(979), 515–525. https://doi.org/10.1086/283181

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure 1. *Limnology and Oceanography*, *18*(6), 897–907.

Melzner, F., Mark, F. C., Seibel, B. A., & Tomanek, L. (2020). Ocean Acidification and Coastal Marine Invertebrates: Tracking CO2 Effects from Seawater to the Cell. *Annual Review of Marine Science*, *12*, 499–523. https://doi.org/10.1146/annurev-marine-010419-010658

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., … Körtzinger, A. (2013a). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology*, *160*(8), 1875–1888.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., … Körtzinger, A. (2013b). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology*, *160*(8), 1875–1888. https://doi.org/10.1007/s00227-012-1954-1

Mendoza-Lera, C., & Mutz, M. (2013). Microbial activity and sediment disturbance modulate the vertical water flux in sandy sediments. *Freshwater Science*, *32*(1), 26–38. https://doi.org/10.1899/11-165.1

Mercado, J. M., & Gordillo, F. J. L. (2011). Inorganic carbon acquisition in algal communities: Are the laboratory data relevant to the natural ecosystemš. *Photosynthesis Research*, *109*(1–3), 257–267. https://doi.org/10.1007/s11120-011-9646-0

Metzker, M. L. (2010). Sequencing technologies — the next generation. *Nature Reviews Genetics*, *11*(1), 31–46. https://doi.org/10.1038/nrg2626

Meysman, F. J. R., Middelburg, J. J., & Heip, C. H. R. (2006). Bioturbation: a fresh look at Darwin's last idea. *Trends in Ecology & Evolution*, *21*(12), 688–695.

Middelburg, J J, & Levin, L. A. (2009). Coastal hypoxia and sediment biogeochemistry. *Biogeosciences*, *6*(7), 1273–1293.

Middelburg, Jack J., Soetaert, K., & Hagens, M. (2020). Ocean Alkalinity, Buffering and Biogeochemical Processes. *Reviews of Geophysics*, *58*(3). https://doi.org/10.1029/2019RG000681

Millero, F. J., Lee, K., & Roche, M. (1998). Distribution of alkalinity in the surface waters of the major oceans. *Marine Chemistry*, *60*(1–2), 111–130.

Monaco, A., & Prouzet, P. (2015). *Vulnerability of Coastal Ecosystems and Adaptation*. John Wiley & Sons.

Mongin, M., Baird, M. E., Tilbrook, B., Matear, R. J., Lenton, A., Herzfeld, M., … Steven, A. D. L. (2016). The exposure of the Great Barrier Reef to ocean acidification. *Nature Communications*, *7*, 1–8. https://doi.org/10.1038/ncomms10732

Monier, A., Findlay, H. S., Charvet, S., & Lovejoy, C. (2014). Late winter under ice pelagic microbial communities in the high Arctic Ocean and the impact of short-term exposure to elevated CO2 levels. *Frontiers in Microbiology*, *5*(SEP), 1–12. https://doi.org/10.3389/fmicb.2014.00490

Morse, J. W., Andersson, A. J., & Mackenzie, F. T. (2006a). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO2 and "ocean acidification": Role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, *70*(23), 5814–5830.

Morse, J. W., Andersson, A. J., & Mackenzie, F. T. (2006b). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO2 and "ocean acidification": Role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, *70*(23), 5814–5830.

Moser, S. C., Jeffress Williams, S., & Boesch, D. F. (2012). Wicked challenges at land's end: Managing coastal vulnerability under climate change. *Annual Review of Environment and Resources*, *37*, 51–78. https://doi.org/10.1146/annurev-environ-021611-135158

Mostofa, K. M. G., Liu, C. Q., Zhai, W. D., Minella, M., Vione, D., Gao, K. S., … Sakugawa, H. (2016). Reviews and Syntheses: Ocean acidification and its potential impacts on marine ecosystems. *Biogeosciences*, *13*(6), 1767–1786. https://doi.org/10.5194/bg-13-1767-2016

Mucci, A. (1983). The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. *American Journal of Science*, *283*(7), 780–799.

Muller-karger, F. E., Varela, R., Thunell, R., Luerssen, R., Hu, C., & Walsh, J. J. (2005). *The importance of continental margins in the global carbon cycle*. *32*(December 2004), 10–13. https://doi.org/10.1029/2004GL021346

Muller, F. L. L., & Bleie, B. (2008). Estimating the organic acid contribution to coastal seawater alkalinity by potentiometric titrations in a closed cell. *Analytica Chimica Acta*, *619*(2), 183–191.

Murrell, P. (2003). *The grid Graphics Package*. 1–6.

Nancy, Boparai, J. K., & Sharma, P. K. (2021). *Metatranscriptomics: A Promising Tool to Depict Dynamics of Microbial Community Structure and Function BT - Microbial Metatranscriptomics Belowground* (M. Nath, D. Bhatt, P. Bhargava, & D. K. Choudhary, Eds.). https://doi.org/10.1007/978-981-15-9758-9_22

Nelson, K. S., Baltar, F., Lamare, M. D., & Morales, S. E. (2020). Ocean acidification affects microbial community and invertebrate settlement on biofilms. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-60023-4

Nixon, S. W. (1981). Remineralization and nutrient cycling in coastal marine ecosystems. In *Estuaries and nutrients* (pp. 111–138). Springer.

Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, *41*(1), 199–219.

Norkko, A., Cummings, V. J., Thrush, S. F., Hewitt, J. E., & Hume, T. (2001). Local dispersal of juvenile bivalves: Implications for sandflat ecology. *Marine Ecology Progress Series*, *212*, 131–144. https://doi.org/10.3354/meps212131

Nunes-Alves, C. (2015). Microbial ecology: Do it yourself nitrification. *Nature Reviews Microbiology*, *14*(2), 61.

O'Malley, M. A. (2008). 'Everything is everywhere: but the environment selects': ubiquitous distribution and ecological determinism in microbial biogeography. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, *39*(3), 314–325.

Obi, C. C., Adebusoye, S. A., Ugoji, E. O., Ilori, M. O., Amund, O. O., & Hickey, W. J. (2016). Microbial communities in sediments of Lagos Lagoon, Nigeria: elucidation of community structure and potential impacts of contamination by municipal and industrial wastes. *Frontiers in Microbiology*, *7*, 1213.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P. M., … & Wagner, H. (2008). The vegan package. *Community Ecology Package*, (May 2014), 190. Retrieved from https://bcrc.bio.umass.edu/biometry/images/8/85/Vegan.pdf

Oliver, A. E., Newbold, L. K., Whiteley, A. S., & van der Gast, C. J. (2014). Marine bacterial communities are resistant to elevated carbon dioxide levels. *Environmental Microbiology Reports*, *6*(6), 574–582. https://doi.org/10.1111/1758-2229.12159

Orem, W. H., Holmes, C. W., Kendall, C., Lerch, H. E., Bates, A. L., Silva, S. R., … Hedgman, C. (1999). Geochemistry of Florida Bay sediments: Nutrient history at five sites in eastern and central Florida Bay. *Journal of Coastal Research*, *15*(4), 1055–1071.

Orr, J. C. (2011). Recent and future changes in ocean carbonate chemistry. *Ocean Acidification*, *1*, 41–66.

Orr, J. C., Epitalon, J.-M., Dickson, A. G., & Gattuso, J.-P. (2018). Routine uncertainty propagation for the marine carbon dioxide system. *Marine Chemistry*, *207*, 84–107.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., … Yool, A. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, *437*(7059), 681–686. https://doi.org/10.1038/nature04095

Ouellette, D., Desrosiers, G., Gagne, J.-P., Gilbert, F., Poggiale, J.-C., Blier, P. U., & Stora, G. (2004). Effects of temperature on in vitro sediment reworking processes by a gallery biodiffusor, the polychaete Neanthes virens. *Marine Ecology Progress Series*, *266*, 185–193.

Ovalle, A. R. C., Rezende, C. E., Lacerda, L. D., & Silva, C. A. R. (1990). Factors affecting the hydrochemistry of a mangrove tidal creek, sepetiba bay, Brazil. *Estuarine, Coastal and Shelf Science*, *31*(5), 639–650. https://doi.org/10.1016/0272-7714(90)90017-L

Paerl, H. W. (1985). Enhancement of marine primary production by nitrogen-enriched acid rain. *Nature (London)*, *315*(6022), 747–749. https://doi.org/10.1038/315747a0

Paliaga, P., Felja, I., Budiša, A., & Ivančić, I. (2019). The Impact of a Fish Cannery Wastewater Discharge on the Bacterial Community Structure and Sanitary Conditions of Marine Coastal Sediments. *Water*, *11*(12), 2566.

Papaspyrou, S., Gregersen, T., Cox, R. P., Thessalou-Legaki, M., & Kristensen, E. (2005). Sediment properties and bacterial community in burrows of the ghost shrimp Pestarella tyrrhena (Decapoda: Thalassinidea). *Aquatic Microbial Ecology*, *38*(2), 181–190. https://doi.org/10.3354/ame038181

Papaspyrou, S., Gregersen, T., Kristensen, E., Christensen, B., & Cox, R. P. (2006). Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes (Nereis diversicolor and N. virens). *Marine Biology*, *148*(3), 541–550. https://doi.org/10.1007/s00227-005-0105-3

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., … Fry, J. C. (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature*, *436*(7049), 390–394. https://doi.org/10.1038/nature03796

Partensky, F., Hess, W. R., & Vaulot, D. (1999). Prochlorococcus, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews*, *63*(1), 106–127.

Pauly, D., & Christensen, V. (1995). Primary production required to sustain global fisheries. *Nature*, *374*(6519), 255–257. https://doi.org/10.1038/374255a0

Pendleton, L., Donato, D. C., Murray, B. C., Crooks, S., Jenkins, W. A., Sifleet, S., … Baldera, A. (2012). Estimating Global "Blue Carbon" Emissions from Conversion and Degradation of Vegetated Coastal Ecosystems. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0043542

Pernetta, J. C., & Milliman, J. D. (1995). Land-Ocean Interactions in the Coastal Zone: implementation plan. *Oceanographic Literature Review*, *42*(9), 801.

Peters, H. (1997). Observations of Stratified Turbulent Mixing in an Estuary: Neap-to-spring Variations During High River Flow. *Estuarine, Coastal and Shelf Science*, *45*(1), 69–88. https://doi.org/10.1006/ecss.1996.0180

Philippot, L., Griffiths, B. S., & Langenheder, S. (2021). Microbial Community Resilience across Ecosystems and Multiple Disturbances. *Microbiology and Molecular Biology Reviews*, *85*(2), 1–24. https://doi.org/10.1128/mmbr.00026-20

Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., & Engel, A. (2010). Acidification increases microbial polysaccharide degradation in the ocean. *Biogeosciences*, *7*(5), 1615–1624.

Polerecky, L., Franke, U., Werner, U., Grunwald, B., & De Beer, D. (2005). High spatial resolution measurement of oxygen consumption rates in permeable sediments. *Limnology and Oceanography: Methods*, *3*(FEB.), 75–85. https://doi.org/10.4319/lom.2005.3.75

Poremba, K., Tillmann, U., & Hesse, K. J. (1999). Tidal impact on planktonic primary and bacterial production in the German Wadden Sea. *Helgoland Marine Research*, *53*(1), 19–27. https://doi.org/10.1007/PL00012133

Poretsky, R., Rodriguez-R, L. M., Luo, C., Tsementzi, D., & Konstantinidis, K. T. (2014). Strengths and Limitations of 16S rRNA Gene Amplicon Sequencing in Revealing Temporal Microbial Community Dynamics. *PLOS ONE*, *9*(4), e93827. Retrieved from https://doi.org/10.1371/journal.pone.0093827

Power, J. F., Carere, C. R., Lee, C. K., Wakerley, G. L. J., Evans, D. W., Button, M., … Stott, M. B. (2018). Microbial biogeography of 925 geothermal springs in New Zealand. *Nature Communications*, *9*(1), 2876. https://doi.org/10.1038/s41467-018-05020-y

Pratt, D. R., Pilditch, C. A., Lohrer, A. M., & Thrush, S. F. (2014). The effects of short-term increases in turbidity on sandflat microphytobenthic productivity and nutrient fluxes. *Journal of Sea Research*, *92*, 170–177. https://doi.org/10.1016/j.seares.2013.07.009

Price, N. N., Hamilton, S. L., Tootell, J. S., & Smith, J. E. (2011). Species-specific consequences of ocean acidification for the calcareous tropical green algae Halimeda. *Marine Ecology Progress Series*, *440*, 67–78.

Primavera, J. H. (2006). Overcoming the impacts of aquaculture on the coastal zone. *Ocean and Coastal Management*, *49*(9–10), 531–545. https://doi.org/10.1016/j.ocecoaman.2006.06.018

Probert P.K. (1984). Disturbance, sediment stability, and trophic structure of soft-bottom communities. *Journal of Marine Research*, *42*, 893–921.

Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A. H., Nieuwdorp, M., & Levin, E. (2020). Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PLOS ONE*, *15*(1), e0227434. Retrieved from https://doi.org/10.1371/journal.pone.0227434

Provoost, P., Heuven, S. van, Soetaert, K., Laane, R., & Middelburg, J. J. (2010). Seasonal and long-term changes in pH in the Dutch coastal zone. *Biogeosciences*, *7*(11), 3869–3878.

Provoost, P., van Heuven, S., Soetaert, K., Laane, R., & Middelburg, J. J. (2010). Long-term record of pH in the Dutch coastal zone: a major role for eutrophication-induced changes. *Biogeosciences Discussions*, *7*(3).

Provoost, P., Van Heuven, S., Soetaert, K., Laane, R. W. P. M., & Middelburg, J. J. (2010). Seasonal and long-term changes in pH in the Dutch coastal zone. *Biogeosciences*, *7*(11), 3869–3878. https://doi.org/10.5194/bg-7-3869-2010

Pytkowicz, R. M., & Atlas, E. (1975). Buffer intensity of seawater 1. *Limnology and Oceanography*, *20*(2), 222–229.

Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics*, *12*(1), 38. https://doi.org/10.1186/1471-2105-12-38

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Correction: Corrigendum: Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, *35*(12), 1211. https://doi.org/10.1038/nbt1217-1211b

Raffaelli, D., Bell, E., Weithoff, G., Matsumoto, A., Cruz-Motta, J. J., Kershaw, P., … Jones, M. (2003). The ups and downs of benthic ecology: Considerations of scale, heterogeneity and surveillance for benthic–pelagic coupling. *Journal of Experimental Marine Biology and Ecology*, *285*, 191–203.

Ralston, D. K., & Stacey, M. T. (2005). Stratification and turbulence in subtidal channels through intertidal mudflats. *Journal of Geophysical Research C: Oceans*, *110*(8), 1–16. https://doi.org/10.1029/2004JC002650

Rasmussen, H., & Jorgensen, B. B. (1992). Microelectrode studies of seasonal oxygen uptake in a coastal sediment: role of molecular diffusion. *Marine Ecology Progress Series*, *81*(3), 289–303. https://doi.org/10.3354/meps081289

Rassmann, J., Lansard, B., Gazeau, F., Guidi-Guilvard, L., Pozzato, L., Alliouane, S., … Rabouille, C. (2018a). Impact of ocean acidification on the biogeochemistry and meiofaunal assemblage of carbonate-rich sediments: Results from core incubations (Bay of Villefranche, NW Mediterranean Sea). *Marine Chemistry*, *203*(May 2017), 102–119. https://doi.org/10.1016/j.marchem.2018.05.006

Rassmann, J., Lansard, B., Gazeau, F., Guidi-Guilvard, L., Pozzato, L., Alliouane, S., …

Rabouille, C. (2018b). Impact of ocean acidification on the biogeochemistry and meiofaunal assemblage of carbonate-rich sediments: Results from core incubations (Bay of Villefranche, NW Mediterranean Sea). *Marine Chemistry*, *203*, 102–119.

Raulf, F. F., Fabricius, K., Uthicke, S., de Beer, D., Abed, R. M. M., & Ramette, A. (2015). Changes in microbial communities in coastal sediments along natural CO2 gradients at a volcanic vent in Papua New Guinea. *Environmental Microbiology*. https://doi.org/10.1111/1462-2920.12729

Ravaglioli, C., Bulleri, F., Rühl, S., McCoy, S. J., Findlay, H. S., Widdicombe, S., & Queirós, A. M. (2019). Ocean acidification and hypoxia alter organic carbon fluxes in marine soft sediments. *Global Change Biology*, *25*(12), 4165–4178. https://doi.org/10.1111/gcb.14806

Ravenschlag, K., Sahm, K., & Amann, R. (2001). Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard). *Applied and Environmental Microbiology*, *67*(1), 387–395. https://doi.org/10.1128/AEM.67.1.387-395.2001

Regnier, P., Friedlingstein, P., Ciais, P., Mackenzie, F. T., Gruber, N., Janssens, I. A., … Andersson, A. J. (2013). Anthropogenic perturbation of the carbon fluxes from land to ocean. *Nature Geoscience*, *6*(8), 597–607.

Reimers, C. E. (1987). An in situ microprofiling instrument for measuring interfacial pore water gradients: methods and oxygen profiles from the North Pacific Ocean. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(12), 2019–2035.

Reimers, C. E., Ruttenberg, K. C., Canfield, D. E., Christiansen, M. B., & Martin, J. B. (1996). Porewater pH and authigenic phases formed in the uppermost sediments of the Santa Barbara Basin. *Geochimica et Cosmochimica Acta*, *60*(21), 4037–4057. https://doi.org/10.1016/S0016-7037(96)00231-1

Reise, K. (2001). *Ecological comparisons of sedimentary shores (Ecological Studies; v 151)*.

Reise, K. (2002). Sediment mediated species interactions in coastal waters. *Journal of Sea Research*, *48*(2), 127–141. https://doi.org/10.1016/S1385-1101(02)00150-8

Reuter, J. A., Spacek, D. V, & Snyder, M. P. (2015). High-Throughput Sequencing Technologies. *Molecular Cell*, *58*(4), 586–597. https://doi.org/https://doi.org/10.1016/j.molcel.2015.05.004

Revelle, R., & Suess, H. E. (1957). Carbon Dioxide Exchange Between Atmosphere and Ocean and the Question of an Increase of Atmospheric CO2 during the Past Decades. *Tellus*, *9*(1), 18–27. https://doi.org/10.3402/tellusa.v9i1.9075

Revsbech, N. P. (1983). In situ measurement of oxygen profiles of sediments by use of oxygen microelectrodes. In *Polarographic oxygen sensors* (pp. 265–273). Springer.

Rhoads, D. C., & Boyer, L. F. (1982). The effects of marine benthos on physical properties of sediments. In *Animal-sediment relations* (pp. 3–52). Springer.

Riebesell, U., Gattuso, J.-P., Thingstad, T. F., & Middelburg, J. J. (2013). Arctic ocean acidification: pelagic ecosystem and biogeochemical responses during a mesocosm study. *Biogeosciences*, *10*, 5619–5626.

Riebesell, U., & Zondervan, I. (2000). *Rie2000a*. *407*(September), 2–5.

Rinta-Kanto, J. M., Sun, S., Sharma, S., Kiene, R. P., & Moran, M. A. (2012). Bacterial community transcription patterns during a marine phytoplankton bloom. *Environmental Microbiology*, *14*(1), 228–239.

Ríos, A. F., Resplandy, L., García-Ibáñez, M. I., Fajar, N. M., Velo, A., Padin, X. A., … Morel, F. M. M. (2015). Decadal acidification in the water masses of the Atlantic Ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(32), 9950–9955. https://doi.org/10.1073/pnas.1504613112

Rodil, I. F., Lohrer, A. M., & Thrush, S. F. (2013). Sensitivity of heterogeneous marine benthic habitats to subtle stressors. *PloS One*, *8*(11), e81646.

Roy, A. S., Gibbons, S. M., Schunck, H., Owens, S., Caporaso, J. G., Sperling, M., … Gilbert, J. A. (2013). Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms. *Biogeosciences*, *10*(1), 555–566. https://doi.org/10.5194/bg-10-555-2013

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/https://doi.org/10.1016/j.gecco.2019.e00547

Ryan, P. C., H., D. A., & M., T. J. (2006). Molecular Evidence for the Broad Distribution of Anaerobic Ammonium-Oxidizing Bacteria in Freshwater and Marine Sediments. *Applied and Environmental Microbiology*, *72*(10), 6829–6832. https://doi.org/10.1128/AEM.01254-06

Rysgaard, S., Fossing, H., & Jensen, M. M. (2001). Organic matter degradation through oxygen respiration, denitrification, and manganese, iron, and sulfate reduction in marine sediments (the Kattegat and the Skagerrak). *Ophelia*, *55*(2), 77–91. https://doi.org/10.1080/00785236.2001.10409475

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., … Rios, A. F. (2004). The Oceanic Sink for Anthropogenic CO<sub>2</sub&gt. *Science*, *305*(5682), 367. Retrieved from http://science.sciencemag.org/content/305/5682/367.abstract

Sabine, C. L., & Tanhua, T. (2010). Estimation of anthropogenic CO2 inventories in the ocean. *Annual Review of Marine Science*, *2*, 175–198.

Sala, M. M., Aparicio, F. L., Balague, V., Boras, J. A., Borrull, E., Cardelus, C., … Marrase, C. (2016). Contrasting effects of ocean acidification on the microbial food web under different trophic conditions. *Ices Journal of Marine Science*, *73*(3), 670–679. https://doi.org/10.1093/icesjms/fsv130

Salisbury, J., Green, M., Hunt, C., & Campbell, J. (2008). Coastal acidification by rivers: A threat to shellfish? *Eos*, *89*(50), 513. https://doi.org/10.1029/2008EO500001

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., … Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, *12*(1), 1–12.

Sandoval-Gil, J. M., Barrote, I., Silva, J., Olivé, I., Costa, M. M., Ruiz, J. M., … Santos, R. (2015). Plant-water relations of intertidal and subtidal seagrasses. *Marine Ecology*, *36*(4), 1294–1310. https://doi.org/10.1111/maec.12230

Sarmiento, J. L., & Gruber, N. (2006). Ocean biogeochemical cycles. *Princeton University*.

Säwström, C., Hyndes, G. A., Eyre, B. D., Huggett, M. J., Fraser, M. W., Lavery, P. S., … Laverock, B. (2016). Coastal connectivity and spatial subsidy from a microbial perspective. *Ecology and Evolution*. https://doi.org/10.1002/ece3.2408

Scala, D. J., & Kerkhof, L. J. (2000). Horizontal Heterogeneity of Denitrifying Bacterial Communities in Marine Sediments by Terminal Restriction Fragment Length Polymorphism Analysis. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, *66*(5), 1980–1986. Retrieved from http://www.es.umb

Schiel, D. R. (1988). Algal interactions on shallow subtidal reefs in northern New Zealand: A review. *New Zealand Journal of Marine and Freshwater Research*, *22*(3), 481–489. https://doi.org/10.1080/00288330.1988.9516317

Schloss, P. D., & Westcott, S. L. (2011). Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.02810-10

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Seitzinger, S. P., Harrison, J. A., Dumont, E., Beusen, A. H. W., & Bouwman, A. F. (2005). Sources and delivery of carbon, nitrogen, and phosphorus to the coastal zone: An overview of Global Nutrient Export from Watersheds (NEWS) models and their application. *Global Biogeochemical Cycles*, *19*(4), 1–11. https://doi.org/10.1029/2005GB002606

Setubal, J. C. (2021). Metagenome-assembled genomes: concepts, analogies, and challenges. *Biophysical Reviews*, *13*(6), 905–909. https://doi.org/10.1007/s12551-021-00865-y

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*(3), 379–423.

Sharma, R., Nagender Nath, B., Parthiban, G., & Jai Sankar, S. (2001). Sediment redistribution during simulated benthic disturbance and its implications on deep seabed mining. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *48*(16), 3363–3380. https://doi.org/10.1016/S0967-0645(01)00046-7

Shetye, S., Sudhakar, M., Jena, B., & Mohan, R. (2013). Occurrence of nitrogen fixing cyanobacterium Trichodesmium under elevated pCO2 conditions in the Western Bay of Bengal. *International Journal of Oceanography*, *2013*.

Shi, D., Hong, H., Su, X., Liao, L., Chang, S., & Lin, W. (2019). The physiological response of marine diatoms to ocean acidification: differential roles of seawater pCO 2 and pH.

Journal of Phycology, *55*(3), 521–533.

SHOKRALLA, S., SPALL, J. L., GIBSON, J. F., & HAJIBABAEI, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology*, *21*(8), 1794–1805. https://doi.org/https://doi.org/10.1111/j.1365-294X.2012.05538.x

Silburn, B., Kröger, S., Parker, E. R., Sivyer, D. B., Hicks, N., Powell, C. F., … Greenwood, N. (2017). Benthic pH gradients across a range of shelf sea sediment types linked to sediment characteristics and seasonal variability. *Biogeochemistry*, *135*(1–2), 69–88. https://doi.org/10.1007/s10533-017-0323-z

Simeone, S., Molinaroli, E., Conforti, A., & De Falco, G. (2018). Impact of ocean acidification on the carbonate sediment budget of a temperate mixed beach. *Climatic Change*, *150*(3), 227–242.

Simone, M., Schulz, K., Oakes, J., & Eyre, B. (2020). Warming and ocean acidification may decrease estuarine dissolved organic carbon export to the ocean. *Biogeosciences Discussions*, 1–22. https://doi.org/10.5194/bg-2020-335

Singer, E., Wagner, M., & Woyke, T. (2017). Capturing the genetic makeup of the active microbiome in situ. *The ISME Journal*, *11*(9), 1949–1963. https://doi.org/10.1038/ismej.2017.59

Sippo, J. Z., Maher, D. T., Tait, D. R., Holloway, C., & Santos, I. R. (2016). Are mangroves drivers or buffers of coastal acidification? Insights from alkalinity and dissolved inorganic carbon export estimates across a latitudinal transect. *Global Biogeochemical Cycles*, *30*(5), 753–766. https://doi.org/10.1002/2015GB005324

Smith, C. J., Dong, L. F., Wilson, J., Stott, A., Osborn, A. M., & Nedwell, D. B. (2015). Seasonal variation in denitrification and dissimilatory nitrate reduction to ammonia process rates and corresponding key functional genes along an estuarine nitrate gradient. *Frontiers in Microbiology*, *6*(542). https://doi.org/10.3389/fmicb.2015.00542

Smith, D. C., Simon, M., Alldredge, A. L., & Azam, F. (1992). Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature*, *359*(6391), 139–142.

Smith, E. M., & Hopkinson, C. S. (2005). *Estuarine respiration: an overview of benthic, pelagic, and whole system respiration*. https://doi.org/10.1093/acprof:oso/9780198527084.003.0008

Snelgrove, P V R, & Butman, C. A. (1995). Animal-sediment relationships revisited: cause versus effect. *Oceanographic Literature Review*, *42*(8), 668.

Snelgrove, Paul V R. (1997). The Importance of Marine Sediment Biodiversity in Ecosystem Processes. *Source: Ambio*, *26*(8), 578–583.

Soetaert, K., Hofmann, A. F., Middelburg, J. J., Meysman, F. J. R., & Greenwood, J. (2007). Reprint of "The effect of biogeochemical processes on pH." *Marine Chemistry*, *106*(1-2 SPEC. ISS.), 380–401. https://doi.org/10.1016/j.marchem.2007.06.008

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., … Herndl, G. J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere."

Proceedings of the National Academy of Sciences, *103*(32), 12115–12120.

Sohm, J. A., Webb, E. A., & Capone, D. G. (2011). Emerging patterns of marine nitrogen fixation. *Nature Reviews Microbiology*, *9*(7), 499–508.

Solomon, S. (2007). The physical science basis: Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. *Intergovernmental Panel on Climate Change (IPCC), Climate Change 2007*, *996*.

Solomon, Susan, Qin, D., Manning, M., Averyt, K., & Marquis, M. (2007). *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC* (Vol. 4). Cambridge university press.

Somero, G. N. (2012). The physiology of global change: Linking patterns to mechanisms. *Annual Review of Marine Science*, *4*, 39–61. https://doi.org/10.1146/annurev-marine-120710-100935

Song, B., Li, Z., Li, S., Zhang, Z., Fu, Q., Wang, S., … Qi, S. (2021). Functional metagenomic and enrichment metatranscriptomic analysis of marine microbial activities within a marine oil spill area. *Environmental Pollution*, *274*, 116555. https://doi.org/https://doi.org/10.1016/j.envpol.2021.116555

Spilling, K. (2007). Dense sub-ice bloom of dinoflagellates in the Baltic Sea, potentially limited by high pH. *Journal of Plankton Research*, *29*(10), 895–901. https://doi.org/10.1093/plankt/fbm067

Spring, S., Scheuner, C., Göker, M., & Klenk, H. P. (2015). A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Frontiers in Microbiology*, *6*(APR), 1–17. https://doi.org/10.3389/fmicb.2015.00281

Ståhl, H., Tengberg, A., Brunnegård, J., Bjørnbom, E., Forbes, T. L., Josefson, A. B., … Hall, P. O. J. (2004). Factors influencing organic carbon recycling and burial in Skagerrak sediments. *Journal of Marine Research*, *62*(6), 867–907. https://doi.org/10.1357/0022240042880873

Steckbauer, A., Ramajo, L., Hendriks, I. E., Fernandez, M., Lagos, N. A., Prado, L., & Duarte, C. M. (2015). Synergistic effects of hypoxia and increasing CO2 on benthic invertebrates of the central Chilean coast. *Frontiers in Marine Science*, *2*(JUL), 1–12. https://doi.org/10.3389/fmars.2015.00049

Stevens, C. L. (2003). Turbulence in an estuarine embayment: Observations from Beatrix Bay, New Zealand. *Journal of Geophysical Research: Oceans*, *108*(2), 1–15. https://doi.org/10.1029/2001jc001221

Stingl, U., Tripp, H. J., & Giovannoni, S. J. (2007). Improvements of high-throughput culturing yielded novel SAR11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. *The ISME Journal*, *1*(4), 361.

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., … Midgley, P. M. (2013). Climate change 2013: the physical science basis. Intergovernmental panel on climate change, working group I contribution to the IPCC fifth assessment report (AR5). *New York*.

Stumm, W., & Morgan, J. J. (2012). *Aquatic chemistry: chemical equilibria and rates in natural waters* (Vol. 126). John Wiley & Sons.

Subramaniam, A., Yager, P. L., Carpenter, E. J., Mahaffey, C., Björkman, K., Cooley, S., … Capone, D. G. (2008). Amazon River Enhances Diazotrophy and Carbon Sequestration in the Tropical North Atlantic Ocean. *Proc Natl Acad Sci U S A*, *105*(30), 10460–10465. https://doi.org/10.1073/pnas.0710279105

Sulpis, O., Boudreau, B. P., Mucci, A., Jenkins, C., Trossman, D. S., Arbic, B. K., & Key, R. M. (2018). Current CaCO3 dissolution at the seafloor caused by anthropogenic CO2. *Proceedings of the National Academy of Sciences*, *115*(46), 11700–11705.

Sulpis, O., Jeansson, E., Dinauer, A., Lauvset, S. K., & Middelburg, J. J. (2021). Calcium carbonate dissolution patterns in the ocean. *Nature Geoscience*, 1–6.

Sunda, W. G., & Cai, W. J. (2012). Eutrophication induced CO2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric P CO2. *Environmental Science and Technology*, *46*(19), 10651–10659. https://doi.org/10.1021/es300626f

Sundquist, E. T., Plummer, L. N., & Wigley, T. M. L. (1979). Carbon dioxide in the ocean surface: the homogeneous buffer factor. *Science*, *204*(4398), 1203–1205.

Suzuki, I., Dular, U., & Kwok, S. C. (1974). Ammonia or ammonium ion as substrate for oxidation by Nitrosomonas europaea cells and extracts. *Journal of Bacteriology*, *120*(1), 556–558. https://doi.org/10.1128/jb.120.1.556-558.1974

Suzuki, Isamu, Dular, U., & Kwok, S. C. (1974). Ammonia or Ammonium Ion as Substrate for Oxidation by Nitrosomonas europaea Cells and Extracts. *Journal of Bacteriology*, *120*(1), 556–558. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC245802/

Taguchi, F., & Fujiwara, T. (2010). Carbon dioxide stored and acidified low oxygen bottom waters in coastal seas, Japan. *Estuarine, Coastal and Shelf Science*, *86*(3), 429–433. https://doi.org/10.1016/j.ecss.2009.07.037

Tait, D. R., Maher, D. T., Macklin, P. A., & Santos, I. R. (2016). Mangrove pore water exchange across a latitudinal gradient. *Geophysical Research Letters*, *43*(7), 3334–3341. https://doi.org/10.1002/2016GL068289

Tait, K., Laverock, B., Shaw, J., Somerfield, P. J., & Widdicombe, S. (2013). Minor impact of ocean acidification to the composition of the active microbial community in an Arctic sediment. *Environmental Microbiology Reports*. https://doi.org/10.1111/1758-2229.12087

Tait, K., Laverock, B., & Widdicombe, S. (2014). Response of an Arctic Sediment Nitrogen Cycling Community to Increased CO2. *Estuaries and Coasts*, *37*(3 LB-Tait2014), 724–735. https://doi.org/10.1007/s12237-013-9709-x

Taylor, P., Lichtschlag, A., Toberman, M., Sayer, M. D. J., Reynolds, A., Sato, T., & Stahl, H. (2015). Impact and recovery of pH in marine sediments subject to a temporary carbon dioxide leak. *International Journal of Greenhouse Gas Control*, *38*, 93–101. https://doi.org/10.1016/j.ijggc.2014.09.006

Thomas, H., Schiettecatte, L. S., Suykens, K., Koné, Y. J. M., Shadwick, E. H., Prowe, A. E. F., … Borges, A. V. (2009). Enhanced ocean carbon storage from anaerobic alkalinity generation in coastal sediments. *Biogeosciences*, *6*(2), 267–274. https://doi.org/10.5194/bg-6-267-2009

Tilman, D. (1996). Biodiversity: population versus ecosystem stability. *Ecology*, *77*(2), 350–363. https://doi.org/10.2307/2265614

Torsvik, V., xd, vre, xe, s, L., & Thingstad, T. F. (2002). Prokaryotic Diversity: Magnitude, Dynamics, and Controlling Factors. *Science*, *296*(5570), 1064–1066. Retrieved from http://www.jstor.org.ezproxy.waikato.ac.nz/stable/3076691

Tseng, C.-H., & Tang, S.-L. (2014). Marine Microbial Metagenomics: From Individual to the Environment. *International Journal of Molecular Sciences*, *15*(5), 8878–8892. https://doi.org/10.3390/ijms15058878

Turk, D., Zappa, C. J., Meinen, C. S., Christian, J. R., Ho, D. T., Dickson, A. G., & McGillis, W. R. (2010). Rain impacts on CO2 exchange in the western equatorial Pacific Ocean. *Geophysical Research Letters*, *37*(23), 1–6. https://doi.org/10.1029/2010GL045520

Turner, S. J., Grant, J., Pridmore, R. D., Hewitt, J. E., Wilkinson, M. R., Hume, T. M., & Morrisey, D. J. (1997). Bedload and water-column transport and colonization processes by post- settlement benthic macrofauna: Does infaunal density matter? *Journal of Experimental Marine Biology and Ecology*, *216*(1–2), 51–75. https://doi.org/10.1016/S0022-0981(97)00090-7

Tynan, S., & Opdyke, B. N. (2011). Effects of lower surface ocean pH upon the stability of shallow water carbonate sediments. *Science of the Total Environment*, *409*(6), 1082–1086.

Ulfsbo, A., Hulth, S., & Anderson, L. G. (2011). pH and biogeochemical processes in the Gotland Basin of the Baltic Sea. *Marine Chemistry*, *127*(1), 20–30. https://doi.org/https://doi.org/10.1016/j.marchem.2011.07.004

Upton, H. F., & Folger, P. (2015). Ocean acidification. *Ocean Acidification Strategic Research and Monitoring Plan*, *22*(4), 1–7.

Valiela, I. (n.d.). *Marine Ecological Processes 1995*. Springer: New York.

Valiela, Ivan. (2009). *Global coastal change*. John Wiley & Sons.

Villanueva, R. A. M., & Chen, Z. J. (2019). ggplot2: Elegant Graphics for Data Analysis (2nd ed.). *Measurement: Interdisciplinary Research and Perspectives*, *17*(3), 160–167. https://doi.org/10.1080/15366367.2019.1565254

Vonnahme, T. R., Molari, M., Janssen, F., Wenzhöfer, F., Haeckel, M., Titschack, J., & Boetius, A. (2020). Effects of a deep-sea mining experiment on seafloor microbial communities and functions after 26 years. *Science Advances*, *6*(18). https://doi.org/10.1126/sciadv.aaz5922

Vonnahme, Tobias R, Molari, M., Janssen, F., Wenzhöfer, F., Haeckel, M., Titschack, J., & Boetius, A. (2020). Effects of a deep-sea mining experiment on seafloor microbial communities and functions after 26 years. *Science Advances*, *6*(18), eaaz5922.

Vopel, K., Del-Río, C., & Pilditch, C. A. (2018). Effects of CO2 enrichment on benthic primary production and inorganic nitrogen fluxes in two coastal sediments. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-017-19051-w

Vopel, K., Laverock, B., Cary, C., & Pilditch, C. A. (2021). Effects of warming and CO2 enrichment on O2 consumption, porewater oxygenation and pH of subtidal silt sediment. *Aquatic Sciences*, *83*(1). https://doi.org/10.1007/s00027-020-00765-5

Vopel, K., Marshall, A., Brandt, S., Hartland, A., Lee, C. K., Cary, S. C., & Pilditch, C. A. (2021). Biogeochemical feedbacks to ocean acidification in a cohesive photosynthetic sediment. *Scientific Reports*, *11*(1), 22867. https://doi.org/10.1038/s41598-021-02314-y

Vopel, K., Vopel, A., Thistle, D., & Hancock, N. (2007). Effects of spatangoid heart urchins on O2 supply into coastal sediment. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps333161

Waldbusser, G. G., & Salisbury, J. E. (2014). Ocean acidification in the coastal zone from an organism's perspective: Multiple system parameters, frequency domains, and habitats. *Annual Review of Marine Science*, *6*, 221–247. https://doi.org/10.1146/annurev-marine-121211-172238

Waldbusser, G. G., Voigt, E. P., Bergschneider, H., Green, M. A., & Newell, R. I. E. (2011). Biocalcification in the Eastern Oyster (Crassostrea virginica) in Relation to Long-term Trends in Chesapeake Bay pH. *Estuaries and Coasts*, *34*(2), 221–231. https://doi.org/10.1007/s12237-010-9307-0

Walker, A. M., Leigh, M. B., & Mincks, S. L. (2021). Patterns in Benthic Microbial Community Structure Across Environmental Gradients in the Beaufort Sea Shelf and Slope. *Frontiers in Microbiology*, *12*(January), 1–21. https://doi.org/10.3389/fmicb.2021.581124

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014a). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, *148*, 1–13. https://doi.org/10.1016/j.ecss.2014.05.027

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014b). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, *148*, 1–13.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Zejie, Deng, H., Chen, L., Xiao, Y., & Zhao, F. (2013). In situ measurements of dissolved oxygen, pH and redox potential of biocathode microenvironments using microelectrodes. *Bioresource Technology*, *132*, 387–390. https://doi.org/10.1016/j.biortech.2012.11.026

Wang, Zhao, Juarez, D. L., Pan, J. F., Blinebry, S. K., Gronniger, J., Clark, J. S., … Hunt, D. E. (2019). Microbial communities across nearshore to offshore coastal transects are

primarily shaped by distance and temperature. *Environmental Microbiology*, *21*(10), 3862–3872. https://doi.org/10.1111/1462-2920.14734

Wanninkhof, R., Lewis, E., Feely, R. A., & Millero, F. J. (1999). The optimal carbonate dissociation constants for determining surface water pCO2 from alkalinity and total inorganic carbon. *Marine Chemistry*, *65*(3–4), 291–301.

Ward, B. B. (1987). Kinetic studies on ammonia and methane oxidation by Nitrosococcus oceanus. *Archives of Microbiology*, *147*(2), 126–133.

Warwick, R. M., Goss-Custard, J. D., Kirby, R., George, C. L., Pope, N. D., & Rowden, A. A. (1991). Static and Dynamic Environmental Factors Determining the Community Structure of Estuarine Macrobenthos in SW Britain: Why is the Severn Estuary Different? *The Journal of Applied Ecology*, *28*(1), 329. https://doi.org/10.2307/2404133

Warwick, R., & Uncles, R. (1980). Distribution of Benthic Macrofauna Associations in the Bristol Channel in Relation to Tidal Stress. *Marine Ecology Progress Series*, *3*, 97–103. https://doi.org/10.3354/meps003097

Washburne, A. D., Morton, J. T., Sanders, J., McDonald, D., Zhu, Q., Oliverio, A. M., & Knight, R. (2018). Methods for phylogenetic analysis of microbiome. *Nature Microbiology*, *3*(6), 652–661. https://doi.org/10.1038/s41564-018-0156-0

Wasmund, K., Pelikan, C., Schintlmeister, A., Wagner, M., Watzka, M., Richter, A., … Loy, A. (2021). Genomic insights into diverse bacterial taxa that degrade extracellular DNA in marine sediments. *Nature Microbiology*, *6*(7), 885–898. https://doi.org/10.1038/s41564-021-00917-9

Watanabe, Y., Tait, K., Gregory, S., Hayashi, M., Shimamoto, A., Taylor, P., … Kita, J. (2015). Response of the ammonia oxidation activity of microorganisms in surface sediment to a controlled sub-seabed release of CO2. *International Journal of Greenhouse Gas Control*, *38*, 162–170. https://doi.org/https://doi.org/10.1016/j.ijggc.2014.11.013

Watson, S. W. (1989). Nitrifying bacteria. *Bergey's Manual of Systematic Bacteriology*, *3*, 1808–1834.

WATSON, S. W. (1971). Taxonomic Considerations of the Family Nitrobacteraceae Buchanan: Requests for Opinions. *International Journal of Systematic Bacteriology*, *21*(3), 254–270. https://doi.org/10.1099/00207713-21-3-254

Webb, A. P., & Eyre, B. D. (2004). The effect of natural populations of the burrowing and grazing soldier crab (Mictyris longicarpus) on sediment irrigation, benthic metabolism and nitrogen fluxes. *Journal of Experimental Marine Biology and Ecology*, *309*(1), 1–19. https://doi.org/10.1016/j.jembe.2004.05.003

Webster, N. S., Negri, A. P., Flores, F., Humphrey, C., Soo, R., Botté, E. S., … Uthicke, S. (2013). Near-future ocean acidification causes differences in microbial associations within diverse coral reef taxa. *Environmental Microbiology Reports*, *5*(2), 243–251. https://doi.org/10.1111/1758-2229.12006

Weinbauer, M. G., Mari, X., & Gattuso, J.-P. (2011). Effect of ocean acidification on the diversity and activity of heterotrophic marine microorganisms. *Ocean Acidification, Edited by: Gattuso, J.-P. and Hansson, L., Oxford University Press, Oxford*, 83–98.

Wenzhöfer, F, Adler, M., Kohls, O., Hensen, C., Strotmann, B., Boehme, S., & Schulz, H. D. (2001). Calcite dissolution driven by benthic mineralization in the deep-sea: in situ measurements of Ca2+, pH, pCO2 and O2. *Geochimica et Cosmochimica Acta*, *65*(16), 2677–2690. https://doi.org/https://doi.org/10.1016/S0016-7037(01)00620-2

Wenzhöfer, Frank, & Glud, R. N. (2004). Small-scale spatial and temporal variability in coastal benthic O 2 dynamics: Effects of fauna activity. *Limnology and Oceanography*, *49*(5), 1471–1481. https://doi.org/10.4319/lo.2004.49.5.1471

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences*, *95*(12), 6578–6583. https://doi.org/10.1073/pnas.95.12.6578

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wickham, H., Francois, R., & Bryan, J. (2020). *Package ' tibble .'*

Wickham, H., Francois, R., & Bryan, J. (2021). *Package ' tibble .'*

Wickham, H., & Wickham, M. H. (2020). Package 'plyr.' *Obtenido Httpscran Rproject Orgwebpackagesdplyrdplyr Pdf*.

Widdicombe, S, Dashfield, S. L., McNeill, C. L., Needham, H. R., Beesley, A., McEvoy, A., … Berge, J. A. (2009). Effects of CO2 induced seawater acidification on infaunal diversity and sediment nutrient fluxes. *Marine Ecology Progress Series*, *379*, 59–75.

Widdicombe, S, & Needham, H. R. (2007). Impact of CO2-induced seawater acidification on the burrowing activity of Nereis virens and sediment nutrient flux. *Marine Ecology Progress Series*, *341*, 111–122.

Widdicombe, Stephen, & Spicer, J. I. (2008). Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology*, *366*(1–2), 187–197. https://doi.org/10.1016/j.jembe.2008.07.024

Widdicombe, Stephen, Spicer, J. I., & Kitidis, V. (2011). Effects of ocean acidification on sediment fauna. *Ocean Acidification. Oxford University Press, Oxford*, 176–191.

Wilde, S. B., & Plante, C. J. (2002). Spatial heterogeneity of bacterial assemblages in marine sediments: The influence of deposit feeding by Balanoglossus aurantiacus. *Estuarine, Coastal and Shelf Science*, *55*(1), 97–107. https://doi.org/10.1006/ecss.2001.0889

Wilms, R., Köpke, B., Sass, H., Chang, T. S., Cypionka, H., & Engelen, B. (2006). Deep biosphere-related bacteria within the subsurface of tidal flat sediments. *Environmental Microbiology*, *8*(4), 709–719. https://doi.org/10.1111/j.1462-2920.2005.00949.x

Witt, V., Wild, C., Anthony, K. R. N., Diaz-Pulido, G., & Uthicke, S. (2011). Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef. *Environmental Microbiology*, *13*(11), 2976–2989. https://doi.org/10.1111/j.1462-2920.2011.02571.x

Wood, H. L., Spicer, J. I., & Widdicombe, S. (2008). Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1644), 1767–1773. https://doi.org/10.1098/rspb.2008.0343

Wootton, J. T., Pfister, C. A., & Forester, J. D. (2008). Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(48), 18848–18853. https://doi.org/10.1073/pnas.0810079105

Xie, M. Y. (2016). *Package 'knitr.'*

Yamada, N., & Suzumura, M. (2010). Effects of seawater acidification on hydrolytic enzyme activities. *Journal of Oceanography*, *66*(2), 233–241. https://doi.org/10.1007/s10872-010-0021-0

Yeo, S. K., Huggett, M. J., Eiler, A., & Rappé, M. S. (2013). Coastal bacterioplankton community dynamics in response to a natural disturbance. *PloS One*, *8*(2), e56207.

Ysebaert, T., Herman, P. M. J., Meire, P., Craeymeersch, J., Verbeek, H., & Heip, C. H. R. (2003). Large-scale spatial patterns in estuaries: Estuarine macrobenthic communities in the Schelde estuary, NW Europe. *Estuarine, Coastal and Shelf Science*, *57*(1–2), 335–355. https://doi.org/10.1016/S0272-7714(02)00359-1

Ysebaert, Tom, & Herman, P. M. J. (2002). Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Marine Ecology Progress Series*, *244*(Levin 1992), 105–124. https://doi.org/10.3354/meps244105

Zeebe, R. E., & Wolf-Gladrow, D. A. (2001). *CO2 in seawater: equilibrium, kinetics, isotopes*. Gulf Professional Publishing.

Zhai, W. D., Zhao, H. De, Zheng, N., & Xu, Y. (2012). Coastal acidification in summer bottom oxygen-depleted waters in northwestern-northern Bohai Sea from June to August in 2011. *Chinese Science Bulletin*, *57*(9), 1062–1068. https://doi.org/10.1007/s11434-011-4949-2

Zhu, Q., Aller, R. C., & Fan, Y. (2006). Two-dimensional pH distributions and dynamics in bioturbated marine sediments. *Geochimica et Cosmochimica Acta*, *70*(19), 4933–4949. https://doi.org/10.1016/j.gca.2006.07.033

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., … Ramette, A. (2011). Global Patterns of Bacterial Beta-Diversity in Seafloor and Seawater Ecosystems. *PLoS ONE*, *6*(9), e24570. https://doi.org/10.1371/journal.pone.0024570

Zondervan, I., Zeebe, R. E., Rost, B., & Riebesell, U. (2001). Decreasing marine biogenic calcification: A negative feedback on rising atmospheric pCO2. *Global Biogeochemical Cycles*, *15*(2), 507–516. https://doi.org/10.1029/2000GB001321

Chapter 1: A Review of The Literature

The Effects of Ocean Acidification on Benthic Microbial Communities from a Coastal Intertidal Sand Flat on the North Island of New Zealand

Shelly Brandt[1], Alexis Marshall[1], Kay Vopel[2], Bonnie Laverock[2], Conrad Pilditch[1], Adam Hartland[1], Charles Lee[1], and Craig Cary[1]

[1]The University of Waikato, Thermophile Research Unit
[2]The Auckland University of Technology

Abstract

The impact of Ocean Acidification (OA) on the carbonate chemistry of the pelagic environment has been extensively analyzed. However, considerably less attention has been allocated to the examination of the benthic marine ecosystem. Intertidal sediments represent a highly stratified and heterogeneous environment that plays host to an abundant and diverse microbial consortium, responsible for driving the coastal ecosystem through vast and complex biogeochemical pathways. The intertidal zone is a productive and dynamic environment that is regularly subjected to significant natural and anthropogenic stress. These systems are shaped by variability and major oscillations in seawater pH over diel or diurnal cycling periods. This indicates that these environments are reactive to changes in the oceanic climate, though simultaneously capable of tolerating natural patterned fluctuations. This led to the hypothesis that resident microbial communities from these more variable environments will possess inherent physiological plasticity to the effects of OA. Here we demonstrate the robustness of bacterial communities from a typical New Zealand intertidal sandflat exposed to different IPCC-based environmental pH regimes. Ion Torrent PGM sequence results of the V4 region of the 16S rRNA gene for the Total Community [DNA] and the Active Community [RNA] showed greater sensitivity and resolution in the Active Community, where select "sentinel" community members were shown to respond to reductions in pH. Although, despite the differing nature of the pH treatments applied, the core intertidal sedimentary community remained structurally and compositionally unaffected. Interestingly, many of the responding low tolerant taxa were shown to belong to a subset of nitrifying bacteria (AOB's), indicating possible linkages to a particular aspect of ecosystem functionality. The identification and classification of these *sensitive* bacteria may provide information regarding the health and susceptibility of the

marine ecosystem to decreases in pH, operating as potential microbial bioindicators. We also report incongruencies between the biogeochemical redox (O_2) profile measurements and the sediment microbial community activity. Where despite the re-establishment of *inferred* redox as determined by the micro profiling data, the microbial community was still in a state of flux when the first pH amendment was applied.

Introduction

The cascading impacts of ocean acidification are projected to radically transform marine ecosystems across all trophic levels at both regional and global scales (Cavicchioli et al., 2019; Doney et al., 2020; Melzner et al., 2020). Over the past 250 years, the effects of ocean acidification (OA) have elicited significant changes in open ocean marine carbonate chemistry, effectively decreasing the average surface seawater pH from 8.2 to 8.1 (Boyd, 2011; Burrell et al., 2016; Zeebe & Wolf-Gladrow, 2001). The Intergovernmental Panel on Climate Change (IPCC) predicts a further decline in open ocean pH from 8.1 to 7.8 by the year 2100 (IGBP, 2013; Doney et al., 2009; Stocker et al., 2013). Much of the current OA research focuses primarily on surveying the pelagic marine ecosystem (Crummett, 2020; Vassilis Kitidis et al., 2011a; Roy et al., 2013), with very little attention devoted to addressing the response of near-shore or benthic sediments to the effects of OA. Of the select few OA studies that do address the benthic environment, the majority of them focus on changes occurring at the macroscale (e.g., foraminifera, urchins, nematodes) rather than the microscale (i.e., bacteria, archaea, viruses) (Hale et al., 2011; Kroeker et al., 2011). Those studies that do assess microbial activity in the benthic sediments rarely come to also include the OA factor in terms of its effects on microbiological response (Cordier, 2020; Walker et al., 2021).

Despite the paucity of information, it is predicted that the greatest impact of OA is likely to occur within coastal and estuarine ecosystems given their proximity to densely populated coastal regions, which then translates to additional increased exposure to human-related disturbances (Lidbury et al., 2012). These ecosystems are some of the most productive regions on the planet (Steven Bouillon et al., 2008; Gill, 2005) and are well regarded for their economic and ecological significance (Maung-Saw-Htoo-Thaw et al., 2017; Primavera, 2006). Coastal zones alone account for an estimated 15% of total global primary productivity and over 50% of carbonate and organic carbon burial, highlighting their prominent role in buffering atmospheric CO_2 within the oceanic ecosystem (Gattuso et al., 1998; Giraud et al., 2008; Muller-karger et al., 2005). Despite their critical role, there remains a prominent gap in knowledge regarding the reactivity of coastal benthic sediments to reductions in overlying water column pH and the secondary influence of this on the resident microbial fraction.

The coastal environment is often recognized as a responsive ecotone between the terrestrial biome and the open ocean system, experiencing strong gradients in natural and

anthropogenic stressors (Doney et al., 2009; IGBP, n.d. 2013; Stocker et al., 2013). These environments can regularly undergo acidification or basification depending on the relationship between the influx of coastal waters by anthropogenic CO_2, watershed export of alkalinity, and organic matter introduction (Duarte, Hendriks, et al., 2013). The regulation of pH within nearshore coastal surface waters is unique in its complexity as pH variation is dependent on watershed inputs of Ca^{2+}, carbonate alkalinity, organic/inorganic carbon, nutrients, ecosystem metabolic activity, and the hydrological processes that govern the mixing of coastal waters (Aufdenkampe et al., 2011; Duarte, Hendriks, et al., 2013). The general impact of increased CO_2 enrichment in the sediments is not clearly defined as the dispersion of CO_2 saturated pore water is subjected to different environmental fates (e.g., chemical diffusion, pore water density gradients, mechanical disturbances, mineral buffering, and CO_2 enriched pore water transport) which collectively increase the variability of pH within the sediment (Taylor et al., 2015). Previous geochemical and oceanographic studies have addressed the concept of oceanic buffering capacity (i.e., Revelle Factor) to examine the sensitivity of pCO_2 to variations in Dissolved Inorganic Carbon (DIC) concentrations, though our understanding of ocean carbon dioxide system sensitivity and the availability of adequate methods to quantify resistance (i.e., buffering) on a benthic microbial community level to changes in pH are still in their infancy (Bolin, 1959; Revelle & Suess, 1957; Sarmiento & Gruber, 2006; Sundquist et al., 1979).

Intense biogeochemical and mineral dissolution precipitation reactions near the seafloor result in the generation of strong pH gradients at the water-sediment interface (Reimers et al., 1996). In marine sediments, the potential to buffer excess CO_2 is influenced by various physicochemical factors such as the increasing or decreasing free proton concentrations in the sediment pore-water, which influences heterotrophic respiration, chemoautotrophic activity, and the subsequent dissolution of calcium carbonate (Silburn et al., 2017). The cohesive versus permeable nature of the sediments also affects diffusive or irrigative processes, where the concentration of calcium carbonate with the sediment directly influences carbonate chemistry and total alkalinity (Yaakov, 1973; Middelburg et al., 2020). Benthic macrofaunal species are also known to affect sediment pH dynamics through physical bioturbation processes, as well as prokaryotic organisms that are capable of utilizing free

hydrogen ions through inward H^+ proton pumping mechanisms (Inoue et al., 2018; Zhu et al., 2006).

Intertidal sediments are highly heterogeneous in nature in terms of vertical sediment stratification. This stratification can directly affect the activity of the microbial community and possible bioturbation, which collectively can impact vertical zonation of oxygen availability, pH, pCO_2, organic matter concentration, and content and nutrient bioavailability (Torsvik et al., 2002; Horner-Devine et al., 2004; Lozupone & Knight, 2007). Within sediments, microbial communities residing in the oxygenated upper layer of the sediment are responsible for driving the transformation of various essential compounds. This biogeochemical cycling provides the critical link between the degradation of organic matter and the regeneration of nutrients, collectively supporting an estimated ~80% of all coastal ecosystem primary production (Nixon, 1981; Graf, 1992; Valiela, 1995; Raffaelli et al., 2003; Bianchi, 2007; Doney et al., 2009). The overall net effect of these microbial transformations in coastal sediments generates sustained reductions in pH that can differ from the overlying water column pH by as much as 1-2 pH units over a short millimeter to centimeter spatial scales (Cai et al., 1995; Fenchel & Riedl, 1970; Marinelli & Boudreau, 1996). Within the intertidal zone, sediments are also tidally influenced on both diel and diurnal cycling patterns, resulting in significant daily fluctuations in both oxygen (O_2) and pH profiles (Joint et al., 2011; Liu et al., 2010a).

In this study, we investigated the compositional and structural response of the Active [RNA] and Total [DNA] intertidal sediment microbial communities to a sustained decrease in overlying water column pH. We hypothesized that the composition of the bacterial communities collected from coastal marine intertidal benthic sediments would not be in response to increased levels of CO_2 reflecting adaptation to the dynamic and robust system from which they are derived. We chose to run this experiment using a complex mesocosm based approach to control for macrofauna, temperature, and light, all of which are known to influence microbial community composition and structure (Goldberg et al., 2017; Laverock et al., 2010; Llirós et al., 2014; Wang et al., 2019)—at the same time, exposing the sediment communities to both a rapid and gradual reduction in seawater pH. The rapid shift treatment was designed to emulate the major pH fluctuations experienced by the variable coastal intertidal zone with the intent of pushing the bacterial communities to the outer limits of their

physiological pH tolerance range. The gradual or stepwise reduction treatment was structured to be comparable to a more stable coastal marine ecosystem that does not observe the same frequency or extreme pH fluctuations. With the intention of capturing the most subtle structural shifts from the microbial communities to very nominal drops in pH using IPCC metrics as a baseline proxy for futuristic pH conditions. Here we describe the compositional and structural reactivity of intertidal sediment microbial communities, profiled from a well-established intertidal sand flat located on the North Island of New Zealand to the effects of ocean acidification.

Methods and Materials

Field Sampling and Site Selection

Tuapiro Point represents a typical intertidal sandflat located in Shelly Bay, Tauranga Harbor, within The Bay of Plenty on the North Island of New Zealand (37° 29'25.1" S' 175° 57'01.3" E'), selected for this study as being a model coastal marine ecosystem (Douglas, Lohrer, & Pilditch, 2019; Pratt, Pilditch, Lohrer, & Thrush, 2014) (**Figure 1**.). Sediment samples for molecular analysis were collected within a 1 x 2 m quadrat (attempting to avoid natural sediment gradients) within the intertidal zone during tidal transition from high to low in early morning (mid-October 2016 (Austral Spring)). Within the quadrat area, sediment was measured and excavated at four sediment depth intervals (0-1; 1-2; 2-5 and 5+ cm) carefully separating each layer into its own individual sterile bucket. Previous work by Pratt et al., 2014, was used to provide estimates of the intertidal sedimentary site conditions within Tuapiro Point (e.g., median grain size, silt/clay content ratio). This study identified an area to be a typical New Zealand intertidal sandflat in relation to sediment composition and physicochemical properties. Resident macrofaunal species were removed by individually passing the contents of each bucket containing the selected sediment layers through a sterilized 1 mm and a 500 μm mesh sieve. This was then followed by manual homogenization of each layer to reduce inherent levels of benthic sediment heterogeneity. All four buckets were then capped with natural seawater and immediately transported to the AUT (Auckland University of Technology Marine Research Facility) for processing. The different sediment layers were then physically reconstructed back into their original sediment profile by their respective depths (e.g., 0-1; 1-2; 2-5 and 5+ cm) into 65 individual 250 ml plastic core containers with the delicate 0-1 cm layer carefully preserved at the surface. Sediment collection for microbial community analysis in the field, described here as "Baseline" sampling, involved four 7 ml vol replicates samples (n=4) to be taken from the upper layer sediment directly from the sandflat that would later serve as control for the microbial community composition and structure prior to physical disturbance. Fifty reconstructed cores were distributed at random into one of three specially designed flow-through mesocosm chambers at the AUT Marine Research Facility under carefully controlled conditions (i.e., temperature, darkness, pH, conductivity, turbidity) maintaining circulated artificial seawater set to a pre-established universal seawater pH value of 8.1 (Vopel, Del-Río, & Pilditch, 2018).
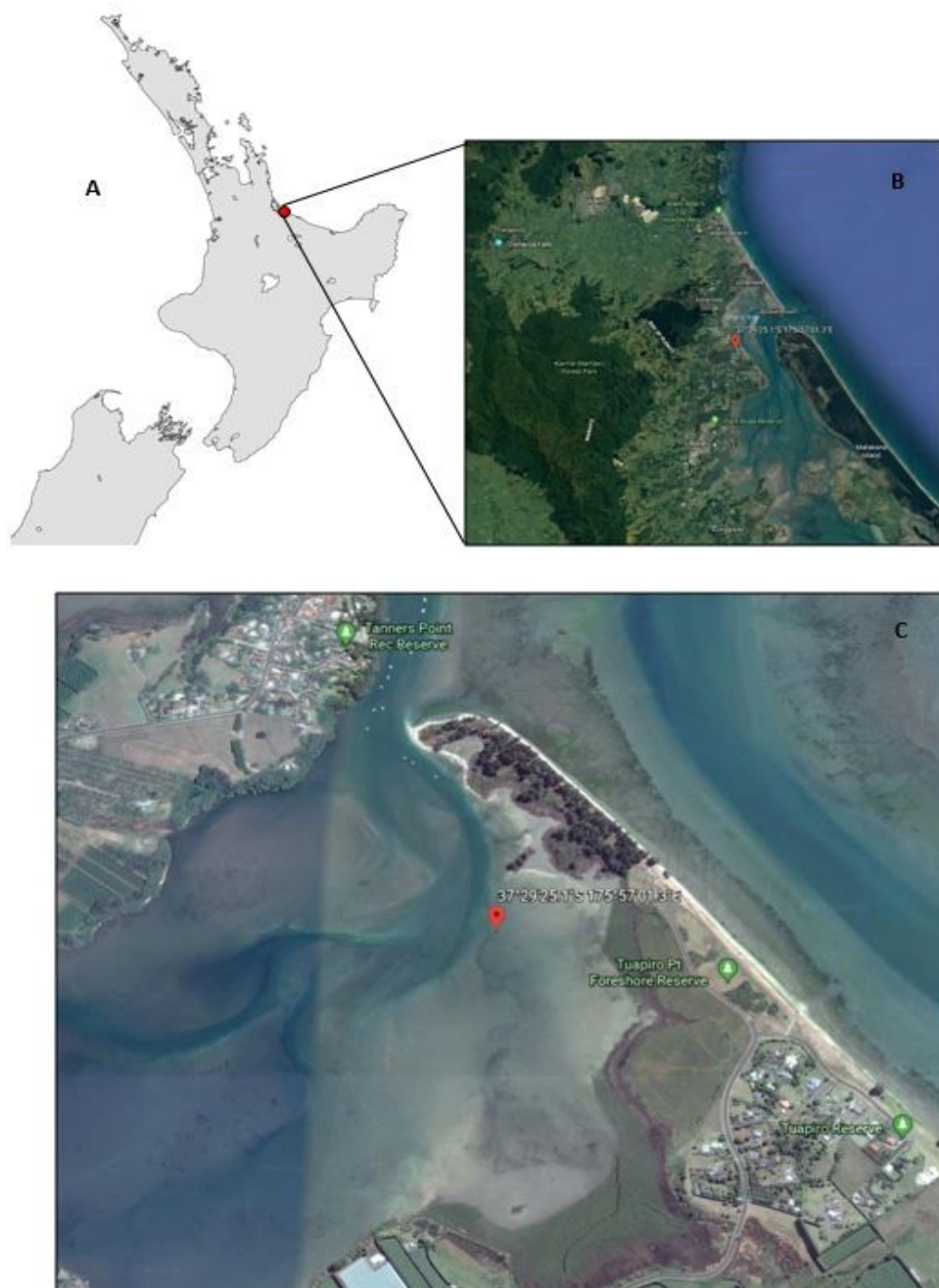
Figure 1. Displays the sampling site location selected for this experiment. A: Shows the sampling site on the east coast of the North Island of New Zealand; B: Gives a broad regional view of the site in Tauranga Harbor near Shelly Bay; C: describes the exact GPS coordinate location of the site relative to the intertidal sandflat off the coast of Tuapiro Point.

Chapter 2: Experiment 1

Flow Chamber System Set-up and Monitoring

Three specially engineered flow-through mesocosm chambers (112 x 72 x 60 cm) were used for the controlled simulation of different OA pH treatments, located at the Auckland University of Technology (AUT) Marine Research Facility. The mesocosm system specifications are as described by Vopel et al. (2018). In brief, the influx of CO_2-enriched air (5%: CO_2, 21%: O_2 in N_2) into each flow chamber was automatically controlled by the CapCtr Software Program (Loligo Systems Aps) for the systematic modulation of interflow chamber pH through the carbonation of the overlying water column. All flow chambers were set at a stabilized initial pH of 8.1. The closed system was continuously recirculated with the same total 560 L volume (per flow chamber) of artificial seawater mixture (Instant Ocean, Spectrum Brands, Blacksburg, VA, USA) with the added contribution of air jet flow to simulate natural advective processes. Artificial seawater (ASW) was selected for this study for the purpose of experimental reproducibility, as synthetic seawater or ASW is made using a standardized chemical formula. Which aids in reducing experimentally introduced variability, unlike natural seawater (Alpert et al., 2015).   The system was maintained in complete darkness for the entire length of the study to control for dominating photosynthetic processes. CO_2 degassing caused by the departure from the seawater air equilibrium resulted in a pH offset of 0.02 pH units between the mixing barrel and mesocosm flow-through chamber below. This variation was algorithmically accounted for using the system software data program, and the enrichment of CO_2 gas was continuously calibrated and subsequently adjusted as necessary.

PH was frequently monitored and controlled using the automatic injection of CO_2-enriched air (5% CO_2, 21% O_2 in nitrogen) to carbonate the seawater and reduce pH by 0.03 units per day. The deposition and rate of CO_2 gas input were automatically regulated using the CapCtr Software Program (Loligo Systems Aps) and SenTix HWD electrode connected to a calibrated pH 3310-meter electrode (WTW) and an additional solenoid valve (Vopel et al., 2018) to monitor the influx. A particle filter was used to artificially achieve a standard level of turbidity. Salinity was measured using a conductivity meter (Knick Portamess® Conductivity Meter) and maintained by adding ultrapure H_2O to regulate salinity at a constant 35 ppt based on filed data readings. A 1 L volume of seawater was collected every 3-6 days from each mesocosm flow chamber over the course of the experiment to measure Dissolved Inorganic Carbon (DIC) using coulometry and total alkalinity (TA) using potentiometric titration. This

was in accordance with the SOP's (2 & 3a) described by Dickson et al., 2007 and Pierrot's adaptation of the CO2Sys.BAS program previously established by Lewis & Wallace, 1998 for seawater pCO_2 and pH (seawater scale, mol kg-SW^{-1}) computation. The associated HSO_4^- dissociation constant was analyzed using the methodology by Dickson, 1990, where the corresponding K_1 and K_2 values for carbonic acid measurements were based on Mehrbach et al., 1973 and then refitted based on Dickson & Millero, 1987. Total alkalinity measurements were constantly taken to monitor carbonation levels using an AS-Alk 2 Total Alkalinity Titrator (Hoppe-Jones, Oldham, & Drewes, 2010) (Apollo SciTech Inc., Georgia, USA) at Plymouth Marine Laboratory.

Experimental Treatment and Sampling Design

An acclimatization period of 11 days was applied to allow redox to re-establish in the reconstructed cores under a pH of 8.1 prior to starting the experimental treatments. Previous biogeochemical studies examining OPD and redox in marine sediments have determined ~10 days to be a sufficient period of time for the re-establishment of biogeochemical redox processes within the sediment (Ferrández-Gómez et al., 2021). Microelectrode profiling of O_2 (Rasmussen & Jorgensen, 1992) was used as a proxy for the re-establishment of redox and to determine the OPD (Oxygen Penetration Depth) in the upper layer of the sediment (**Supp. Figure 1**.). Once a stable oxygen profile was realized, the individual pH treatments were undertaken. Two of the flow-through chambers were used to simulate two different ocean acidification environmental scenarios (Rapid shift and Stepwise Reduction), both based on current global IPCC data projections (**Figure 2**.). The third chamber served as a control (RE3: Control (C)) and was maintained at a pH of 8.1 for the duration of the experiment. The first experimental treatment (RE2: Rapid Shift (RaS)) involved a sudden decrease in overlying water column seawater pH from 8.1 down to 7.6 at the T0 (0-11 days *in mesocosm*) sampling time point. The second treatment (RE4: Stepwise Reduction (StR)) utilized a gradual reduction in pH over time, starting at 8.1 and decreasing to 7.6 pH at a rate of 0.03 pH units per day. The entire experiment was run over 34 days, including the acclimation period. Sediment samples were collected across 5 time points (T0-11: Day 0 (n = 12 (4 cores per treatment)), T1: Day 4 (n = 12) T2 Day 8 (n = 12), T3: Day 16 (n = 12), T4: Day 24 (n = 12) after being placed into the flow chambers (**Figure 2. and Supp. Table 1.**). 7 g of sediment from the surface sediment (0-1cm). Each sample was preserved with MoBio Lifeguard^{TM} RNA Preservation

Agent (Qiagen Inc.: Germantown, MD, USA) to prevent the degradation of total DNA and RNA using a 1:1 (7 g: 7 ml) ratio and then stored at -20°C until nucleic acid extraction.

Sediment Physicochemical Analysis

Intact, duplicate sediment core samples were randomly selected from each of the three mesocosm flow-through chambers at each of the five sampling time points to be used for geochemical analysis. Sediment cores were capped with a layer of seawater, immediately frozen, and stored at -80°C prior to analysis. Cores were then later deconstructed and sectioned at the previously mentioned reconstructed sediment depth intervals whilst frozen, transferred to sterile 50 ml falcon tubes, thawed on ice, and centrifuged at 10,000 RPM for 5 min to separate off pore water. Sediment physicochemical analyses were conducted for sediment grain size, water content/bulk density, porosity, chlorophyll-α, and phaeopigments, total organic carbon content, total $CaCO_3$ content, and alkalinity as previously described Drylie et al., 2019. In brief, water content was determined by loss of weight using combustion analysis of the upper 1 cm of the intertidal sediment layer where it was first dried at 60°C and then combusted at 550°C to determine pore-water, wet weight, dry weight, organic content, calcium carbonate content, and pigments (Chlorophyll a/Phaeopigments). Particle size fractionation (Grain size = 63 μm) determination was conducted using ~10 ml sample of sediment and analyzed using the Malvern-Mastersizer-2000.
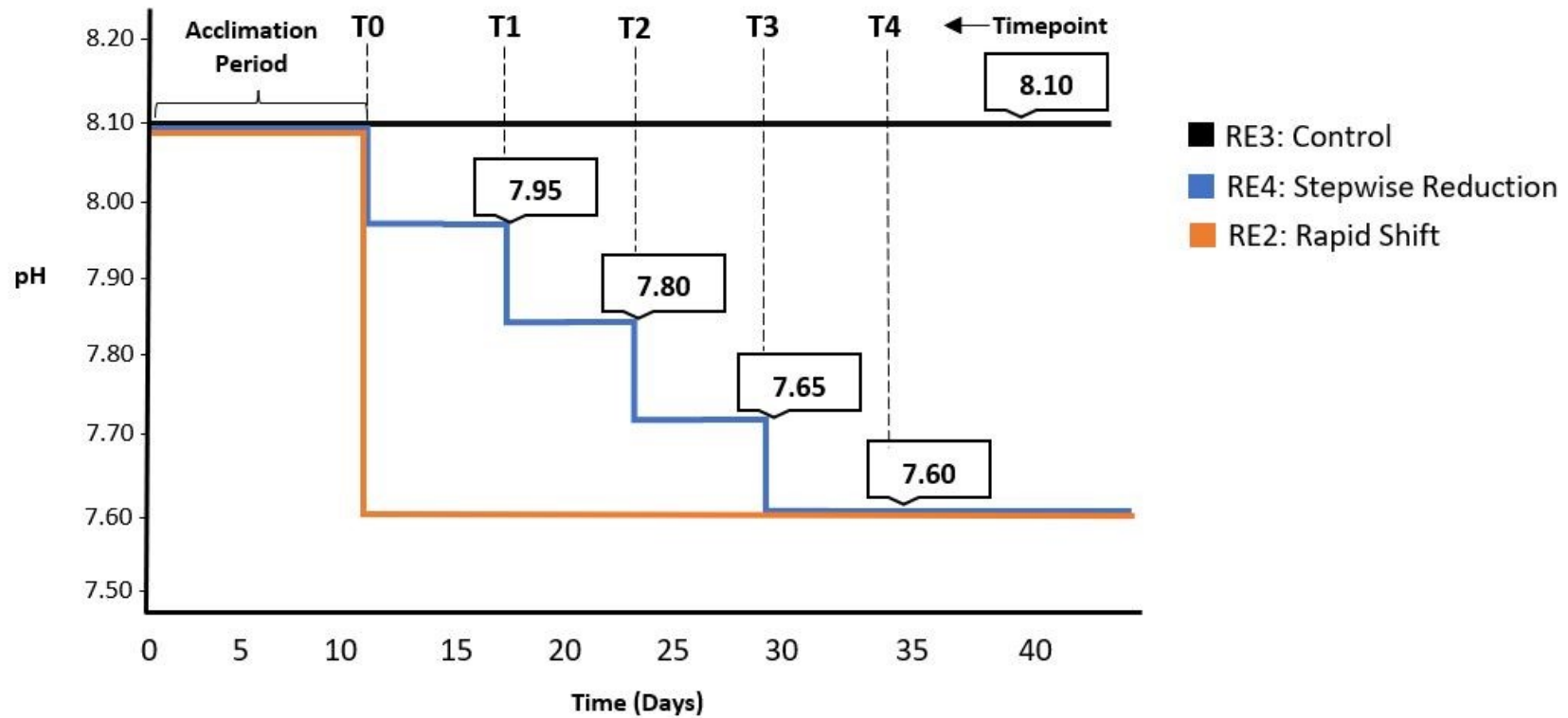
Figure 2. Describes the experimental design for each of the pH treatments employed over the course of the study. RE3: Control (Black) the Experimental Control where the pH was maintained at a pH of 8.1; RE4: Stepwise Reduction (Blue) Stepwise Reduction Treatment where the pH was incrementally reduced over time; and RE2: Rapid Shift (Orange) the Rapid Shift Treatment where the pH is radically reduced from 8.1 down to 7.6 at the first sampling time point (T1).

Chapter 2: Experiment 1

Sediment nucleic acid extraction

Each of the preserved sediment samples was thawed on ice, centrifuged at 2,500 RPM for 5 min, and the resulting supernatant of porewater and Lifegaurd Preservation agent were decanted. DNA and RNA from 3.0 g of wet sediment were co-extracted using the MoBio RNA Powersoil Total RNA Isolation Kit (Qiagen Inc.: Germantown, MD, USA) and the DNA Elution Accessory Kit following the manufacturer's instructions (Qiagen Inc.: Germantown, MD, USA). Total DNA and Total RNA were quantified using the Quant-it ^{TM} PicoGreen High Sensitivity DNA and HS RNA Assay Kits, respectively (Invitrogen, Thermo Fisher Scientific, Basingstoke, UK). Total DNA and Total RNA quality were also determined using the 260:280 ratio from the Nanodrop^{TM} 1000 Spectrophotometer (Thermo Fisher Scientific). Total RNA was first treated with the Turbo DNA-Free^{TM} Kit (Thermo Fisher Scientific) to remove any residual DNA prior to cDNA synthesis, using random hexamers and the SuperScript® IV First-Strand Synthesis System (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. DNA and cDNA were stored at -20°C until further downstream processing.

PCR and Ion Torrent PGM Sequencing

The polymerase chain reaction (PCR) was used to amplify the V4 region of the 16S rRNA gene, with the application of the Earth Microbiome Ion Torrent Fusion primers (Parada et al., 2016; Quince et al., 2011). A concentration of 5 ng of cDNA and DNA were amplified in triplicate per each sediment core sample (i.e., 3 PCR reactions for every 1 core sample) using a PCR reaction volume of 25 µl total volume per reaction (Volume ratio per reaction using original concentrations: H_2O; 0.8 µl BSA (Bovine Serum Albumin) (0.4 mg/ml)(Promega Corporations, USA); 3 µl of DNTP's (2 mM) (Invitrogen Ltd. New Zealand); 3 µl of 10X PCR Buffer (Invitrogen Ltd. New Zealand); 3 µl of M_gCl_2 (50 mM); 0.12 µl of Taq Polymerase Enzyme (0.12 µl) (Invitrogen Ltd. New Zealand); 0.5 µl of Forward Primer (10 mM) and 0.5 µl of Reverse Primer (10 mM) (Integrated DNA Technologies Inc.); 2 µl of respective cDNA or DNA (5ng/µl). Thermocycler conditions were set at an initial hot start incubation step at 94°C for 3 minutes followed by 30 cycles of 94°C for 45 seconds, 50°C for 1 min and 72°C for 1.5 minutes, and a final extension step of 10 minutes at 72°C with an infinite hold at 4°C. The resulting products (previously run in triplicate) were then pooled back together for each core sample for greater coverage and community diversity representation and visually inspected on a 1% TAE agarose gel with a 1 KB+ ladder and a 0.01% volume of SYBR Safe fluorescent DNA gel stain. Individual

PCR products were then normalized using SequelPrep™ Normalization Kit, 2 µl of each sample were then pooled together as a library, re-quantified using the Quant-it ™ PicoGreen High Sensitivity RNA and DNA Assay Kits, and then stored at 4°C ready for sequencing. Sequencing library preparation and amplification was performed using the Ion PGM™ IA 500 Kit (Thermo Fisher Scientific, Massachusetts, USA) and sequenced on the Ion Torrent PGM™ System using an Ion 318v2 chip (Thermo Fisher Scientific, Massachusetts, USA) at The University of Waikato Sequencing Facility, Hamilton, New Zealand. Raw sequences (FASTQ Format) were trimmed and quality filtered using Mothur (Version 1.40.5) (Schloss et al., 2009) for the removal of unusually short reads (<275bp) and long reads (>345bp) as well as homopolymers (>6). Quality filtering was conducted using USEARCH (Version 10.0.240 (Linux 64-bit) pipelines (Edgar, 2010). High-quality sequence reads were obtained following QC and then clustered into Operational Taxonomic Units (OTU) using a similarity threshold of 97%. Identification of operational taxonomic units (OTU's) from raw sequence data using the 97% clustering approach is currently maintained as a standard bioinformatic binning method (Schloss & Westcott, 2011), though we acknowledge the application of other bioinformatic approaches (i.e., ASV: Amplicon Sequence Variants)(Prodan et al., 2020). For this study, and it's bioinformatic analysis, the use of OTU's as differentiating taxonomic units for community analysis proved to be more than sufficient for complete annotation. QC protocol, sequence trimming, and quality filtering were conducted based on the methodology described in Power et al. (2018). The resulting RNA (cDNA) and DNA sequenced reads were bioinformatically merged back together which allowed for a cohesive overlap between the two datasets where the representing OTU's were synchronous between both the RNA and DNA. This allowed for a direct comparison between both total and active community datasets, prompting a more effective and accurate method of community analysis.

Bioinformatic Analyses

Following 16S rRNA amplicon sequencing, the data were taxonomically classified using a standard RDP classifier database (Michigan State University Ribosomal Database Project) for the raw 16S rRNA sequences (Wang et al., 2007) where all dominant OTU's from the sediment community could be effectively annotated. Taxonomic assignments which resulted in a confidence value of less than 80% were classified as "Unknown." Sequence data was statistically analysed using R Core Team 2020-10-10 (version 4.0.3) and additional R packages

for microbiome analysis: Phyloseq (McMurdie & Holmes, 2013) for the importation, analysis and visualization of the phylogenetic sequencing data (OTU Table); ggplot2 (Villanueva & Chen, 2019) for graphically displaying the complex data generated from the data frame; vegan (Oksanen et al., 2008) for conducting multivariate analyses for ecological communities; tidyverse (Wickham et al., 2019) for cohesive overlap between other R packages to functionally model, transform and visualize the data; viridis (Garnier, 2017) for visually colour mapping the data for colour blind interpretation; adespatial (Jombart et al., 2020) for providing tools for building spatial predictors for multivariate analysis; ggpubr(Alboukadel Kassambara & Kassambara, 2020) for producing publication ready figures; PhILR (Washburne et al., 2018) for the phylogenetic isometric log transformation of the compositional data; ape (Paradis et al., 2004) for the analysis of phylogenetics and evolution function of phylogenetic tree data; CoDaSeq (Fernandes et al., 2018) for compositional analysis of high throughput sequencing data that takes into account phylogenetic relatedness of microbial data using PhILR. Alpha diversity indices: Shannon (Shannon, 1948); Observed Species Richness (Brose & D. Martinez, 2004), Chao1 (Chao, 1984) were conducted using vegan and the phyloseq package in R Studio in order to assess changes within each sample over time in response to reductions in pH. Bacterial abundance and richness were analyzed using a pairwise ANOVA (Analysis of Variance) in MicrobiomSeq for select alpha diversity indices, followed by a Tukey HSD test for post-hoc comparison in R Studio (Version 4.0.3) using a fitted aov, and a 95% family-wise confidence level to check for true significance, and additional QQ (Quantile-Quantile) plots to confirm normal distribution assumptions within the data." The ANCOM analysis works by applying a comprehensive statistical framework for analyzing microbial compositional data. This method is designed to take into account the underlying community structural information within the data and then allows for two or more populations of interest to be compared. The ANCOM approach compared to other standard statistical tests (i.e., t test, ZIG (Zero Inflated Gaussian)) showed that ANCOM effectively controlled for the false discovery rate (FDR), and additionally helped improve statistical power. Whereas both the t-test and ZIG methods exhibited inflated FDR's by as much as 68% (Mandal et al., 2015).

Results

Sediment Geochemistry

After the initial acclimatization period of 11 days, both $[H^+]$ and O_2 concentrations displayed characteristic depth profiles expected of redox re-establishment (**Supp. Fig. 1. and Supp. Fig. 2**.). Oxygen Penetration Depth (OPD) decreased rapidly at the sediment-water interface, with O_2 detectable to 1-2 mm in depth. The OPD became shallower over the course of the experiment, suggesting that O_2 consumption increased due to microbial degradative processes. Due to an unexpected program error during the final microelectrode O_2 profiling measurement for the Stepwise Treatment, the data output could not be used. The shape of the O_2 profiles did not change markedly between the start and completion of the experiment, implying that redox was stable at the onset of the experiment despite reconstructing the cores after sieving the sediments. The concentration of $[H^+]$ in the over-laying seawater was higher in both treatment tanks compared to the Control, where concentrations were notably higher at end of the experiment than at the start (**Supp. Figure 2**.).

In the top 1 mm of sediment in both treatment tanks $[H^+]$ consumption, alkalinity or carbonate dissolution was observed. At a depth of 1 mm, the concentration of $[H^+]$ increased during the experiment with 3-4x higher concentrations of $[H^+]$ at 8 mm depth by the end of the experiment. This increased concentration of $[H^+]$ occurred in all experimental treatment tanks but was interestingly most pronounced in the Control, possibly as a result of the physical sampling disturbance which may have inadvertently introduced heterogeneous microniches at the sediment surface. These profiles demonstrate a consistent increase in $[H^+]$ concentration followed by a subsequent decrease in O_2 consumption in the sediment throughout the experiment. This is to be expected given the natural microbial metabolic processes taking place, and the additional sustained saturation of excess CO_2 to draw down seawater pH.

Sediment Physicochemical Analysis

Sediment water content/bulk density, porosity, chlorophyll-α and phaeopigments, total organic carbon content, total $CaCO_3$ content, and alkalinity did not differ significantly between the experimental treatments and control (**Supp. Table 2. and 3.**). Sediment grain size (63/Dx (50)) differed between the Control and StR Treatment (*t-test: p≤0.0*5) and

between the Control and RaS Treatment (*t*-test: *p≤0.0*5). Differences in sediment grain size between cores sampled from the different treatments may also have contributed to variations in [H⁺] ion concentrations between treatments, with the greatest variation detected in the control, where differences in grain size can alter dissolution processes through variability in pore water within the top 0-1 mm of sediment.

Molecular Analysis

Following Bokulich et al., 2013 a pre-treatment 0.005% abundance cull was applied to the raw counts sequence data with any OTU with cumulatively less than 247 reads being removed from the dataset. Of the original read count of 13,877 OTU's, only 1,621 were preserved post 0.005% cull. This stringent approach effectively reduced the total read count from 5,236,606 down to 4,844,488, where 93% of the count data was contained within 12.7% of the OTU's. Samples were rarefied without replacement, and a "set seed (1234)" function was used for statistical reproducibility (**Supp. Figure 9a**.). Taxonomic information was imported in to Phyloseq, and the phylogenetic tree was imported using the "greengenes" import function from the MUSCLE program generated output (semi colon delimited file). Metadata, taxonomic information, the OTU table and the phylogenetic tree were merged in to a Phyloseq object and processed using the established bioinformatic pipeline. To minimize the risk of false discovery, the OTU matrix was subsampled without replacement to the minimum read count (15,274 reads).

Determination of the requirement for rarefaction was based on the read count variation score calculated from the differences between the highest (139,866 reads) and lowest read counts. The resulting read count variation score (9.15x) was used to decide between rarefying or non-rarefying the data. The risk of false discovery is considered highest when the read count variation is 10x. Given that our read count variation score fell into the category of medium to high risk we ran preliminary statistical analyses to compare between a rarefied and non-rarefied data set (**Supp. Figure 9a-c**). No major differences were identified between the two datasets, nor could any variations in statistical results be observed. Though arguments can be made for both the need for rarefying or not rarefying the dataset, we opted for *non-rarefaction* based on the principles outlined by McMurdie & Holmes, 2014. which highlights the risks of introducing unnecessary variation into the analytical process, potentially skewing the interpretation of the data. Taxonomic classification of the generated

OTU's were identified as *Bacteria* (1,566 OTU's) and *Archaea* (11 OTU's). All taxa assigned as *Chloroplasts* (37 OTU's) at the Order level were removed from the dataset. This filtering approach reduced the overall OTU read count from 1,621 down to 1,577. An R-generated rarefaction curve of the OTU data matrix demonstrated that all samples were sequenced to full taxonomic saturation and were well represented, where increasing the depth of sequencing did not add substantially more OTU diversity **(Supp. Figure 9a-c.).** Post rarefication, the "raw" reads counts were transformed into relative abundance and hierarchical clustering methods were used to visually quality check the data and check for potential sample outliers. The sediment microbial community was shown to be dominated by *Gammaproteobacteria* (DNA: 18.3%; RNA: 21.6%), *Unclassified* (DNA: 13.4%; RNA: 10.6%), *Deltaproteobacteria* (DNA: 11%; RNA: 10.1%), *Alphaproteobacteria* (DNA: 9.2%; RNA: 9.4%), *Flavobacteria* (DNA: 8.4%; RNA: 8.6%) (**Supp. Figure(s) 3 & 4**.).

Differences in Community Structure Between pH Treatments

Alpha diversity metric analyses used to assess changes in structure, species richness and evenness (i.e., distribution and relative abundances) between the two pH treatments (Ras and StR), and the experimental Control identified that at each sampling time point, no significant differences were detected in the species diversity between the Control (RE3: 8.1 pH) and pH Treatment tanks (RE2: RaS; RE4: StR) in either the Total (**Figure 3**.) or Active sediment Communities (**Figure 4)**. Over time, species richness and diversity within the Control and Stepwise Reduction (StR) Treatments increased whereas, it decreased in the Rapid Shift (RaS) Treatment. Within the Control (8.1 pH), the largest increases in diversity in the Total community (**Figure 3A**.) occurred between T0 and T3 (0 days: 16 Days (8.1 pH)), T0 and T4 (0 Days: 24 Days (8.1 pH)); T1 and T2 (4 Days: 8 Days (8.1 pH)), and T1 and T4 (4 Days: 24 Days (8.1 pH)) (ANOVA: $p{\leq}0.05$). In the Active Control (**Figure 4A**.), the community diversity changed between T0 and T2 (0 Days: 8 Days (8.1 pH)), T0 and T3 (0 Days: 16 Days (8.1 pH)), T1 and T3 (4 Days: 16 Days (8.1 pH)), T1 and T4 (4 Days: 24 Days (8.1 pH)), and between T3 and T4 (16 Days: 24 Days (8.1 pH)). Within the StR, diversity of the Total community increased between T0 (8.1 pH) and T3 (7.65 pH) (0 Days: 16 Days) and T3 (7.65 pH) and T4 (7.6 pH) (16 Days: 24 Days) (**Figure 3B**.). Within the Active StR Community, diversity of the Active community increased between T0 (8.1 pH) and T4 (7.6 pH) (0 Days: 24 Days) (**Figure 4C**.). The increase in species diversity observed in the *Active* community StR treatment, indicates

possible intermediate disturbance-like responses, where the effects of transient or periodic ecological disturbances in the environment impact sediment microbial diversity. Within the RaS Treatment, decreases in the Total community diversity occurred between T0 (8.1 pH) and T3 (7.6 pH) (0 Days: 16 Days) and between T0 (8.1 pH) and T4 (7.6 pH) (0 Days: 24 Days) (**Figure 3B**.) over time. No significant differences were detected within the Active Community RaS Treatment (**Figure 4B.**) over time.
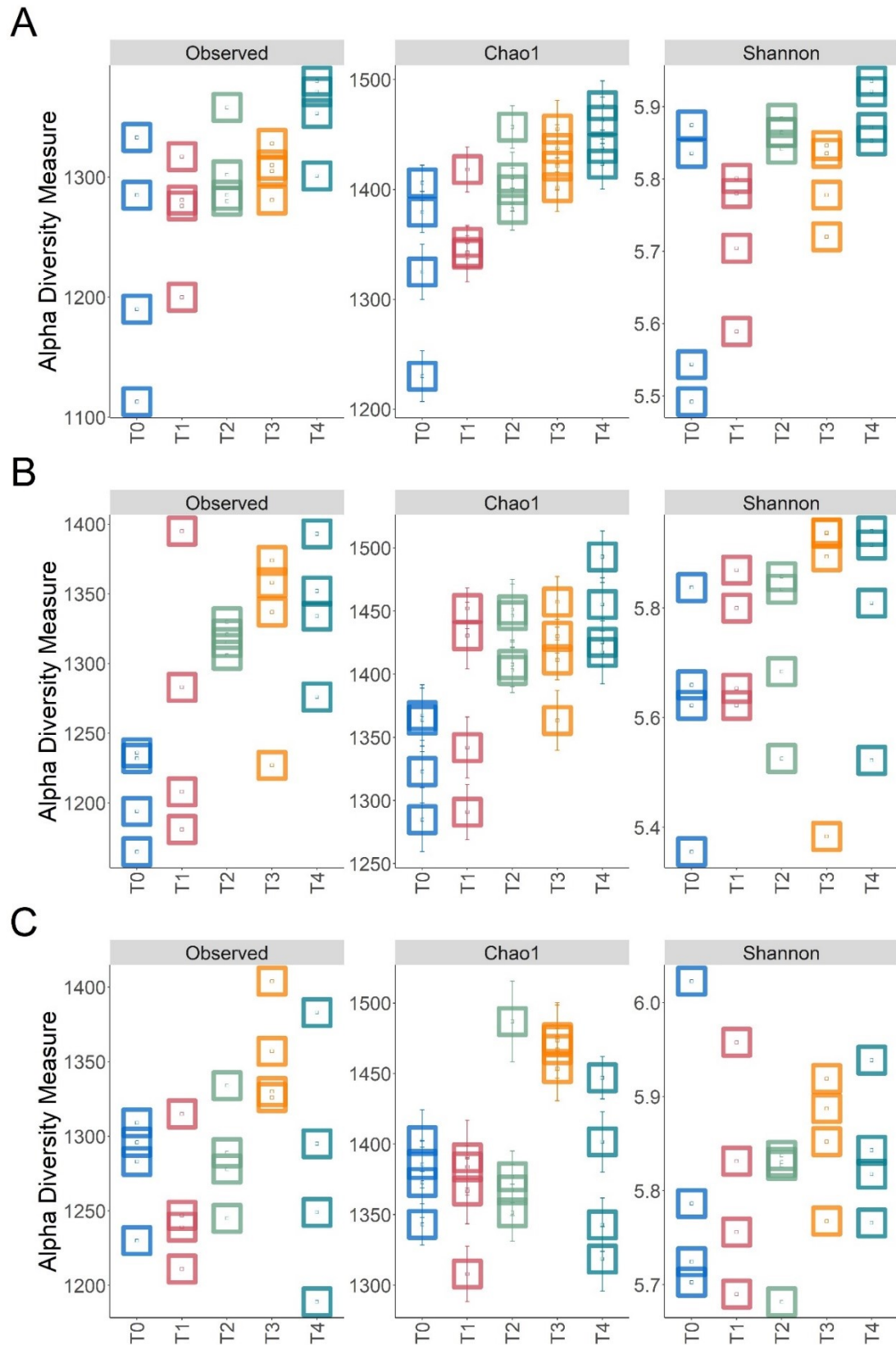
Figure 3. Total Community [DNA] Observed, Chao1, and Shannon alpha diversity metrices for each of the different experimental pH treatments (Top to Bottom: A: Control (Circle); B: Rapid Shift (RaS); C: Stepwise Reduction (StR)) over course of the experiment (across time: (T0-T4).
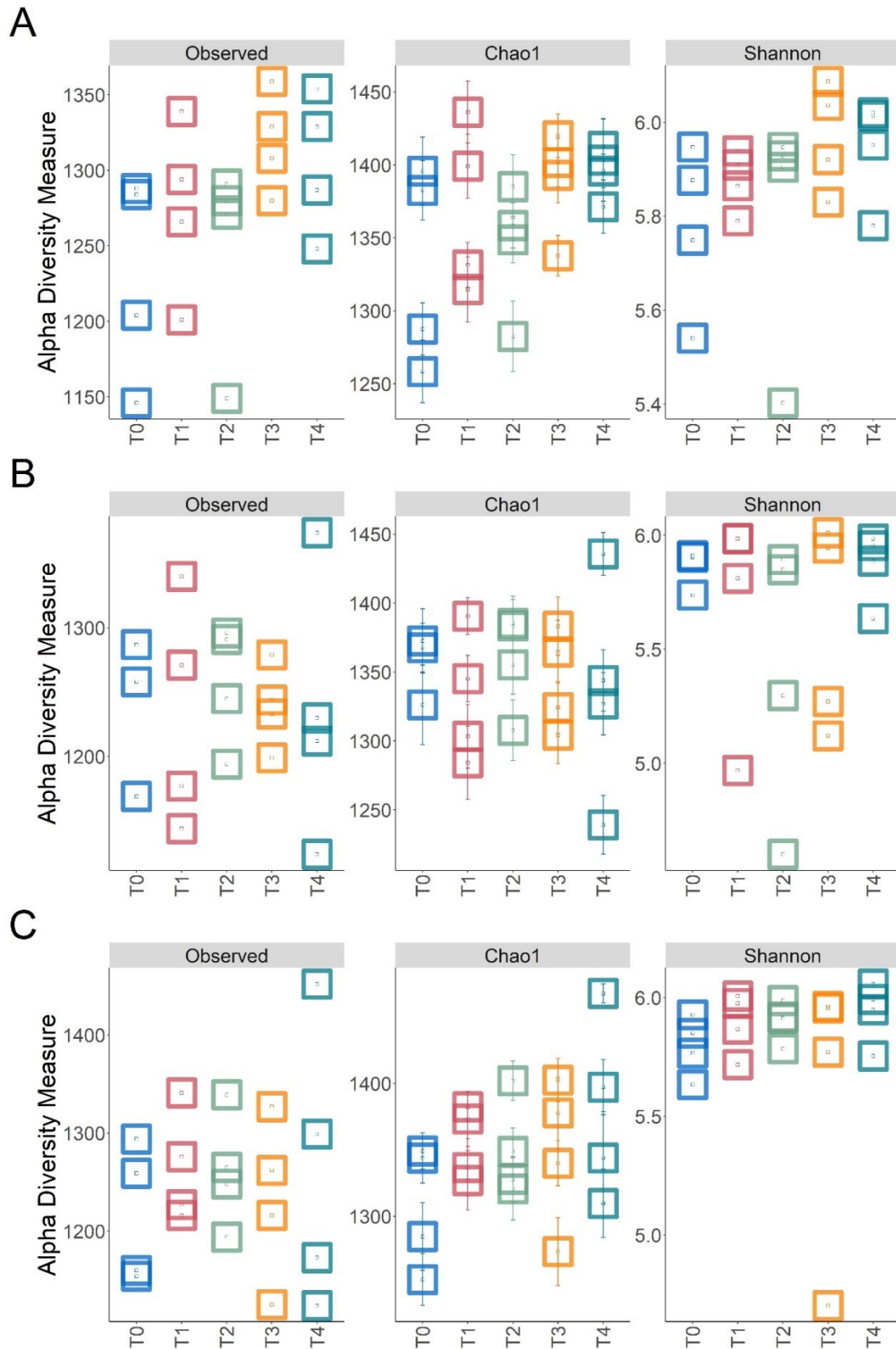
Figure 4. Active Community [RNA] Observed, Chao1, and Shannon alpha diversity metrices for each of the different experimental pH treatments (Top to Bottom: A: Control (Circle); B: Rapid Shift (RaS); C: Stepwise Reduction (StR)) over course of the experiment (across time:(T0-T4).

Variances in Community Composition and Relative Abundances

Beta diversity metric analyses calculated using phylogenetic tree branch distances were used to explain differences in community composition between the experimental pH treatments (RaS and StR) in terms of relative abundance. Results showed that the Total and Active community composition displayed significant structural changes over time (**Figure 5.**). The largest variation occurred between field samples (B: Baseline) and post sieved reconstructed cores, with community structure then increasing from T0 to T4, where 38% of the detected variation could be explained within the first two axes. Both the Total and Active Communities were shown to structurally separate across the primary axis by *time*, where samples clustered together at each of their respective time points from the initial "Baseline field sampling" to the final *in-situ* sampling time point (T4: 24 Days: Control pH: 8.1; Treatment pH: 7.6) (PhILR: Total Community: ANOSIM: $p \leq 0.05$; R: 0.5671; Active Community: ANOSIM: $p \leq 0.05$; R: 5532) (**Figure 5.**) (**Supp. Tables 4a. & 4b.**).

Structural variation within the microbial community observed at T0 (0_{-11}days; 8.1 pH) was considerably higher than the variation detected between the Baseline field sample replicates (8.1 pH). After 16 days *in mesocosm,* microbial communities from each of the different Treatments and the Control had markedly less variation than at the start of the experiment, whereby at T3 (16 days; C: 8.1 pH; RaS: 7.6 pH; StR: 7.65 pH) and T4 (24 days; C: 8.1 pH; RaS: 7.6 pH; StR: 7.6 pH) the communities were shown to reconverge. This is clearly demonstrated in **Figure 5.** where the Active (A.) and Total (B.) communities had a drastic structural response to the physical disturbance captured between the Baseline samples (B) and the first timepoint (T0) where the high level of variation in both communities is evident, where the T0 communities are highly dissimilar from the non-disturbed Baseline samples. However, despite the impact of this disturbance and the effects of pH treatment on both the Active and Total sediment communities, there is a pronounced structural recovery effect after 8 days in the system, whereby day 16 (T3) both communities displayed *near* Baseline (i.e., pre disturbance) levels of community structural stabilization (**Figure 5.**).

To better understand and explain the interaction between pH treatments, the dominating temporal effect had to be addressed, where "time" was selectively removed as a factor in the analysis of community level response. By examining the relationship of both treatments compared against the control along a linear timeline, demonstrated that community composition was progressing along the same temporal axis, making

interpretation of microbial response to pH as a disturbance, a moving target. By assessing the variations between pH treatments within an individual timepoint, effectively removed the confounding temporal effect. Where the observed nuances between the two treatments were a result of pH and not of "time." Results of this Individual time point analysis used to assess the effects of reduced pH on the different pH treatments compared back against the control, showed high levels of variation (explained by the primary and secondary axes) in the initial Baseline sampling (8.1 pH) time point across both Total (**Figure 6**.) and Active Communities (**Figure 6**.), with slightly higher variation represented in the Active Community (Total Community Baseline: 71.1%; Active Community Baseline: 74.4%). Sampling at T0 (0_{-11}: Days *in mesocosm*) (**Supp. Figure 7**.) and T1 (4 Days *in mesocosm*) (**Supp. Figure 7**.) captured the second and third highest variation levels in both Total and Active Communities (Total Community T0: 56%; T1: 46.6%; Active Community T0: 59%; T1: 48.4%) **(Supp. Figure 7.)**. In both the Total and Active communities sampling at T2 (8 Days *in mesocosm*) **(Supp. Figure 7.)**, showed markedly less variation, and by T3 (16 Days in mesocosm) (**Figure 6**.) and T4 (24 Days in mesocosm) only very subtle variation could be identified (**Figure 6**.).

Significant differences between the pH Treatments were identified at T3 (16 days; C: 8.1 pH; RaS: 7.6 pH; StR: 7.65 pH) **(Figure 6.)** and T4 (24 days; C: 8.1 pH; RaS: 7.6 pH; StR: 7.6 pH) (**Figure 6**.) for the Active Community and T4 for the Total Community **(Figure 6.)** (**Supp. Table(s) 5D-E. & 6E.**) (Active: T3: ANOSIM: $p \leq 0.05$; R: 2338; T4: ANOSIM: $p \leq 0.05$; R: 0.3171; Total: T4: (ANOSIM: $p \leq 0.05$; R: 0.3287).
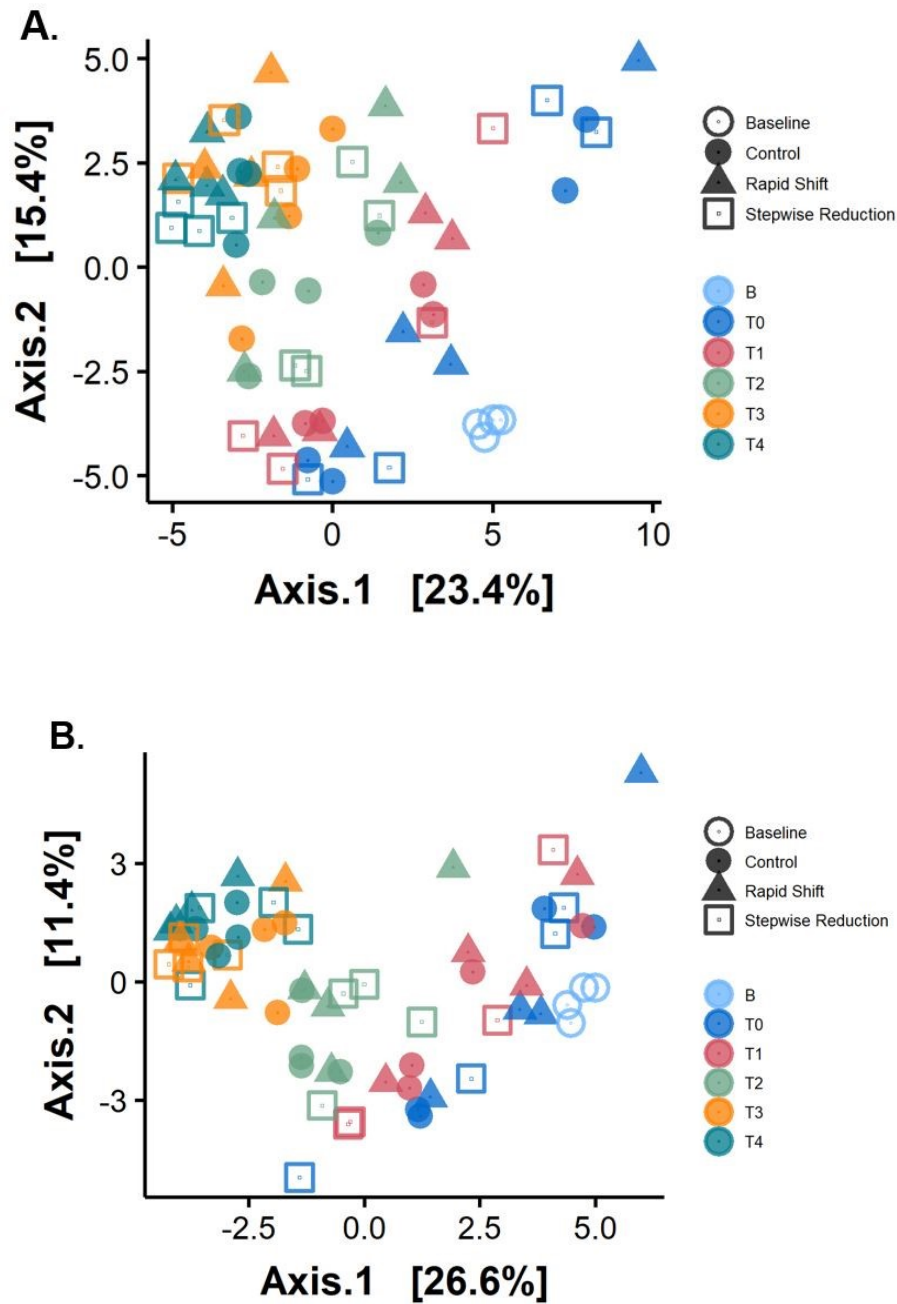
Figure 5. Shows the PhILR Beta Diversity Metric Analyses for both the Active [RNA] (A.) and the Total [DNA] (B.) communities for all pH treatments (Baseline (B): Open Circle; Control (C): Closed Circle; Rapid Shift (RaS): Triangle; Stepwise Reduction (StR): Square) at each of the sampling timepoints from Baseline (pre disturbance/treatment) to T4 (24 Days in the Mesocosm). In both A. [RNA] and B. [DNA] ordinations, there is a clear distinction from the Baseline samples and the first sampling timepoint T0 (0 Days in the Mesocosm) in terms of dissimilarity and inter-sample variation. By the final two sampling timepoints (T3: 16 Days) and T4 (24 Days) both A. [RNA] and B. [DNA] communities are shown to restructure.
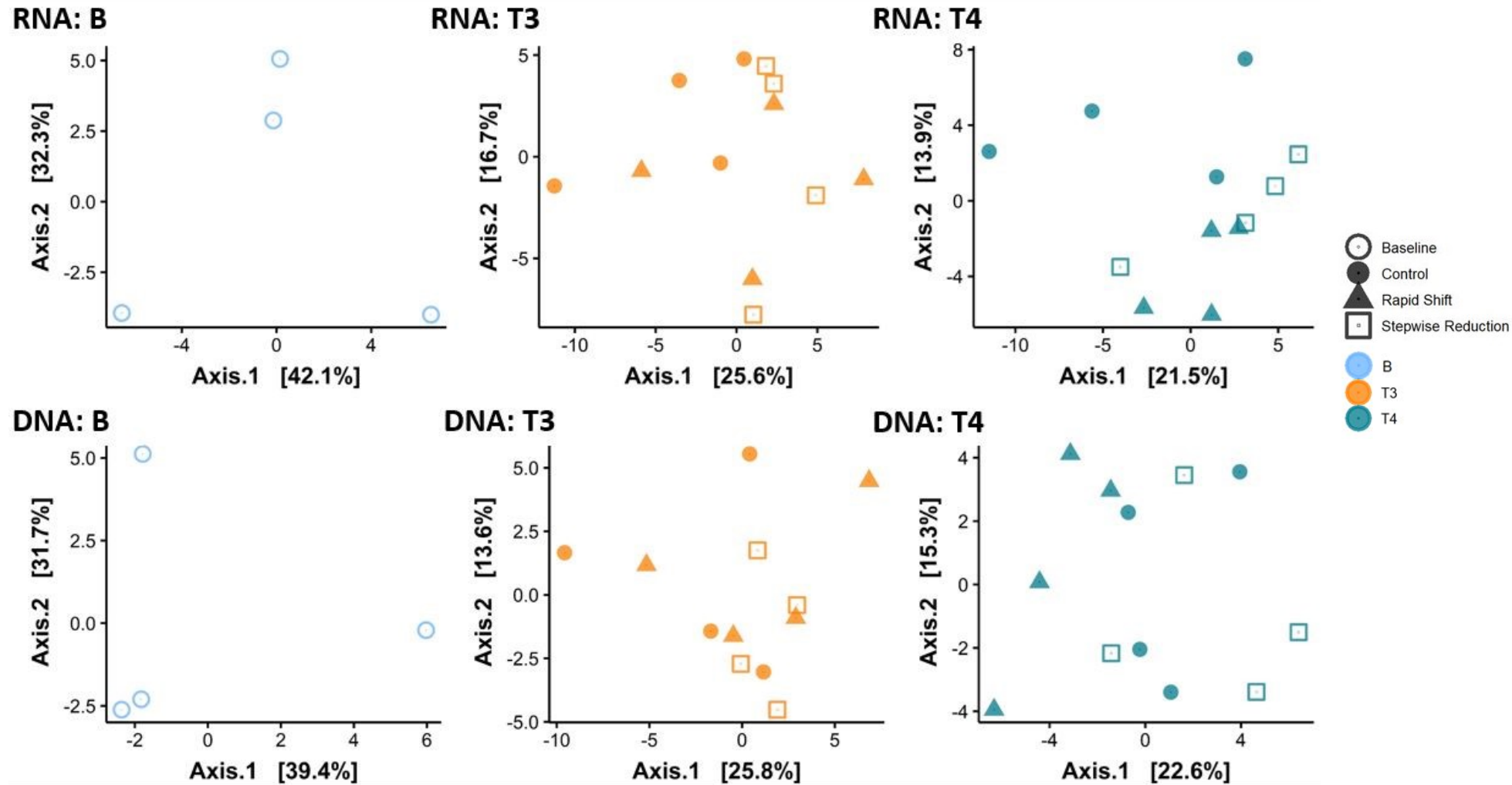
Figure 6. Shows the Active [RNA] (Top) and the Total [DNA] (Bottom) community PhILR Beta Diversity Metric Analyses for each of the pH treatments (Baseline (B): Open Circle; Control (C): Closed Circle; Rapid Shift: RaS: Triangle; Stepwise Reduction (StR): Square) at the sampling timepoints where significant differences between pH treatments were detected (T3: 16 Days and T4: 24 Days) compared back against the Baseline (B) pre-disturbance /treatment community structure.

Chapter 2: Experiment 1

Compositional Analysis of Whole Community Resistance

As anticipated, results of the taxonomic compositional analyses of both the Active [RNA] and Total [DNA] communities as shown in the (**Supp. Figure 3. and 4.**) identified that though the structure of both communities were shown to change in response to the different pH treatments (as well as the physical disturbance), the *composition* of the communities remained unchanged. A total of 99.4% of the *whole* (RNA and DNA) community did not compositionally respond to the pH treatment, displaying clear community-wide resistance to changes in seawater pH. The relative abundance of certain taxa within the top 10 most abundant bacteria across both treatments (RaS and StR) and the Control for both the Active [RNA] and Total [DNA] communities displayed no clear patterns in relative taxonomic abundance across time (T0-T4).

Identification of Responding *Sentinel* Taxa Under Low pH

Analysis of composition of microbiomes (ANCOM) was applied here to statistically account for naturally introduced compositional constraints endemic to microbial ecological analyses. This approach detects differentially abundant OTU's or taxon across the different pH treated sediment communities (Rapid/Stepwise), while maintaining high levels of statistical power (Mandal et al., 2015). ANCOM is traditionally used for assessing compositional data (i.e., 16S rDNA: total DNA), though here we also use it to measure differences in the *relative activity* of in the active cDNA community under low pH conditions. Over time, throughout the 34-day experiment, much of the community remained unaffected by reductions in pH within the different pH Treatments (RaS/StR) and the control where <0.6% of the whole community (1,577 OTU's) changed in relative abundance (%). In the Total [DNA] community, 19 sensitive/responding taxa were identified between each of the Treatments and the Control. Eight were detected in the RaS Treatment: *Porphyrobacter* (OTU361); *Gimesia* (OTU429); *Nitrosomonas* (OTU524); *Aestuariibacter* (OTU59); *Family VIII*(OTU45); *Gimesia* (OTU339); *Rhodobacterales* (OTU181); and *Alteromonadales* (OTU184) (**Figure 9B**.) (**Supp. Table 7.**). Five OTUs were identified within the StR Treatment: *Dasania* (OTU450), *Bacteroidetes* (OTU1374), *Firmicutes* (OTU284), *Gimesia* (OTU339), and *Rhodobacterales* (OTU181) (**Figure 9A**.) (**Supp. Table 7.**). Six OTU's were detected in the Control: *Proteobacteria* (OTU750); *Planctomicrobium* (OTU882), *Gimesia* (OTU339); *Rhodobacterales* (OTU181); *Alteromonadales* (OTU184); and *Saprospinaceae* (OTU145) (**Supp. Figure 6.**).

The Active [RNA] Community identified 25 sensitive/responding taxa over time within the two pH Treatments and the Control. Nine significant taxa were detected within the RaS Treatment: *Porphyrobacter* (OTU361); *Gimesia* (OTU429); *Aestuariibacter* (OTU59); *Fluviicola* (OTU160); *Proteobacteria* (OTU152); *Planctomycetes* (OTU182); *Nitrosomonas* (OTU248); *Family XIII* (OTU362) and *Nitrosomonas* (OTU524) (**Figure 9D**.) (**Supp. Table 8.**). Eight OTU's were identified in the StR Treatment: *Desulfobacterales* (OTU1193)*; Oceanospirillales* (OTU99); *Dasania* (OTU450); *Proteobacteria* (OTU152); *Planctomycetes* (OTU182); *Nitrosomonas* (OTU524); *Rhodobacterales* (OTU181), and *Subdivision 3 Genera Incertae Sedis* (OTU481) (**Figure 9C.**) (**Supp. Table 8**.). Eight OTU's were detected within the Control: *Subdivision 3 Genera Incertae Sedis* (OTU481); *Candidate Division WPS-2* (OTU1466); *Nitrosomonas* (OTU524); *Family XIII* (OTU362); *Rhodobacterales* (OTU181); *Nitrosomonas* (OTU248); *Gimesia* (OTU178), and *Family VIII* (OTU45) (**Supp. Figure 5**.). The relative abundance of OTU's affiliated with the Family *Nitrosomonadaceae* (OTU524; OTU248) under low pH conditions from T0 to T4 in all pH Treatments where the relative abundance was highest at the lowest pH (T4: RaS: 7.6 pH; StR: 7.6 pH) (**Supp. Figures 6a. & 6b**.).

At each of the time points sampled, the bulk of the Total and Active microbial communities (1,577 Total OTU's <0.15%) was undisturbed under the applied pH treatments (RaS and StR), (Figure 10 and 11). However, by applying the analysis of the composition of microbiome algorithm (ANCOM) individual OTU's could be identified as responding to pH treatment at each time point sampled. Within the Total Community at T0 (0 Days; pH: 8.1), 2 OTUs were shown to differ across all treatments (**Supp. Table 9**.).

The relative abundance of a single OTU identified at the phylum level as *Proteobacteria* was reduced in the StR and RaS treatments compared to the control. In contrast, the taxonomic relative abundance of a single *Proteobacteria* OTU identified to the level of genus as *Alteromonas* was significantly higher in both the StR and RaS Treatments relative to the control (Figure 10A). Within the Active Community, at T0 (0 Days; pH: 8.1), 2 responsive OTU's identified within the family *Rhodothermaceae* genus *Rubricoccus*, increased for both the StR and RaS treatments and a single Archaeal OTU identified as *Crenarchaeota* increased in relative abundance for the StR Treatment and decreased for the RaS Treatment (**Figure 7A.**).

At T1 (4 Days; RaS pH: 7.6; StR: 7.95) for the Total Community, the relative abundance of 2 OTUs identified within the genus *Rubricoccus* increased in both the StR and RaS Treatments, and a single Archaeal OTU identified as *Crenarchaeota,* increased for the StR Treatment and decrease for the RaS Treatment relative to the control (**Figure 7B**.). For the Active Community, the relative abundance of a single OTU identified to the phylum level as *Acidobacteria* increased in relative abundance for the StR Treatment and decreased in relative abundance for the RaS Treatment (**Figure 8B.**).

At T2 (8 Days; RaS pH: 7.6; StR pH: 7.8) for the Total Community, a single OTU identified to the phylum level as *Proteobacteria* decreased in relative abundance for both StR and RaS Treatments (**Figure 7C**.). For the Active Community, a single OTU identified to the genus level as *Algicola*, decreased for the StR Treatments and increased for the RaS Treatments; a single OTU identified to the phylum level as *Proteobacteria* (OTU1229), increased in relative abundance in the StR Treatment and decreased in the RaS Treatment; and a single OTU identified as the phylum *Proteobacteria* (OTU39), decreased for both StR and RaS treatments, relative to the control (**Figure 8C.**).

At T3 (16 Days: RaS pH: 7.6; StR pH: 7.65) in the Total Community, a single OTU identified as the genus *Mangrovibacterium*, decreased in relative abundance for both StR and RaS Treatments (**Figure 7D**.). In the Active Community, the relative abundance of a single OTU identified as the genus *Oceanospirillales* increased for both StR and RaS Treatments, relative to the control (**Figure 8D.**).

At the final sampling time point, T4 (24 Days: RaS pH: 7.6; StR: 7.6), in both the Total and Active community a single OTU identified as the genus *Dasania* increased in both the StR and RaS Treatments and a single OTU identified as the genus *Porphyrobacter* decreased in relative abundance in the StR Treatment and increased in relative abundance for the RaS Treatment, relative to the Control (**Figure 7E and Figure 8E).**

For greater clarity on the significance of these changes in taxonomic relative abundance between treatments, we applied a heatmap approach of the responding sentinel taxa previously identified from the ANCOM analysis of the Active [RNA] and Total [DNA] Communities by pH treatments (RaS and StR), mapped back against the whole analyzed (RNA and DNA) OTU table (1,577 OTU's (**Figure 9**.). The resulting output was used to represent the

relative abundances (%) of these sensitive bacteria of interest compared against the complete sediment microbial community and how these abundances change relative to time (T0-T4). In the Total [DNA] community, in the RaS treatment, bacteria from the family *Nitrosomonadaceae* and *Alteromonadaceae* were identified as increasing in relative abundance (*Nitrosomonadaceae*: 0.25% increase (T4); *Alteromonadaceae*: 1.00% increase (T4)) over time. No bacteria from either family (*Nitrosomonadaceae/Alteromonadaceae*) were identified in the Total [DNA] community StR treatment (**Figure 9**.). In the Active [RNA] community RaS treatment, the relative abundance of *Nitrosomonadaceae* was shown to markedly increase (0.5%) from T2 (8 Days) to T4 (24 Days). This response was also shown to occur in the Active [RNA] community StR treatment where *Nitrosomonadaceae* increased by 0.65% (relative abundance) from T3 (16 Days) to T4 (24 Days) (**Figure 9**.). The detection of the same family of responding taxa (*Nitrosomonadaceae*) between both pH treatments in the Active [RNA] community data reinforces the significance of these bacteria in reacting to changes in water column pH, which is then further corroborated by a similar response from the Total [DNA] community which is considered to be infinitely less sensitive in its detection capabilities than the Active community. No taxa from either family of *Nitrosomonadaceae* or *Alteromonadaceae* was detected in either Total [DNA] or Active [RNA] Controls (**Supp. Figure 5**.).
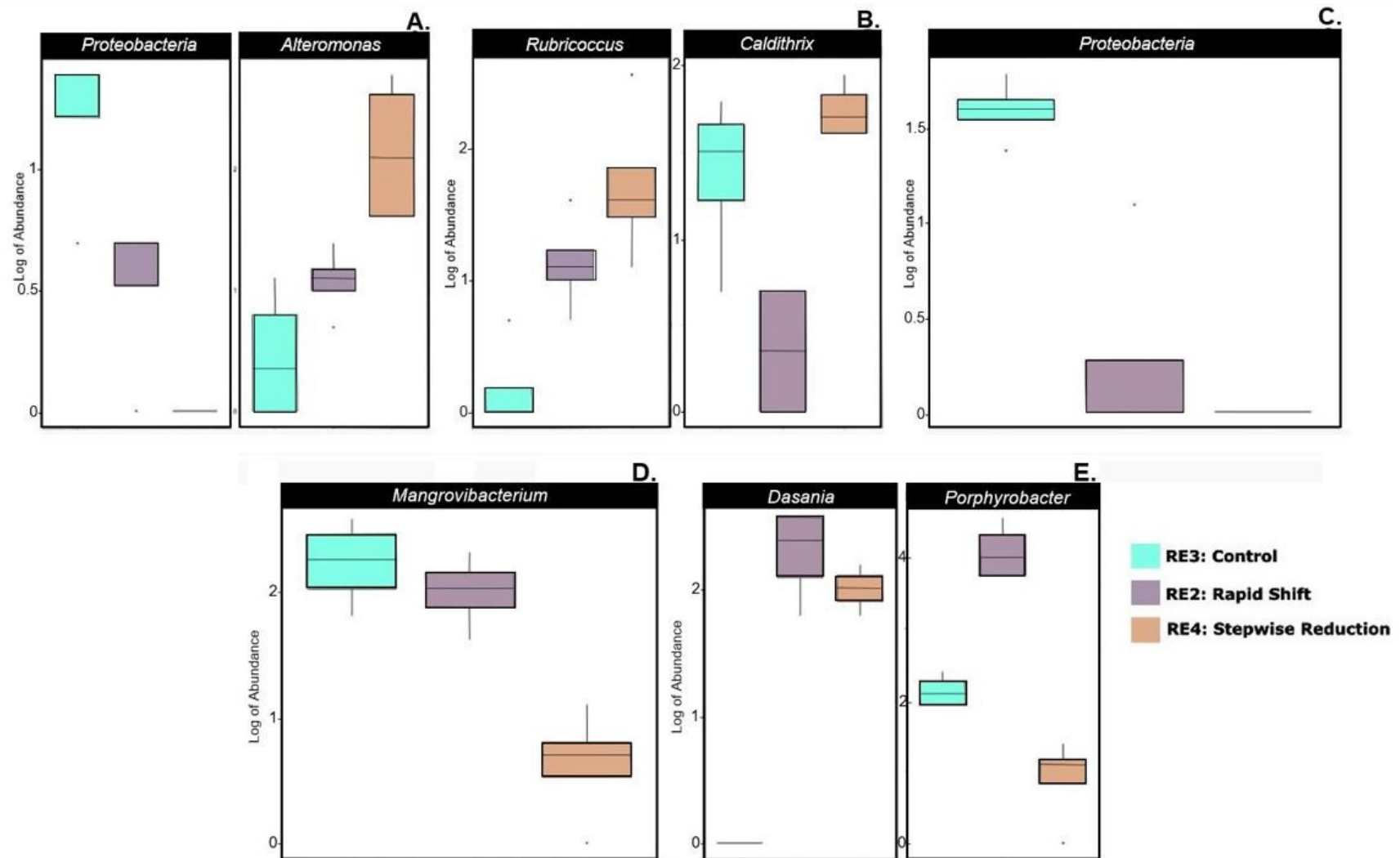
Figure 7. Shows the Total [DNA] Community (B.) Compositional Analyses using ANCOM Statistics for each of the different experimental pH treatments (Control: Blue (Left side of each plot); Rapid Shift (RaS): Purple; Stepwise Reduction (StR): Orange) for each of the individual sampling time points (A: T0 (0 Days); B: T1 (4 Days); C: T2 (8 Days); D: T3 (16 Days); and E: T4 (24 Days)).
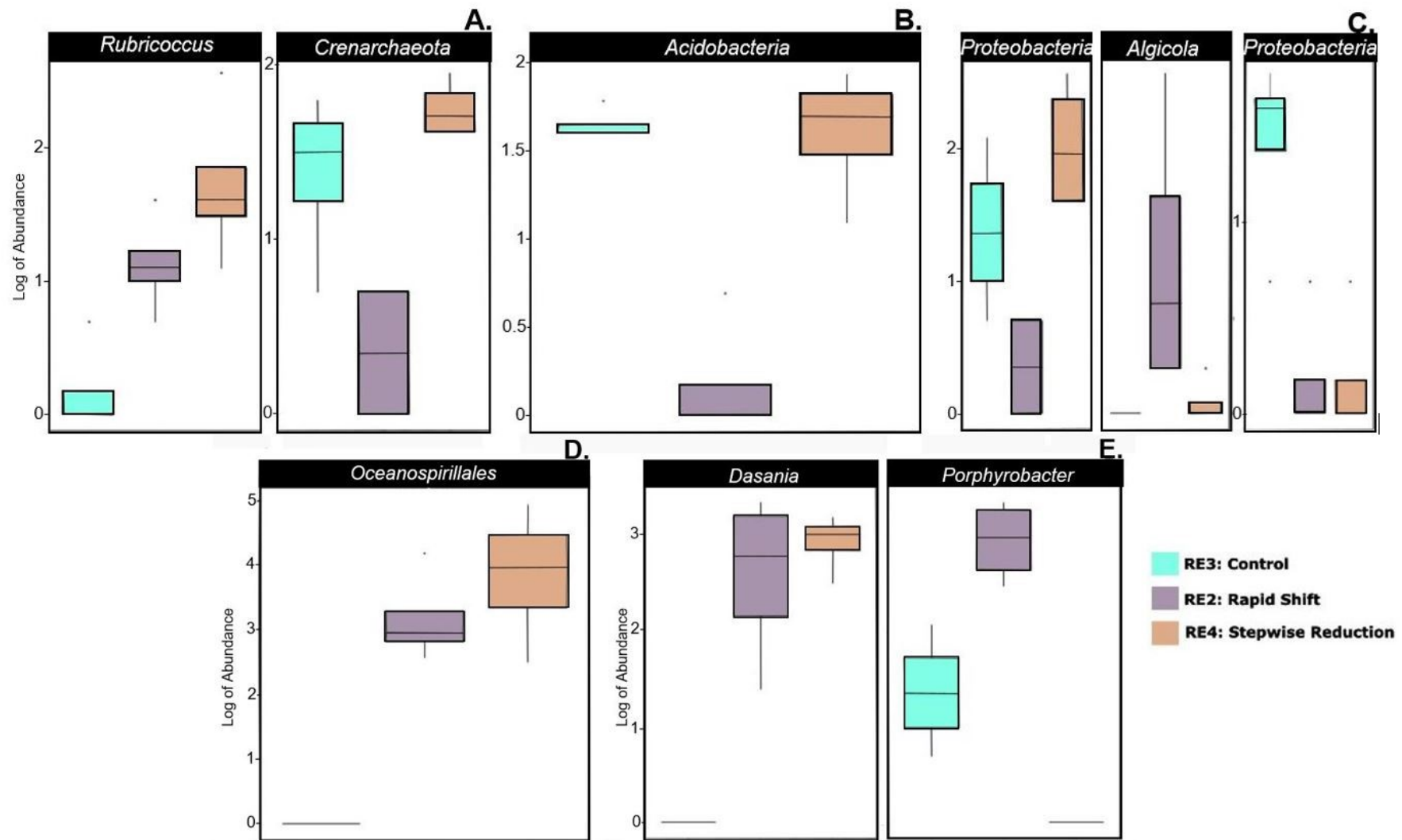
Figure 8. Shows the Active [RNA] Community (A.) Compositional Analyses using ANCOM Statistics for each of the different experimental pH treatments (Control: Blue (Left side of each plot); Rapid Shift (RaS): Purple; Stepwise Reduction (StR): Orange) for each of the individual sampling time points (A: T0 (0 Days); B: T1 (4 Days); C: T2 (8 Days); D: T3 (16 Days); and E: T4 (24 Days)).
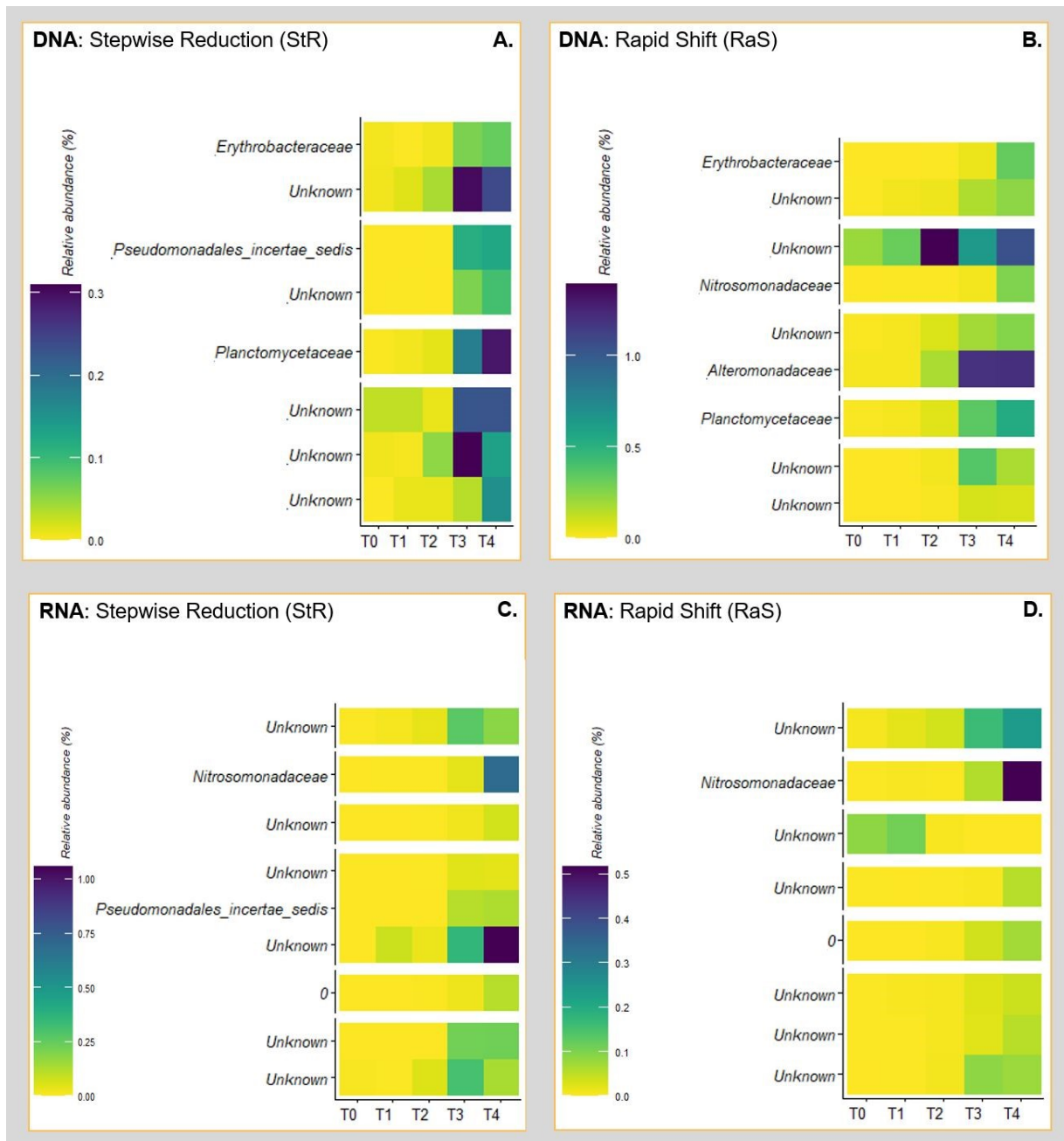
Figure 9. A heatmap of the responding sentinel taxa identified using ANCOM (Analysis of Composition of Microbiomes) for the Total [DNA] and Active [RNA] communities, mapped back against the complete OTU dataset using taxonomic relative abundance measures in % at the family level for each of the pH treatments (A. Stepwise Reduction (StR): DNA; B. Rapid Shift (RaS): DNA; C. Stepwise Reduction (StR): RNA; D. Rapid Shift (RaS): RNA) across each sampling timepoint (T0-T4).

Discussion

The intertidal zone represents one of the most complex and productive marine ecosystems in the world, operating at the interface between the terrestrial biome and open ocean (Stocker et al., 2013). These regions experience frequent and significant pH oscillations across a standard tidal cycling event that are intrinsic characteristics of coastal intertidal ecosystems (Duarte et al., 2013; Hofmann et al., 2011; Jansen et al., 2009; Mercado & Gordillo, 2011; Wootton et al., 2008). While recent studies have focused primarily on understanding the impact and importance of OA on the functioning of marine subtidal systems (Crummett, 2020; Tait et al., 2014), few studies have directly addressed OA impact on intertidal sediments (Kerfahi et al., 2020; Simone et al., 2020). We hypothesized that the microbial communities from typical benthic intertidal sediments would exhibit compositional plasticity and innate tolerability to low pH exposure as predicted from ocean acidification. Here we show that our study corroborates these findings where the bulk of the sediment community remained compositionally resistant when subjected to both rapid and gradual reductions in water column pH. Though we also report findings of individual taxa that demonstrate increased sensitivity to changes in water column pH. Additionally, we also identified that O_2 profiles as a proxy for redox do not reflect true structural stability within the intertidal sediment microbial community.

Benthic Sediment Microbial Resistance

Our results clearly demonstrated that the microbial community remained structurally and compositionally undisturbed despite the delivered highly contrasting pH treatments. Under both treatment regimes, less than 0.6% of the total community diversity responded to the perturbations. This supports the concept that microbial communities from dynamic intertidal systems are to some degree pre-disposed for changes in water column pH compared to their potentially less tolerant *subtidal* counterparts (Currie et al., 2017). This finding is consistent with other studies which have also demonstrated microbiological resistance and community-wide structural stability to changes in overlying seawater pH in other similar marine environments (e.g., subtidal sediments and pelagic) (Tait et al., 2013; Joint et al., 2011; Liu et al., 2010b; Oliver et al., 2014). For instance, Tait et al., 2013 observed only minor differences in the composition of microbial communities in subtidal Arctic surface

sediment using intact sediment cores, where the composition was only significantly different between the lowest (380 µatm) and highest (3000 µatm) pCO_2 treatments over a 14-day exposure period. Oliver et al., 2014 demonstrated that pelagic bacterial communities exposed to elevated CO_2 did not have a significant effect on community abundance, structure, or composition over a period of 18 treatment days, indicating no intrusion of low pH on bacterial communities within the water column.

In contrast, a number of other marine-based pelagic studies have reported significant changes in microbial community composition in response to high CO_2 environments over much shorter periods of low pH exposure (Crummett, 2020; Currie et al., 2017; Nelson et al., 2020; Sala et al., 2016; Webster et al., 2013). To illustrate, Crummett, 2020 identified a significant decrease in microbial community diversity over a 21-day *in-situ* period under high pCO_2 exposure in a sub-tidal pelagic system that traditionally experiences regular high pCO_2 and low pH (~7.8 pH) events. This suggests that *some* microbial communities that are consistently exposed to elevated pCO_2 environments still exhibit sensitivity to low pH. Nelson et al., 2020 also observed that a reduction in pH from 8.1 to 7.8 to 7.4 resulted in major shifts in biofilm microbial community structure and composition, transitioning from an autotrophically dominated community to a heterotrophically dominated one. In our study, the impact of the disturbance when reconstructing the cores was likely profound, creating the significant lag time needed for the microbial communities to re-establish. Despite these initial delays, minor taxa sensitive to the low pH treatments were still detected.

Sentinel taxa respond to changes in pH

Though the majority (99.4%) of the benthic sediment microbial community appeared to be resistant to a reduction in pH, we identified several minor groups, including a series of Ammonia Oxidizing Bacteria (AOB) and different taxa from the Class *Gammaproteobacteria,* there were shown to be highly responsive to changes in surrounding water column pH. *Nitrosomonadaceae* from the Genus *Nitrosomonas* were among some of the most responsive taxa, where they coincidentally displayed associations with a distinct group of previously identified nitrite/ammonia-oxidizing bacteria(AOB) (Watson, 1989; Watson, 1971). Here we show that a significant subset of the responding *low tolerant* microbial individuals (<0.6% of the Total 1,577 OTU's) were strongly affiliated with previously described AOB's, with 20% of the responding Active Community OTU's taxonomically identified as belonging to the Genus

Nitrosomonas (Hagopian & Riley, 1998; Raulf et al., 2015; Watson, 1971). Given that chemoautotrophic taxa play an important functional role in carbon fixation (Watson, 1989; Watson, 1971), the selective removal of these important nitrifying species under low pH could have far-reaching consequences for the effective operation and productivity of the coastal marine ecosystem (Beman et al., 2011b). It is likely that other sedimentary bacteria can fill the role of these different nitrifying species through functional redundancy. However, AOA and AOB are the only taxa that preform the first step of nitrification, along with annamox they are critical to nitrogen cycling within marine sediments (Byoung-Joon et al., 2010). Without them, the flux of fixed nitrogen back to the water column will likely increase the impact and risk of eutrophication (J. Hou, Song, Cao, & Zhou, 2013). What we identify here is that specific genera of bacteria show clear sensitivity to low pH, where continual or progressive disturbance in pH may chronically destabilize these communities, beginning with nitrifying microorganisms. This could also suggest that the ecological process of functional redundancy remains effective for only a short period of time and under short periods of disturbance. Various studies have demonstrated that a reduction in seawater pH has a direct effect on the nitrification process by way of altering ammonia oxidation rates and inhibiting AOB. Bowen et al., 2013 demonstrated that across two highly contrasting marine environmental treatments, AOB, particularly *Nitrosomonas,* was shown to increase (10-23% depending on environment) under acidified conditions. Our study corroborates this finding, where we also observed an exponential increase in the relative abundance of *Nitrosomonas* across both differing pH treatments (RaS/StR) within the Active Community. However, Vopel et al., 2018 demonstrated that excess CO_2 favored benthic microalgae and subsequently suppressed AOB by decreasing N_2 recycling and reducing the capacity for coupled nitrification-denitrification within the sediment. The coupling of denitrification and nitrification promotes the loss and removal of nitrogen from the environment, respectively demonstrating the importance of AOB in governing intertidal nitrogen dynamics. AOB are mediators for the first step in the nitrification process, where primary productivity within the intertidal zone is strongly determined by different sources and sinks of various nitrifying species (Bernhard et al., 2005). This study reinforces the potential microbiological consequences of CO_2 enriched seawater on the biogeochemical regulatory processes of the marine ecosystem. Where shifts in the relative abundance of nitrifying taxa within the sediment community can result in the loss of ecosystem processes (i.e., primary productivity) which could lead to total ecosystem

destabilization(Hou et al., 2014). The identification of these sensitive taxa may provide a useful proxy for monitoring nearshore ecosystem health as a system bioindicator under low pH regimes

The Class *Gammaproteobacteria* are traditionally one of the most abundant marine taxa, often equating to >50% of the marine microbial community and making up a large part of the bacterial biomass within the benthic sediment (Bowman & McCuaig, 2003; Ravenschlag et al., 2001). In our study, we show that of the responding sensitive taxa within the combined Active and Total Community datasets, 27% of the OTU's belonged to the Class *Gammaproteobacteria* (*Pseudoalteromonadaceae; Alteromonadaceae; Oceanospirillales*). A study by Spring et al., 2015 phylogenetically identified clades of oligotrophic marine *Gammaproteobacteria* (Including the following higher taxa: *Pseudomonadaceae, Alteromonadaceae*, and *Oceanospirillaceae*) that could be correlated with distinct and important phenotypic traits which illustrated their adaptive potential to different environmental factors and making them an ecologically valuable marine taxon. *Pseudomonadaceae* are also known to be important heterotrophic nitrifying bacteria (Bonin et al., 1994). Alteromonadaceae have been shown to play an important role in the breakdown of and turnover of organic matter (McCarren et al., 2010). *Oceanospirillales* have been identified as effective bioremediating taxa in response to marine oil spills (Jaekel, Zedelius, Wilkes, & Musat, 2015; Obi et al., 2016). Interestingly, all three taxonomic groups (*Pseudoalteromonadaceae, Alteromonadaceae, Oceanospirillales*) have been seen to play an important role in various marine ecosystems as critical keystone taxa of their respective microbiomes (Liao et al., 2020). The resistibility of these important taxa identified by Spring et al., 2015 refers to maintained cell growth under limited nutrient availability but does not directly assess the impact of low pH on these keystone bacteria. Their sensitivity to sustained low pH, as demonstrated by this and the previously mentioned studies, suggests that the loss of these individuals under low pH regimes would have significant collateral effects on the remainder of the microbial community under future acidified ocean conditions.

Persistence of benthic sediment heterogeneity

Pore-water pH has been known to vary by as much as 1 pH unit between the sediment and overlying water column at depths of up to 30 cm (T. Fenchel & Riedl, 1970). This suggests that despite homogenizing the sediment, high levels of geochemical variability persisted

within the sediments. This variation could also be attributed to differences in alkalinity, which plays an essential role in buffering CO_2 in sediments (Egleston, Sabine, & Morel, 2010; Hu & Cai, 2011; Thomas et al., 2009). Significant differences were detected in sediment grain size between the different treatments. Despite efforts to standardize and homogenize sediment cores, there still exists inherent entropic complexity and heterogeneity in these benthic sediments. We maintain that benthic sediments are incredibly stratified habitats (Barnes & Hughes, 1999; Kessler et al., 2019) confounded by many different environmental factors, including sediment type, permeability, pore-water advection, and hydrological dynamics (Kessler et al., 2019; Boudreau et al., 2001; M. Huettel, Ziebis, & Forster, 1996). Highlighting the intrinsic spatiotemporal variation of benthic sediments and emphasizing the importance of sediment to water-column interface contiguity (e.g., permeability, stratigraphy, and buffering capacity ($CaCO_3$ content, and high alkalinity), which ultimately governs CO_2 impact.

O_2 as a proxy for redox and the stability of microbial community structure

In this study, we used microelectrode profiling of O_2 as a proxy for redox - an established and unchanging O_2 profile would be indicative of an established redox (Donald Eugene Canfield et al., 1993; Reimers, 1987)(Reimers 1987; Canfield 1993). Our experimental design allowed ten days of pre-shock treatment to re-establish redox as predicted by the O2 profiling. The pre-shock profiles displayed a characteristic redox profile, starting with high O_2 levels followed by a quick O_2 drawdown typical of carbon-rich intertidal sediments (De Beer et al., 2005; Kim & Kim, 2007; Polerecky et al., 2005). We posited that the pronounced drawdown in O_2 indicated microbial mediated redox had established (i.e., rapid microbial remineralization), which then informed the start of the experimental pH treatments. O_2 profiling is a well-accepted method to determine biogeochemical reformation that indicates biological system recovery following a disturbance (Hicks et al., 2017; Jorgensen, Revsbech, & Cohen, 1983; Rasmussen & Jorgensen, 1992; Revsbech, 1983; Zejie Wang et al., 2013). However, our amplicon data clearly show that the composition and activity of the microbial community were still destabilized even after 21 days following the initial physical disturbance when the first pH amendment was applied, a finding that was also consistent with previous microbiological sediment disturbance studies (Findlay et al., 1990; Vonnahme et al., 2020). Retroactively, a quick compositional comparison of the active (cDNA) bacterial community at baseline (pre-disturbance) and after the 11-day acclimatization period would have provided

invaluable insight in to whether the community was truly re-established biologically following the physical manipulation, and if it was appropriate to initiate the start of the experiment. Therefore, despite the well-known doctrine, 'biology dictates chemistry' we suggest exercising caution when utilizing microelectrode data as a sole determinant of biological reconstitution, as here, we identified a considerable lag time between microbial recovery and the re-establishment of redox processes.

A variety of biogeochemical processes influence the pH of marine sediments in terms of increasing or decreasing free proton pore-water concentrations (Silburn et al., 2017). This is typically linked to the different cycles of carbon, oxygen, nitrogen, phosphate, iron, silicate, manganese, and sulfur, which are then associated with important heterotrophic respiration processes and chemoautotrophic activity (Soetaert et al., 2007). The post-shock $[H^+]$ (post-treatment) profiles in this study demonstrated a 3-4x higher H+ concentration at depths of 8 mm, where both experimental treatment flow chambers were operating at a pH of 7.6. This indicates that the sediment chemistry was shifting away from CO_3^{2-} dominated system towards an environment with greater HCO_3^- and CO_2 proportionality, altering TOC concentrations. This would then result in the potential saturation of the sediment with free H+ ions as pH decreases and CO_3^{2-} is no longer able to uptake free H+. This change in sediment biogeochemistry could be used to explain the final observed pH profiles. Where the ability of seawater to buffer changes in pH with CO_2 enrichment is dependent on the concentration of CO_3^{2-}, and where the proportion of CO_2 in seawater that remains as aqueous $CO_2(_{aq})$ increases with more CO_2 enrichment (Revelle & Suess, 1957). Silburn et al., 2017 also demonstrated that variability in the depth of sediment pH minima could be determined spatially by sediment type and OPD (Oxygen Penetration Depth). Here, the transport of solutes through the sediment is dictated by diffusive properties, and as pH lowers due to oxidation of organic matter and the re-oxidation of Mn^{2+}, Fe^{2+}, NH_4^+ or HS^- species (Cai & Reimers, 1993).

Impacts of physical disturbance

In this experiment, we applied a physically destructive sampling strategy to minimize any natural sediment heterogeneity introduced by bioturbating infauna. The rationale behind our approach sought to create a level of uniformity between sediment cores and to reduce the natural variation common within the benthic environments – especially in intertidal systems (Jessup et al., 2005; Jessup et al., 2004). However, we observed that the naturally

occurring heterogeneity within the sediments collected within the field was markedly less, and that induced by disturbing the sediment. This initial disturbance had a pronounced impact on the structure of the microbial community. Previous studies have demonstrated that physical disturbance is an essential determinant of benthic microbial community ecology in marine sediments, given its ability to influence physical and chemical processes (Findlay et al., White, 1985; Findlay et al., 1990). Physical manipulation of the sediment can result in immediate changes to grain size, water content, and sediment compaction and affect various diagenetic reactions and pore-water profiles (i.e., reactants and products), effectively altering the niche space inhabited by microbes and resulting in direct impacts to microbial community composition (Aller, 1982; Atkinson et al., 2008; Findlay et al., 1985; Gray, 1974; Marinelli et al., 2002; Mendoza-Lera & Mutz, 2013; Rhoads & Boyer, 1982). The increase in species diversity identified in the Active community in the stepwise reduction treatment suggests an intermediate disturbance like response due to transient/periodic ecological disturbances in the environment which directly influence the sediment microbial diversity. Temporary disturbances are shown to influence community diversity, where microbial diversity tends to be low when the level of disturbance is low and increases when the level of disturbance also increases in either strength or frequency (Allison & Martiny, 2008; Banks et al., 2013; Berga, Székely, & Langenheder, 2012; Hughes, Inouye, Johnson, Underwood, & Vellend, 2008; Yeo, Huggett, Eiler, & Rappé, 2013). However, persistent, or sustained disturbances will have an inverse effect on community diversity, decreasing significantly with continual or prolonged impact (Mackey & Currie, 2001). The observed decrease in diversity shown here is likely attributed to changes in anaerobic degradation of organic matter (i.e., remineralization) due to disturbances to the sediment-water interface, where physical manipulation of the substrate unknowingly increased the rate of bacterial remineralization. A process that was similarly corroborated by Galand et al., 2016. This unintentional impact within our study emphasizes the potential risks associated with any form of benthic sediment disturbances and the potential cascading impacts that result (Christiansen et al., 2020; Sharma et al., 2001; Vonnahme et al., 2020). Despite the effects caused by physical disruption, the microbial communities in this study were shown to restructure and stabilize after 16 days. Other manipulation studies have shown that when given adequate time, the benthic microbial communities are capable of homeostatic re-establishment (Allison & Martiny, 2009; Alongi, 1985; Findlay et al., 1990; Galand et al., 2016; Pimm, 1985; Probert, 1984). However, in this

study, we determined that even after 24 days *in the mesocosm* at the final sampling time point (T4), the variation detected within the community was still greater than the variation that was measured prior to the field sampling disturbance (Baseline). This means more variation was introduced through treatment experimentation and physically destructive sampling than what was already naturally occurring within the active intertidal zone. Though the effect of physical disturbance as an experimental finding in this study remains entirely accidental, we attest that is perhaps one of the more important observations concluded from this manipulation experiment.

Conclusions

In this study, we sought to understand the response of intertidal benthic microbial communities to the effects of ocean acidification. Our objective was to provide a more comprehensive estimate of the overall structural and compositional resistance of sedimentary microbial communities from dynamic coastal environments to futuristic sustained low pH conditions. The intertidal sandflat located in Tauranga Harbour is regularly exposed to high levels of natural and anthropogenic stress. This predisposition to environmental variance appears to have conditioned the benthic microbial communities, where the supposed selective pressures of this coastal region resulted in producing an inherently robust and highly resistant core community. Under both gradual and drastic reductions in pH, the community exhibited substantial tolerability, where the structure and composition of the sediment core community remained stable. Individual members operating on the fringes of the microbial community consistently responded to shifts in seawater pH. These bio-indicative taxa represent an important grouping of nitrifying and keystone bacteria within the marine sedimentary microbiome. Their functional role within the coastal ecosystem, as well as their potential deletion under future acidified ocean conditions, is of critical importance for understanding the stability and integrity of the core sediment microbial community under OA impacts.

We caution against the use of physically destructive sampling methodologies when working with microbiological samples, as this approach may introduce greater biological noise in the data in the form of secondary external effects. We also attest that the sole application of microelectrode profiling techniques (O_2) as a reference for microbial re-establishment

following a disturbance is not truly reflective of community recovery. Ecological manipulation studies may wish to include both biogeochemical profiling data as well as microbiological amplicon data as an inference of structural reconstitution. We also address one of the major limitations of mesocosm studies where the use of a closed, though experimentally controlled system reduces or removes natural system turnover, where the sediment communities are subjected to an unavoidable temporal gradient. This was clearly reflected in our dataset, as the community structure shifted with time, not necessarily as a result of the pH treatment. It is for that purpose that we applied in this study, and encourage in the future, inter-timepoint comparisons in the analysis process to avoid removing important ecological response data. Examining the response of microbial communities along an environmental tolerance gradient, ranging from a dynamic marine ecosystem to a more stabilized one, would provide more detailed information regarding how OA will affect different benthic coastal systems on regional and localized scales. The use of metagenomic and metatranscriptomic analyses would provide greater resolution when determining the extent of the physiological and genetic [RNA] impacts of ocean acidification on the benthic microbial community. Our knowledge and comprehension of how microorganisms in coastal and nearshore sediments respond to imminent acidified ocean conditions are of critical importance for determining wholesale ecosystem resilience under a changing oceanic climate.

Chapter 2: Experiment 1

References

Aller, R. C. (1982). The effects of macrobenthos on chemical properties of marine sediment and overlying water. In *Animal-sediment relations* (pp. 53–102). Springer.

Allison, S. D., & Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, *105*(Supplement 1), 11512–11519.

Allison, S. D., & Martiny, J. B. H. (2009). Resistance, resilience, and redundancy in microbial communities. *In the Light of Evolution*, *2*, 149–166. https://doi.org/10.17226/12501

Alongi, D. M. (1985). Effect of physical disturbance on population dynamics and trophic interactions among microbes and meiofauna. *Journal of Marine Research*, *43*(2), 351–364. https://doi.org/10.1357/002224085788438694

Alpert, P. A., Kilthau, W. P., Bothe, D. W., Radway, J. C., Aller, J. Y., & Knopf, D. A. (2015). The influence of marine microbial activities on aerosol production: A laboratory mesocosm study. *Journal of Geophysical Research: Atmospheres*, *120*(17), 8841–8860. https://doi.org/https://doi.org/10.1002/2015JD023469

Atkinson, B. L., Grace, M. R., Hart, B. T., & Vanderkruk, K. E. N. (2008). Sediment instability affects the rate and location of primary production and respiration in a sand-bed stream. *Journal of the North American Benthological Society*, *27*(3), 581–592. https://doi.org/10.1899/07-143.1

Aufdenkampe, A. K., Mayorga, E., Raymond, P. A., Melack, J. M., Doney, S. C., Alin, S. R., … Yoo, K. (2011). Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, *9*(1), 53–60.

Banks, S. C., Cary, G. J., Smith, A. L., Davies, I. D., Driscoll, D. A., Gill, A. M., … Peakall, R. (2013). How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, *28*(11), 670–679.

Barnes, R. S. K., & Hughes, R. N. (1999). *An introduction to marine ecology*. John Wiley & Sons.

Beman, J. M., Chow, C. E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., … Hutchins, D. A. (2011). Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(1), 208–213. https://doi.org/10.1073/pnas.1011053108

Ben-Yaakov, S. (1973). pH BUFFERING OF PORE WATER OF RECENT ANOXIC MARINE SEDIMENTS. *Limnology and Oceanography*, *18*(1), 86–94. https://doi.org/10.4319/lo.1973.18.1.0086

Berga, M., Székely, A. J., & Langenheder, S. (2012). Effects of disturbance intensity and frequency on bacterial community composition and function. *PLoS One*, *7*(5), e36959.

Bernhard, A. E., Donn, T., Giblin, A. E., & Stahl, D. A. (2005). Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. *Environmental Microbiology*, *7*(9), 1289–1297. https://doi.org/10.1111/j.1462-

2920.2005.00808.x

Bianchi, C. N. (2007). Biodiversity issues for the forthcoming tropical Mediterranean Sea. *Hydrobiologia*, *580*(1 LB-Bianchi2007), 7. https://doi.org/10.1007/s10750-006-0469-5

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., … Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods*, *10*(1), 57–59. https://doi.org/10.1038/nmeth.2276

Bolin, B. (1959). *atmosphere and the sea in motion: scientific contributions to the Rossby memorial volume*.

Bonin, P., Ranaivoson, E. R., Raymond, N., Chalamet, A., & Bertrand, J. C. (1994). Evidence for denitrification in marine sediment highly contaminated by petroleum products. *Marine Pollution Bulletin*, *28*(2), 89–95.

Boudreau, B. P., Huettel, M., Forster, S., Jahnke, R. A., McLachlan, A., Middelburg, J. J., … Sundby, B. (2001). Permeable marine sediments: Overturning an old paradigm. *Eos*, *82*(11), 133–136. https://doi.org/10.1029/EO082i011p00133-01

Bouillon, S., Borges, A. V., Castañeda-Moya, E., Diele, K., Dittmar, T., Duke, N. C., … Twilley, R. R. (2008). Mangrove production and carbon sinks: A revision of global budget estimates. *Global Biogeochemical Cycles*, *22*(2), 1–12. https://doi.org/10.1029/2007GB003052

Bowen, J. L., Kearns, P. J., Holcomb, M., & Ward, B. B. (2013). Acidification alters the composition of ammonia-oxidizing microbial assemblages in marine mesocosms. *Marine Ecology Progress Series*, *492*, 1–8.

Bowman, J. P., & McCuaig, R. D. (2003). Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Applied and Environmental Microbiology*, *69*(5), 2463–2483.

Boyd, P. W. (2011). Beyond ocean acidification. *Nature Geoscience*, *4*, 273. https://doi.org/10.1038/ngeo1150

Brose, U., & D. Martinez, N. (2004). Estimating the richness of species with variable mobility. *Oikos*, *105*(2), 292–300.

Burrell, R. B., Keppel, A. G., Clark, V. M., & Breitburg, D. L. (2016). An automated monitoring and control system for flow-through co-cycling hypoxia and pH experiments. *Limnology and Oceanography: Methods*, *14*(3), 168–185.

Byoung-Joon, P., Soo-Je, P., Dae-No, Y., Stefan, S., S., S. D. J., & Sung-Keun, R. (2010). Cultivation of Autotrophic Ammonia-Oxidizing Archaea from Marine Sediments in Coculture with Sulfur-Oxidizing Bacteria. *Applied and Environmental Microbiology*, *76*(22), 7575–7587. https://doi.org/10.1128/AEM.01478-10

Cai, W.-J., Reimers, C. E., & Shaw, T. (1995). Microelectrode studies of organic carbon degradation and calcite dissolution at a California continental rise site. *Geochimica et Cosmochimica Acta*, *59*(3), 497–511.

Cai, W., & Reimers, C. E. (1993). The development of pH and pCO2 microelectrodes for

studying the carbonate chemistry of pore waters near the sediment-water interface. *Limnology and Oceanography*, *38*(8), 1762–1773.

Canfield, D. E., Jørgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., … Hall, P. O. J. (1993). Pathways of organic carbon oxidation in three continental margin sediments. *Marine Geology*, *113*(1–2), 27–40.

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., … Webster, N. S. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, *17*(9), 569–586. https://doi.org/10.1038/s41579-019-0222-5

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 265–270.

Christiansen, B., Denda, A., & Christiansen, S. (2020). Potential effects of deep seabed mining on pelagic and benthopelagic biota. *Marine Policy*, *114*(September 2018), 103442. https://doi.org/10.1016/j.marpol.2019.02.014

Cordier, T. (2020). Bacterial communities' taxonomic and functional turnovers both accurately predict marine benthic ecological quality status. *Environmental DNA*, *2*(2), 175–183. https://doi.org/10.1002/edn3.55

Crummett, L. T. (2020). Acidification decreases microbial community diversity in the Salish Sea, a region with naturally high pCO2. *PLoS ONE*, *15*(10 October), 1–17. https://doi.org/10.1371/journal.pone.0241183

Currie, A. R., Tait, K., Parry, H., de Francisco-Mora, B., Hicks, N., Mark Osborn, A., … Stahl, H. (2017). Marine microbial gene abundance and community composition in response to ocean acidification and elevated temperature in two contrasting coastal marine sediments. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.01599

De Beer, D., Wenzhöfer, F., Ferdelman, T. G., Boehme, S. E., Huettel, M., Van Beusekom, J. E. E., … Dubilier, N. (2005). Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. *Limnology and Oceanography*, *50*(1), 113–127. https://doi.org/10.4319/lo.2005.50.1.0113

Dickson, A., Sabine, C., & Christian, J. (2007). Guide to Best Practices for Ocean C02 Measurments. In *North Pacific Marine Science Organization* (Vol. 3). Retrieved from www.pices.int

Dickson, A G, & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(10), 1733–1743.

Dickson, Andrew G. (1990). Standard potential of the reaction: AgCl (s)+ 12H2 (g)= Ag (s)+ HCl (aq), and and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, *22*(2), 113–127.

Doney, S. C., Busch, D. S., Cooley, S. R., & Kroeker, K. J. (2020). The Impacts of Ocean Acidification on Marine Ecosystems and Reliant Human Communities. *Annual Review of Environment and Resources*, *45*(1), 1–30. https://doi.org/10.1146/annurev-environ-012320-083019

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean Acidification: The Other CO_2 Problem. *Annual Review of Marine Science*. https://doi.org/10.1146/annurev.marine.010908.163834

Douglas, E. J., Lohrer, A. M., & Pilditch, C. A. (2019). Biodiversity breakpoints along stress gradients in estuaries and associated shifts in ecosystem interactions. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-54192-0

Drylie, T. P., Needham, H. R., Lohrer, A. M., Hartland, A., & Pilditch, C. A. (2019). Calcium carbonate alters the functional response of coastal sediments to eutrophication-induced acidification. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-48549-8

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., … McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts*, *36*(2), 221–236.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Egleston, E. S., Sabine, C. L., & Morel, F. M. M. (2010). Revelle revisited: Buffer factors that quantify the response of ocean chemistry to changes in DIC and alkalinity. *Global Biogeochemical Cycles*, *24*(1), 1–9. https://doi.org/10.1029/2008GB003407

Fenchel, T. M., & Riedl, R. J. (1970). The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *Marine Biology*, *7*(3), 255–268. https://doi.org/10.1007/BF00367496

Fenchel, T., & Riedl, R. (1970). The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *International Journal on Life in Oceans and Coastal Waters*, *7*(3), 255–268. https://doi.org/10.1007/BF00367496

Fernandes, A. D., Vu, M. T. H. Q., Edward, L. M., Macklaim, J. M., & Gloor, G. B. (2018). A reproducible effect size is more useful than an irreproducible hypothesis test to analyze high throughput sequencing datasets. *ArXiv*, 1–15.

Ferrández-Gómez, B., Sánchez, A., Jordá, J. D., Fonfría, E. S., Bordehore, C., & Cerdán, M. (2021). Effectiveness of Oxygen-Saturated Seawater Injections and Air Sparging Technologies in Remediation of Coastal Marine Sediments from Sludge. *Environmental Geochemistry and Health*, *43*(12), 4975–4986. https://doi.org/10.1007/s10653-021-00952-9

Findlay, R. H., Pollard, P. C., Moriarty, D. J. W., & White, D. C. (1985). Quantitative determination of microbial activity and community nutritional status in estuarine sediments: evidence for a disturbance artifact. *Canadian Journal of Microbiology*, *31*(5), 493–498. https://doi.org/10.1139/m85-091

Findlay, R., Trexler, M., Guckert, J., & White, D. (1990). Laboratory study of disturbance in marine sediments: response of a microbial community. *Marine Ecology Progress Series*, *62*(Aller 1982), 121–133. https://doi.org/10.3354/meps062121

Galand, P. E., Lucas, S., Fagervold, S. K., Peru, E., Pruski, A. M., Vétion, G., … Guizien, K. (2016). Disturbance increases microbial community diversity and production in marine

sediments. *Frontiers in Microbiology*, *7*(DEC), 1–11.
https://doi.org/10.3389/fmicb.2016.01950

Garnier, S. (2017). viridis: Default Color Maps from "matplotlib." *R Package Version 0.5.1*, 1–6. Retrieved from https://cran.r-project.org/package=viridis

Gattuso, J. P., Frankignoulle, M., & Wollast, R. (1998). Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics*, *29*, 405–434. https://doi.org/10.1146/annurev.ecolsys.29.1.405

Gill, A. B. (2005). Offshore renewable energy: Ecological implications of generating electricity in the coastal zone. *Journal of Applied Ecology*, *42*(4), 605–615. https://doi.org/10.1111/j.1365-2664.2005.01060.x

Giraud, X., Le Quéré, C., & da Cunha, L. C. (2008). Importance of coastal nutrient supply for global ocean biogeochemistry. *Global Biogeochemical Cycles*, *22*(2), 1–15. https://doi.org/10.1029/2006GB002717

Goldberg, S. J., Nelson, C. E., Viviani, D. A., Shulse, C. N., & Church, M. J. (2017). Cascading influence of inorganic nitrogen sources on DOM production, composition, lability and microbial community structure in the open ocean. *Environmental Microbiology*, *19*(9), 3450–3464. https://doi.org/10.1111/1462-2920.13825

Graf, G. (1992). Benthic-pelagic coupling: a benthic view. *Oceanography and Marine Biology Annual Review*, *30*, 149–190.

Gray, J. S. (1974). Animal-sediment relationships. Oceanogr. *Mar. Biol. Ann. Rev.*, *12*, 223–261.

Hagopian, D. S., & Riley, J. G. (1998). *A closer look at the bacteriology of nitrification*. *18*(May), 223–244.

Hale, R., Calosi, P., Mcneill, L., Mieszkowska, N., & Widdicombe, S. (2011). Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos*, *120*(5), 661–674. https://doi.org/10.1111/j.1600-0706.2010.19469.x

Hicks, N., Ubbara, G. R., Silburn, B., Smith, H. E. K., Kröger, S., Parker, E. R., … Stahl, H. (2017). Oxygen dynamics in shelf seas sediments incorporating seasonal variability. *Biogeochemistry*, *135*(1–2), 35–47. https://doi.org/10.1007/s10533-017-0326-9

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., … Takeshita, Y. (2011). High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PloS One*, *6*(12), e28983.

Hoppe-Jones, C., Oldham, G., & Drewes, J. E. (2010). Attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems. *Water Research*, *44*(15), 4643–4659. https://doi.org/10.1016/j.watres.2010.06.022

Horner-Devine, M. C., Lage, M., Hughes, J. B., & Bohannan, B. J. M. (2004). A taxa–area relationship for bacteria. *Nature*, *432*(7018), 750.

Hou, J., Song, C., Cao, X., & Zhou, Y. (2013). Shifts between ammonia-oxidizing bacteria and

archaea in relation to nitrification potential across trophic gradients in two large Chinese lakes (Lake Taihu and Lake Chaohu). *Water Research*, *47*(7), 2285–2296. https://doi.org/https://doi.org/10.1016/j.watres.2013.01.042

Hou, M., Xiong, J., Wang, K., Ye, X., Ye, R., Wang, Q., … Zhang, D. (2014). Communities of sediment ammonia-oxidizing bacteria along a coastal pollution gradient in the East China Sea. *Marine Pollution Bulletin*, *86*(1–2), 147–153. https://doi.org/10.1016/j.marpolbul.2014.07.031

Hu, X., & Cai, W. J. (2011). An assessment of ocean margin anaerobic processes on oceanic alkalinity budget. *Global Biogeochemical Cycles*, *25*(3), 1–11. https://doi.org/10.1029/2010GB003859

Huettel, M., Ziebis, W., & Forster, S. (1996). Flow-induced uptake of particulate matter in permeable sediments. *Limnology and Oceanography*, *41*(2), 309–322. https://doi.org/10.4319/lo.1996.41.2.0309

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609–623.

Igbp, I. O. C. (n.d.). SCOR (2013) Ocean Acidification Summary for Policymakers–Third Symposium on the Ocean in a High-CO2 World. *International Geosphere-Biosphere Programme. Stockolm, Sweden*.

Inoue, S., Yoshizawa, S., Nakajima, Y., Kojima, K., Tsukamoto, T., Kikukawa, T., & Sudo, Y. (2018). Spectroscopic characteristics of: Rubricoccus marinus xenorhodopsin (Rm XeR) and a putative model for its inward H+ transport mechanism. *Physical Chemistry Chemical Physics*, *20*(5), 3172–3183. https://doi.org/10.1039/c7cp05033j

Jaekel, U., Zedelius, J., Wilkes, H., & Musat, F. (2015). Anaerobic degradation of cyclohexane by sulfate-reducing bacteria from hydrocarbon-contaminated marine sediments. *Frontiers in Microbiology*, *6*, 116.

Jansen, S., Walpersdorf, E., Werner, U., Billerbeck, M., Böttcher, M. E., & De Beer, D. (2009). Functioning of intertidal flats inferred from temporal and spatial dynamics of O2, H2S and pH in their surface sediment. *Ocean Dynamics*, *59*(2), 317–332. https://doi.org/10.1007/s10236-009-0179-4

Jessup, C. M., Forde, S. E., & Bohannan, B. J. M. (2005). Microbial Experimental Systems in Ecology. *Advances in Ecological Research*, *37*(04), 273–307. https://doi.org/10.1016/S0065-2504(04)37009-1

Jessup, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., & Bohannan, B. J. M. (2004). Big questions, small worlds: Microbial model systems in ecology. *Trends in Ecology and Evolution*, *19*(4), 189–197. https://doi.org/10.1016/j.tree.2004.01.008

Joint, I., Doney, S. C., & Karl, D. M. (2011). Will ocean acidification affect marine microbes. *ISME Journal*. https://doi.org/10.1038/ismej.2010.79

Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2020). *Package ' adespatial .'* https://doi.org/10.1890/11-1183.1>.Maintainer

Jorgensen, B. B., Revsbech, N. P., & Cohen, Y. (1983). Photosynthesis and structure of

benthic microbial mats: Microelectrode and SEM studies of four cyanobacterial communities1. *Limnology and Oceanography*, *28*(6), 1075–1093.

Kassambara, A., & Kassambara, M. A. (2020). *Package 'ggpubr.'*

Kerfahi, D., Harvey, B. P., Agostini, S., Kon, K., Huang, R., Adams, J. M., & Hall-Spencer, J. M. (2020). Responses of Intertidal Bacterial Biofilm Communities to Increasing p CO 2. *Marine Biotechnology*, 1–12.

Kessler, A. J., Chen, Y. J., Waite, D. W., Hutchinson, T., Koh, S., Popa, M. E., … Greening, C. (2019). Bacterial fermentation and respiration processes are uncoupled in anoxic permeable sediments. *Nature Microbiology*. https://doi.org/10.1038/s41564-019-0391-z

Kim, K. H., & Kim, D. (2007). *Seasonal and spatial variability of sediment oxygen fluxes in the Beobsan*. *11*(4), 323–329.

Kitidis, V., Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., … Widdicombe, S. (2011). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters*. https://doi.org/10.1029/2011GL049095

Kroeker, K. J., Micheli, F., Gambi, M. C., & Martz, T. R. (2011). Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(35), 14515–14520. https://doi.org/10.1073/pnas.1107789108

L.Pimm, S. (1985). The complexity and stability of ecosystems. *Nature*, *315*(6021), 635–636.

Laverock, B., Smith, C. J., Tait, K., Osborn, A. M., Widdicombe, S., & Gilbert, J. A. (2010). Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. *The ISME Journal*. https://doi.org/10.1038/ismej.2010.86

Lewis, E. R., & Wallace, D. W. R. (1998). *Program developed for CO2 system calculations*. Environmental System Science Data Infrastructure for a Virtual Ecosystem.

Liao, H., Lin, X., Li, Y., Qu, M., & Tian, Y. (2020). Reclassification of the taxonomic framework of orders cellvibrionales, oceanospirillales, pseudomonadales, and alteromonadales in class gammaproteobacteria through phylogenomic tree analysis. *Msystems*, *5*(5), e00543-20.

Lidbury, I., Johnson, V., Hall-Spencer, J. M., Munn, C. B., & Cunliffe, M. (2012). Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. *Marine Pollution Bulletin*, *64*(5), 1063–1066. https://doi.org/https://doi.org/10.1016/j.marpolbul.2012.02.011

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J.-P. (2010a). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*, *61*(3), 291–305.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J. P. (2010b). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*. https://doi.org/10.3354/ame01446

Llirós, M., Inceołlu, Ö., García-Armisen, T., Anzil, A., Leporcq, B., Pigneur, L. M., … Servais, P. (2014). Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. *PLoS ONE*, *9*(12), 1–27. https://doi.org/10.1371/journal.pone.0116145

Lozupone, C. A., & Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences*, *104*(27), 11436–11440.

Mackey, R. L., & Currie, D. J. (2001). The diversity–disturbance relationship: is it generally strong and peaked? *Ecology*, *82*(12), 3479–3492.

Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease*, *26*(0). https://doi.org/10.3402/mehd.v26.27663

Marinelli, R. L., & Boudreau, B. P. (1996). An experimental and modeling study of pH and related solutes in an irrigated anoxic coastal sediment. *Journal of Marine Research*, *54*(5), 939–966.

Marinelli, R. L., Lovell, C. R., Wakeham, S. G., Ringelberg, D. B., & White, D. C. (2002). Experimental investigation of the control of bacterial community composition in macrofaunal burrows. *Marine Ecology Progress Series*, *235*, 1–13. https://doi.org/10.3354/meps235001

Maung-Saw-Htoo-Thaw, Ohara, S., Matsuoka, K., Yurimoto, T., Higo, S., Khin-Ko-Lay, … Koike, K. (2017). Seasonal dynamics influencing coastal primary production and phytoplankton communities along the southern Myanmar coast. *Journal of Oceanography*, *73*(3), 345–364. https://doi.org/10.1007/s10872-016-0408-7

McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R., … DeLong, E. F. (2010). Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proceedings of the National Academy of Sciences*, *107*(38), 16420–16427.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

McMurdie, P. J., & Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLOS Computational Biology*, *10*(4), e1003531. Retrieved from https://doi.org/10.1371/journal.pcbi.1003531

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure 1. *Limnology and Oceanography*, *18*(6), 897–907.

Melzner, F., Mark, F. C., Seibel, B. A., & Tomanek, L. (2020). Ocean Acidification and Coastal Marine Invertebrates: Tracking CO2 Effects from Seawater to the Cell. *Annual Review of Marine Science*, *12*, 499–523. https://doi.org/10.1146/annurev-marine-010419-010658

Mendoza-Lera, C., & Mutz, M. (2013). Microbial activity and sediment disturbance modulate the vertical water flux in sandy sediments. *Freshwater Science*, *32*(1), 26–38.

https://doi.org/10.1899/11-165.1

Mercado, J. M., & Gordillo, F. J. L. (2011). Inorganic carbon acquisition in algal communities: Are the laboratory data relevant to the natural ecosystemš. *Photosynthesis Research*, *109*(1–3), 257–267. https://doi.org/10.1007/s11120-011-9646-0

Middelburg, J. J., Soetaert, K., & Hagens, M. (2020). Ocean Alkalinity, Buffering and Biogeochemical Processes. *Reviews of Geophysics*, *58*(3). https://doi.org/10.1029/2019RG000681

Muller-karger, F. E., Varela, R., Thunell, R., Luerssen, R., Hu, C., & Walsh, J. J. (2005). *The importance of continental margins in the global carbon cycle*. *32*(December 2004), 10–13. https://doi.org/10.1029/2004GL021346

Nelson, K. S., Baltar, F., Lamare, M. D., & Morales, S. E. (2020). Ocean acidification affects microbial community and invertebrate settlement on biofilms. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-60023-4

Nixon, S. W. (1981). Remineralization and nutrient cycling in coastal marine ecosystems. In *Estuaries and nutrients* (pp. 111–138). Springer.

Obi, C. C., Adebusoye, S. A., Ugoji, E. O., Ilori, M. O., Amund, O. O., & Hickey, W. J. (2016). Microbial communities in sediments of Lagos Lagoon, Nigeria: elucidation of community structure and potential impacts of contamination by municipal and industrial wastes. *Frontiers in Microbiology*, *7*, 1213.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P. M., … & Wagner, H. (2008). The vegan package. *Community Ecology Package*, (May 2014), 190. Retrieved from https://bcrc.bio.umass.edu/biometry/images/8/85/Vegan.pdf

Oliver, A. E., Newbold, L. K., Whiteley, A. S., & van der Gast, C. J. (2014). Marine bacterial communities are resistant to elevated carbon dioxide levels. *Environmental Microbiology Reports*, *6*(6), 574–582. https://doi.org/10.1111/1758-2229.12159

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Polerecky, L., Franke, U., Werner, U., Grunwald, B., & De Beer, D. (2005). High spatial resolution measurement of oxygen consumption rates in permeable sediments. *Limnology and Oceanography: Methods*, *3*(FEB.), 75–85. https://doi.org/10.4319/lom.2005.3.75

Pratt, D. R., Pilditch, C. A., Lohrer, A. M., & Thrush, S. F. (2014). The effects of short-term increases in turbidity on sandflat microphytobenthic productivity and nutrient fluxes. *Journal of Sea Research*, *92*, 170–177. https://doi.org/10.1016/j.seares.2013.07.009

Primavera, J. H. (2006). Overcoming the impacts of aquaculture on the coastal zone. *Ocean*

and Coastal Management, *49*(9–10), 531–545.
https://doi.org/10.1016/j.ocecoaman.2006.06.018

Probert P.K. (1984). Disturbance, sediment stability, and trophic structure of soft-bottom communities. *Journal of Marine Research*, *42*, 893–921.

Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A. H., Nieuwdorp, M., & Levin, E. (2020). Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PLOS ONE*, *15*(1), e0227434. Retrieved from https://doi.org/10.1371/journal.pone.0227434

Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics*, *12*(1), 38. https://doi.org/10.1186/1471-2105-12-38

Raffaelli, D., Bell, E., Weithoff, G., Matsumoto, A., Cruz-Motta, J. J., Kershaw, P., … Jones, M. (2003). The ups and downs of benthic ecology: Considerations of scale, heterogeneity and surveillance for benthic–pelagic coupling. *Journal of Experimental Marine Biology and Ecology*, *285*, 191–203.

Rasmussen, H., & Jorgensen, B. B. (1992). Microelectrode studies of seasonal oxygen uptake in a coastal sediment: role of molecular diffusion. *Marine Ecology Progress Series*, *81*(3), 289–303. https://doi.org/10.3354/meps081289

Raulf, F. F., Fabricius, K., Uthicke, S., de Beer, D., Abed, R. M. M., & Ramette, A. (2015). Changes in microbial communities in coastal sediments along natural CO_2 gradients at a volcanic vent in Papua New Guinea. *Environmental Microbiology*. https://doi.org/10.1111/1462-2920.12729

Ravenschlag, K., Sahm, K., & Amann, R. (2001). Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard). *Applied and Environmental Microbiology*, *67*(1), 387–395. https://doi.org/10.1128/AEM.67.1.387-395.2001

Reimers, C. E. (1987). An in situ microprofiling instrument for measuring interfacial pore water gradients: methods and oxygen profiles from the North Pacific Ocean. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(12), 2019–2035.

Reimers, C. E., Ruttenberg, K. C., Canfield, D. E., Christiansen, M. B., & Martin, J. B. (1996). Porewater pH and authigenic phases formed in the uppermost sediments of the Santa Barbara Basin. *Geochimica et Cosmochimica Acta*, *60*(21), 4037–4057. https://doi.org/10.1016/S0016-7037(96)00231-1

Revelle, R., & Suess, H. E. (1957). Carbon Dioxide Exchange Between Atmosphere and Ocean and the Question of an Increase of Atmospheric CO_2 during the Past Decades. *Tellus*, *9*(1), 18–27. https://doi.org/10.3402/tellusa.v9i1.9075

Revsbech, N. P. (1983). In situ measurement of oxygen profiles of sediments by use of oxygen microelectrodes. In *Polarographic oxygen sensors* (pp. 265–273). Springer.

Rhoads, D. C., & Boyer, L. F. (1982). The effects of marine benthos on physical properties of sediments. In *Animal-sediment relations* (pp. 3–52). Springer.

Roy, A. S., Gibbons, S. M., Schunck, H., Owens, S., Caporaso, J. G., Sperling, M., … Gilbert, J. A. (2013). Ocean acidification shows negligible impacts on high-latitude bacterial

community structure in coastal pelagic mesocosms. *Biogeosciences*, *10*(1), 555–566. https://doi.org/10.5194/bg-10-555-2013

Sala, M. M., Aparicio, F. L., Balague, V., Boras, J. A., Borrull, E., Cardelus, C., … Marrase, C. (2016). Contrasting effects of ocean acidification on the microbial food web under different trophic conditions. *Ices Journal of Marine Science*, *73*(3), 670–679. https://doi.org/10.1093/icesjms/fsv130

Sarmiento, J. L., & Gruber, N. (2006). Ocean biogeochemical cycles. *Princeton University*.

Schloss, P. D., & Westcott, S. L. (2011). Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.02810-10

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Sharma, R., Nagender Nath, B., Parthiban, G., & Jai Sankar, S. (2001). Sediment redistribution during simulated benthic disturbance and its implications on deep seabed mining. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *48*(16), 3363–3380. https://doi.org/10.1016/S0967-0645(01)00046-7

Silburn, B., Kröger, S., Parker, E. R., Sivyer, D. B., Hicks, N., Powell, C. F., … Greenwood, N. (2017). Benthic pH gradients across a range of shelf sea sediment types linked to sediment characteristics and seasonal variability. *Biogeochemistry*, *135*(1–2), 69–88. https://doi.org/10.1007/s10533-017-0323-z

Simone, M., Schulz, K., Oakes, J., & Eyre, B. (2020). Warming and ocean acidification may decrease estuarine dissolved organic carbon export to the ocean. *Biogeosciences Discussions*, 1–22. https://doi.org/10.5194/bg-2020-335

Soetaert, K., Hofmann, A. F., Middelburg, J. J., Meysman, F. J. R., & Greenwood, J. (2007). Reprint of "The effect of biogeochemical processes on pH." *Marine Chemistry*, *106*(1-2 SPEC. ISS.), 380–401. https://doi.org/10.1016/j.marchem.2007.06.008

Spring, S., Scheuner, C., Göker, M., & Klenk, H. P. (2015). A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Frontiers in Microbiology*, *6*(APR), 1–17. https://doi.org/10.3389/fmicb.2015.00281

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., … Midgley, P. M. (2013). Climate change 2013: the physical science basis. Intergovernmental panel on climate change, working group I contribution to the IPCC fifth assessment report (AR5). *New York*.

Sundquist, E. T., Plummer, L. N., & Wigley, T. M. L. (1979). Carbon dioxide in the ocean surface: the homogeneous buffer factor. *Science*, *204*(4398), 1203–1205.

Tait, K., Laverock, B., & Widdicombe, S. (2014). Response of an Arctic Sediment Nitrogen Cycling Community to Increased CO2. *Estuaries and Coasts*, *37*(3 LB-Tait2014), 724–

735. https://doi.org/10.1007/s12237-013-9709-x

Taylor, P., Lichtschlag, A., Toberman, M., Sayer, M. D. J., Reynolds, A., Sato, T., & Stahl, H. (2015). Impact and recovery of pH in marine sediments subject to a temporary carbon dioxide leak. *International Journal of Greenhouse Gas Control*, *38*, 93–101. https://doi.org/10.1016/j.ijggc.2014.09.006

Thomas, H., Schiettecatte, L. S., Suykens, K., Koné, Y. J. M., Shadwick, E. H., Prowe, A. E. F., … Borges, A. V. (2009). Enhanced ocean carbon storage from anaerobic alkalinity generation in coastal sediments. *Biogeosciences*, *6*(2), 267–274. https://doi.org/10.5194/bg-6-267-2009

Torsvik, V., xd, vre, xe, s, L., & Thingstad, T. F. (2002). Prokaryotic Diversity: Magnitude, Dynamics, and Controlling Factors. *Science*, *296*(5570), 1064–1066. Retrieved from http://www.jstor.org.ezproxy.waikato.ac.nz/stable/3076691

Valiela, I. (n.d.). *Marine Ecological Processes 1995*. Springer: New York.

Villanueva, R. A. M., & Chen, Z. J. (2019). ggplot2: Elegant Graphics for Data Analysis (2nd ed.). *Measurement: Interdisciplinary Research and Perspectives*, *17*(3), 160–167. https://doi.org/10.1080/15366367.2019.1565254

Vonnahme, T. R., Molari, M., Janssen, F., Wenzhöfer, F., Haeckel, M., Titschack, J., & Boetius, A. (2020). Effects of a deep-sea mining experiment on seafloor microbial communities and functions after 26 years. *Science Advances*, *6*(18). https://doi.org/10.1126/sciadv.aaz5922

Vonnahme, Tobias R, Molari, M., Janssen, F., Wenzhöfer, F., Haeckel, M., Titschack, J., & Boetius, A. (2020). Effects of a deep-sea mining experiment on seafloor microbial communities and functions after 26 years. *Science Advances*, *6*(18), eaaz5922.

Vopel, K., Del-Río, C., & Pilditch, C. A. (2018). Effects of CO2 enrichment on benthic primary production and inorganic nitrogen fluxes in two coastal sediments. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-017-19051-w

Walker, A. M., Leigh, M. B., & Mincks, S. L. (2021). Patterns in Benthic Microbial Community Structure Across Environmental Gradients in the Beaufort Sea Shelf and Slope. *Frontiers in Microbiology*, *12*(January), 1–21. https://doi.org/10.3389/fmicb.2021.581124

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Zejie, Deng, H., Chen, L., Xiao, Y., & Zhao, F. (2013). In situ measurements of dissolved oxygen, pH and redox potential of biocathode microenvironments using microelectrodes. *Bioresource Technology*, *132*, 387–390. https://doi.org/10.1016/j.biortech.2012.11.026

Wang, Zhao, Juarez, D. L., Pan, J. F., Blinebry, S. K., Gronniger, J., Clark, J. S., … Hunt, D. E. (2019). Microbial communities across nearshore to offshore coastal transects are primarily shaped by distance and temperature. *Environmental Microbiology*, *21*(10),

3862–3872. https://doi.org/10.1111/1462-2920.14734

Washburne, A. D., Morton, J. T., Sanders, J., McDonald, D., Zhu, Q., Oliverio, A. M., & Knight, R. (2018). Methods for phylogenetic analysis of microbiome. *Nature Microbiology*, *3*(6), 652–661. https://doi.org/10.1038/s41564-018-0156-0

Watson, S. W. (1989). Nitrifying bacteria. *Bergey's Manual of Systematic Bacteriology*, *3*, 1808–1834.

WATSON, S. W. (1971). Taxonomic Considerations of the Family Nitrobacteraceae Buchanan: Requests for Opinions. *International Journal of Systematic Bacteriology*, *21*(3), 254–270. https://doi.org/10.1099/00207713-21-3-254

Webster, N. S., Negri, A. P., Flores, F., Humphrey, C., Soo, R., Botté, E. S., … Uthicke, S. (2013). Near-future ocean acidification causes differences in microbial associations within diverse coral reef taxa. *Environmental Microbiology Reports*, *5*(2), 243–251. https://doi.org/10.1111/1758-2229.12006

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Widdicombe, S., & Spicer, J. I. (2008). Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology*, *366*(1–2), 187–197. https://doi.org/10.1016/j.jembe.2008.07.024

Wootton, J. T., Pfister, C. A., & Forester, J. D. (2008). Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(48), 18848–18853. https://doi.org/10.1073/pnas.0810079105

Yeo, S. K., Huggett, M. J., Eiler, A., & Rappé, M. S. (2013). Coastal bacterioplankton community dynamics in response to a natural disturbance. *PloS One*, *8*(2), e56207.

Zeebe, R. E., & Wolf-Gladrow, D. A. (2001). *CO2 in seawater: equilibrium, kinetics, isotopes*. Gulf Professional Publishing.

Zhu, Q., Aller, R. C., & Fan, Y. (2006). Two-dimensional pH distributions and dynamics in bioturbated marine sediments. *Geochimica et Cosmochimica Acta*, *70*(19), 4933–4949. https://doi.org/10.1016/j.gca.2006.07.033

Chapter 2: Experiment 1

Supplementary Information and Data:
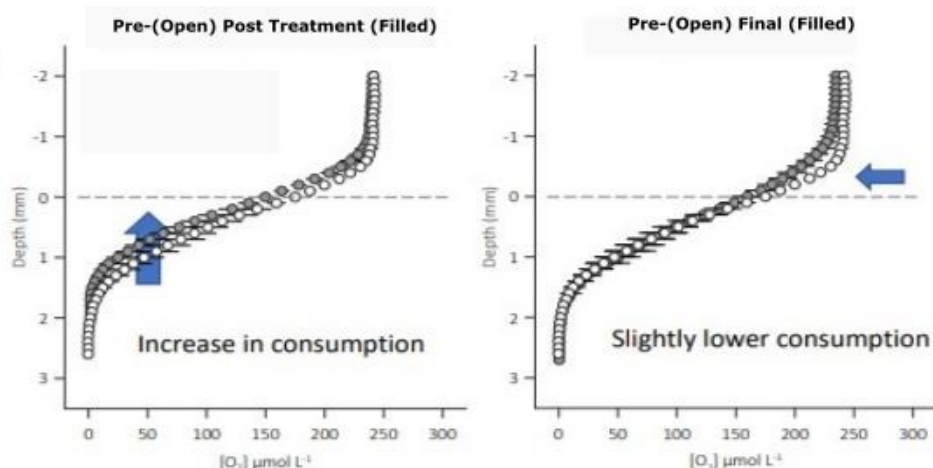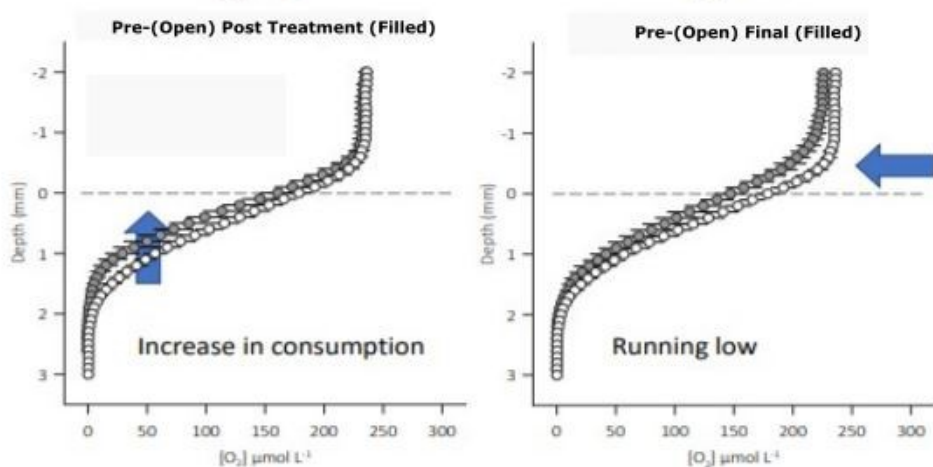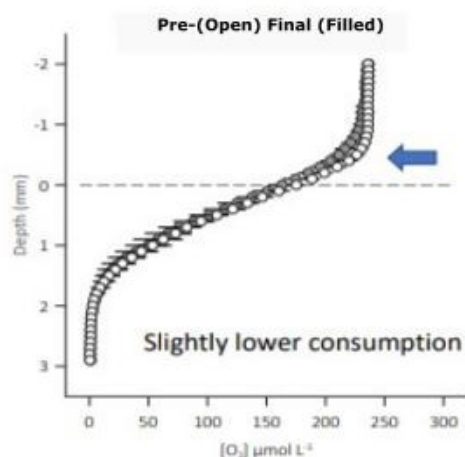
Supplementary Figure 1. Microelectrode oxygen profiles for the sediment porewater for the different experimental pH treatments (Control (C), Stepwise Reduction (StR)), and Rapid Shift (RaS)) from the pre-treatment measurements (Left) compared to the final post-treatment measurements at the conclusion of the experiment (right). Dashed lines indicate the sediment-water interface.

Rapid Shift (RaS) RE2
Pre & Post Treatment
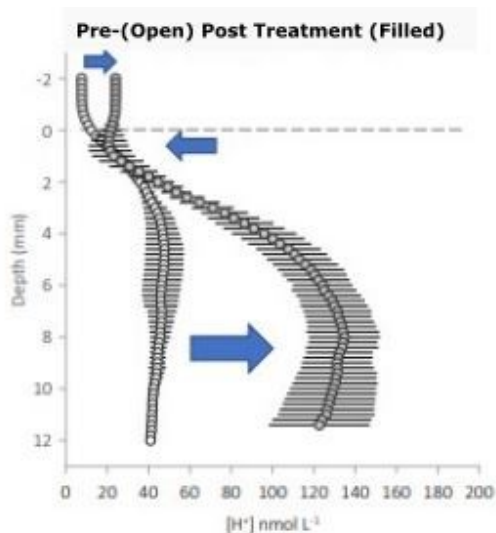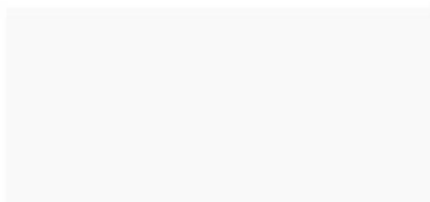
Control (C) RE3
Pre & Post Treatment
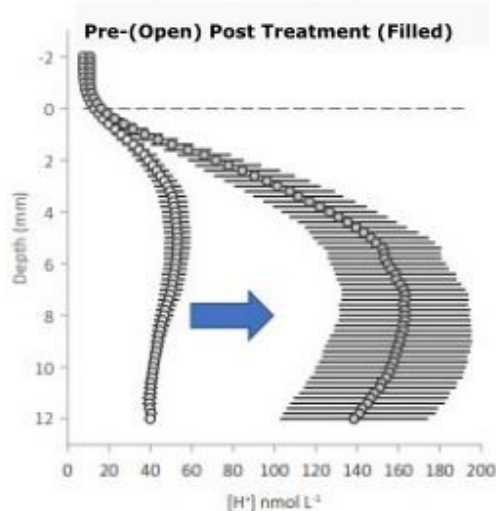
Stepwise Reduction (StR) RE4
Pre-Treatment & Final
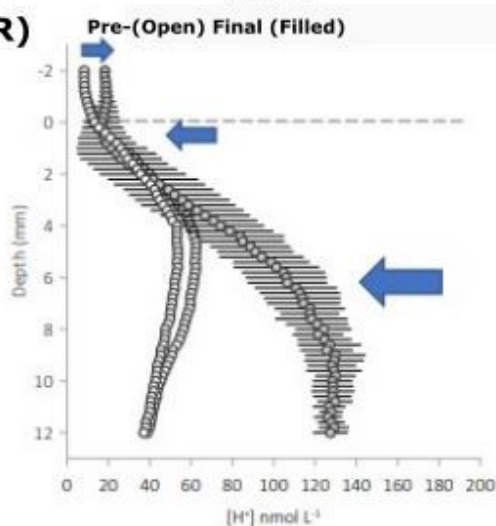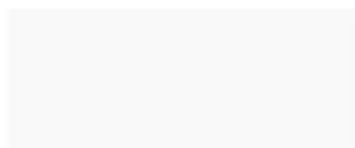
Supplementary Figure 2. pH [H^+] profiles of the different pH treatments (Control (C); Stepwise Reduction (StR); and Rapid Shift (RaS)) demonstrating the pre-treatment measurement profiles in the open circles compared to the final post-treatment measurements depicted in the filled circles. Dashed lines indicate the sediment-water interface. Measurable changes in the Control are thought to be a result of physical disturbance during the sampling process which may have produced microniches.

Supplementary Table 1. AUT Mesocosm sampling timeline for mesocosm system profiling across the different experimental treatments. Redox establishment lasted 11 days. Experimental clock begins post redox establishment. The period during redox establishment is classified as "pre disturbance" or Baseline. Baseline samples were collected from the top 0-1cm (pH: 8.10) homogenized and sieved sediment depth profile and were used as a pre disturbance proxy. T0 sampling (22/11/2016) post redox establishment, begins the start of the pH treatment (Tuesday: 12 samples collected, all tanks at a pH of 8.10) pH reduced after sampling. T1 (Saturday: 12 samples collected; Control: 8.10; STR: 7.95; RS: 7.60) pH reduced after sampling. T2 (Wednesday: 12 samples collected: Control: 8.10; STR: 7.80; RS: 7.60) pH reduced after sampling. T3 (Thursday: 12 samples collected: Control: 8.10; STR: 7.65; RS: 7.60) pH reduced for the last time. T4 final sampling time point (Friday: 12 samples collected: Control: 8.10; STR: 7.65; RS: 7.60) experiment concluded.

Time point	Days *In-Situ*	Hours *In-Situ*	Date Sampled	Control [C]: RE3	Stepwise Reduction [StR]: RE4	Rapid Shift [RaS]:RE2
Baseline	T-11 *Redox Est.*	T-264 *Redox Est.*	11/11/2016	8.1	8.1	8.1
T0	0	0	22/11/2016	8.1	8.1	8.1
T1	4	96	26/11/2016	8.1	7.95	7.6
T2	8	192	30/11/2016	8.1	7.8	7.6
T3	16	*384*	8/12/2016	8.1	7.65	7.6
T4	24	576	16/12/2016	8.1	7.6	7.6

Supplementary Table 2. Sediment core chemistry analyses and results showing the averaged values and standard deviations for each physicochemical measurement from each time point and experimental treatment. Each value is the calculated average of each duplicate/replicate sample from each experimental treatment at each sampling time point.

Time point: Days in Mesocosm	Treatment: Flow Chamber	pH	Chlorophyll a (µg/g dw)	Phaeo Pigments (µg/g dw)	Chlorophyll a & Phaeo	Wet Weight (g)	Dry Weight (g)	Organic Content (%)	CaCO$_3$ Content (%)	Grain Size (63)	Grain Size (Dx (50))
T0:0	Control: RE3	8.1	10.62±0.0	4.78±0.6	2.25±0.3	11.05±0.2	8.32±0.3	1.93±0.2	0.51±0.1	2.99±0.1	182.00±1.0
T0:0	Rapid Shift:RE2	8.1	10.71±0.2	4.83±0.1	2.22±0.0	11.19±0.1	8.68±0.2	1.69±0.2	0.45±0.0	4.29±0.2	179.00±2.0
T0:0	Stepwise Reduction:RE4	8.1	10.60±0.1	4.03±0.0	2.63±0.0	5.76±0.1	4.37±0.0	1.51±0.0	0.30±0.1	2.90±0.2	179.00±2.0
T1:4	Control:RE3	8.1	11.24±0.1	5.46±0.4	2.07±0.2	15.74±0.3	10.40±0.2	1.66±0.2	0.67±0.0	7.65±3.4	161.00±14.0
T1:4	Rapid Shift:RE2	7.6	9.42±0.2	4.36±0.2	2.17±0.2	14.31±0.2	9.53±0.2	1.71±0.0	0.66±0.0	3.86±0.7	177.50±1.5
T1:4	Stepwise Reduction:RE4	7.95	11.72±1.0	5.53±0.2	2.13±0.3	14.42±0.3	8.15±0.2	2.26±0.1	1.11±0.0	5.08±0.9	154.50±1.5
T2:8	Control:RE3	8.1	7.29±0.9	2.72±0.1	2.67±0.2	11.78±0.0	5.98±0.3	2.90±1.2	1.56±0.2	3.13±1.1	201.00±9.0
T2:8	Rapid Shift:RE2	7.6	8.21±0.4	3.41±0.2	2.41±0.0	13.10±0.4	7.96±0.4	1.71±0.0	0.84±0.0	3.27±0.2	192.50±2.5
T2:8	Stepwise Reduction:RE4	7.8	9.58±0.9	3.90±0.2	2.45±0.1	12.15±1.7	6.80±1.5	1.89±0.0	1.03±0.2	3.43±0.6	181.00±12.0
T3:16	Control:RE3	8.1	8.90±0.1	3.95±0.1	2.26±0.1	14.50±0.5	9.06±0.5	1.61±0.0	0.74±0.1	4.22±0.2	177.50±0.5
T3:16	Rapid Shift:RE2	7.6	9.82±0.6	4.57±0.4	2.15±0.0	14.89±0.6	10.00±0.2	1.79±0.1	0.65±0.0	4.37±0.4	171.50±5.5
T3:16	Stepwise Reduction:RE4	7.65	10.22±0.5	5.04±0.8	2.07±0.2	14.30±0.3	8.74±0.2	1.96±0.4	0.82±0.0	6.60±1.9	162.50±9.5
T4:24	Control:RE3	8.1	9.01±0.5	4.34±0.1	2.07±0.1	10.07±0.4	7.70±0.3	1.62±0.0	0.29±0.0	4.83±0.3	166.50±1.5
T4:24	Rapid Shift:RE2	7.6	9.29±0.1	5.16±0.4	1.81±0.1	11.78±0.8	8.89±0.5	1.64±0.1	0.46±0.1	4.29±0.2	169.50±2.5
T4:24	Stepwise Reduction:RE4	7.6	10.38±1.1	5.87±0.2	1.76±0.1	14.37±0.5	10.43±0.4	1.79±0.1	0.50±0.0	5.16±0.0	160.00±2.0

Supplementary Table 3. Inferential *t*-Test Statistical analysis on the physicochemical data sampled from the intact sediment cores. Here we classify p≤0.05 as a measure of statistical significance.

Physicochemical Analysis	Control and Stepwise Reduction Comparison (p≤0.05)	Control and Rapid Shift Comparison (p≤0.05)	Stepwise Reduction and Rapid Shift Comparison (p≤0.05)
Total CaCO₃ Content	0.991	0.399	0.228
Total Organic Carbon Content	0.844	0.394	0.204
Wet Weight	0.794	0.479	0.546
Dry Weight	0.637	0.204	0.229
Chlorophyll a	0.052	0.886	0.070
Phaeopigments	0.213	0.575	0.282
Chlorophyll a & Phaeopigments	0.678	0.177	0.575
Grain Size	0.031	0.932	0.044

	Baseline	Control					Rapid Shift					Stepwise Reduction				
Proteobacteria; Gammaproteobacteria	17.8	17.2	17.7	18.1	18.6	18.7	19.4	18.1	17.8	18.8	18.8	19.2	18.2	17.8	18	18.2
Unclassified; Unclassified	12.2	12.8	12.8	14.4	13.4	14.3	12	12.5	13.4	14.3	13.5	12.7	13.2	12.8	15.3	14.7
Bacteroidetes; Flavobacteriia	11	12	10.7	10.7	11.3	10.8	10.7	10.7	10.8	10.6	10.4	11.4	11.3	11.4	10.8	11.5
Proteobacteria; Alphaproteobacteria	7.7	9.5	8.7	8.3	9.5	9.6	10	8.8	9.4	10.2	9.9	9.2	8.3	9	10.2	9.1
Proteobacteria; Deltaproteobacteria	9.3	8.3	9.1	8.9	8.4	8	8.1	9	8.4	7.4	8.5	8.3	9	8.6	7.7	8
Cyanobacteria/Chloroplast; Chloroplast	9.2	7.9	7.7	7.6	7.1	7.5	7.6	7.9	7.7	7.3	7.7	7.4	8	7.5	7.7	7.1
Planctomycetes; Planctomycetia	6.4	6.8	6.8	5.8	6.1	6.4	7.3	7	6.7	6.5	6.5	6.3	6.3	6.2	6.2	6.5
Cyanobacteria/Chloroplast; Cyanobacteria	4.4	3.7	4.5	3.8	3.7	3.4	3.6	4.3	3.9	3.8	4.1	3.9	3.8	3.8	4	3.1
Bacteroidetes; Sphingobacteriia	4.1	3.9	3.7	4.1	3.9	3.6	3.6	3.8	3.8	3.6	3.6	3.5	4	3.9	3.3	3.8
Bacteroidetes; Unclassified	2.9	3.2	3.4	3.8	3.6	3.2	2.8	3.4	3.5	3.4	3.4	3.3	3.7	3.9	3.1	3.6
Bacteroidetes; Cytophagia	3.5	3.2	3.2	3.5	3.1	2.9	3	3.3	3	3	2.9	3.4	3.4	3.5	3	3
Verrucomicrobia; Verrucomicrobiae	2.2	2.1	2.2	1.7	1.9	2	2.4	2.3	2.2	2.1	1.6	2	2.1	1.9	2	2.1
Actinobacteria; Actinobacteria	2.1	1.8	1.8	1.5	1.5	1.9	2.1	1.6	1.6	1.4	1.6	2.1	1.7	1.7	1.4	1.5
Bacteroidetes; Bacteroidia	1.1	1	1.2	1.3	1.3	1.1	0.9	1.2	1.2	1	1	1	1.1	1.3	0.9	1.3
	T0	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4

Supplementary Figure 3. R generated heatmap depicting taxonomic abundance across each of the different experimental treatments for the Active (RNA-> cDNA) community.

	Baseline	Control					Rapid Shift					Stepwise Reduction				
Proteobacteria; Gammaproteobacteria	20.2	22	21.1	22.3	21.1	23.3	22	21.4	19	21.6	22.6	20.7	21.5	20.9	22.1	23.3
Unclassified; Unclassified	9.1	10.9	10	11.3	10.1	11.2	9.9	9.8	11.8	11	10.7	10.4	10.3	11	10.8	11.9
Proteobacteria; Deltaproteobacteria	10.4	9	11.8	9.7	10.9	9.6	9.8	10.7	10.2	9.9	10.7	10	10.8	10.2	9.6	9.4
Proteobacteria; Alphaproteobacteria	10	9.7	9.4	8.6	9.8	9.1	10.1	9.7	8.5	9.7	9.1	10.2	9.2	8.3	10.4	8.8
Bacteroidetes; Flavobacteriia	9.9	8.3	8.9	7.5	9.7	7.6	8.8	8.4	8.7	9.5	8.1	8.6	8.9	9.1	8.7	7.7
Cyanobacteria/Chloroplast; Chloroplast	7.9	8	8.2	8.2	7.9	8	7.4	8.5	8.4	8.7	9.3	8.6	8.2	8	9.3	7.8
Cyanobacteria/Chloroplast; Cyanobacteria	6.6	6.2	5.6	5.7	5	4.9	5.8	6.6	5.6	5.3	5.8	6	6	5.9	5.3	5.5
Bacteroidetes; Unclassified	4.4	4.3	4.5	4.6	4.1	3.7	4	4.5	4.3	4.2	3.5	4.2	5	4.5	3.7	4.1
Planctomycetes; Planctomycetia	3.6	3.6	3.5	3.6	4.7	3.5	4	3.5	3.7	3.3	3.8	3.9	3.1	3.7	3.3	3.2
Actinobacteria; Actinobacteria	2.7	3.4	2.8	3.9	2.8	3.7	3.1	3.1	4.5	2.6	2.5	2.8	2.9	3.7	2.4	3.2
Bacteroidetes; Cytophagia	2.6	2.1	2.1	2	1.9	1.8	1.8	1.9	2	1.9	1.8	1.9	2.2	2.2	1.8	2
Verrucomicrobia; Verrucomicrobiae	2.3	2.2	1.3	1.7	1.3	1.9	2.4	1.7	1.7	1.5	1.3	2.2	1.4	1.7	1.9	1.7
Proteobacteria; Unclassified	1.3	1.6	1.5	1.7	1.8	2	1.5	1.5	1.7	2.1	1.7	1.5	1.7	1.4	1.9	2.3
Bacteroidetes; Sphingobacteriia	2.2	1.7	1.7	1.8	1.5	1.5	2	1.8	1.8	1.5	1.5	1.9	1.9	1.6	1.5	1.5
	T0	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4

Supplementary Figure 4. R generated heatmap depicting taxonomic abundance across each of the different experimental treatments for the Total [DNA] community.

Chapter 2: Experiment 1

Supplementary Data: Beta Diversity Metrics

PhILR Beta Diversity Analysis (Active [RNA]; Total [DNA])

Supplementary Table 4a. : Active Community (Over 34 Days)

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.003*	0.1094
pH	0.166	0.04948
Time	0.001*	0.5532

Supplementary Table 4b. : Total Community (Over 34 Days)

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.006	0.09645
pH	0.353	0.0166
Time	0.001*	0.5671

****

Supplementary Table 5a. : Active Community T0

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.85	-0.1458
Label	0.802	-0.1458

Supplementary Table 5b. : Active Community T1

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.699	-0.07639
pH	0.687	-0.07639
Label	0.706	-0.07639

Supplementary Table 5c. : Active Community T2

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.591	-0.0463
pH	0.687	-0.07639
Label	0.594	-0.0463

Chapter 2: Experiment 1

Supplementary Table 5d. : Active Community T3

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.056	0.2338
pH	0.056	0.2338
Label	0.043*	0.2338

Supplementary Table 5e. : Active Community T4

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.021*	0.3171
pH	0.008*	0.4982
Label	0.021*	0.3171

****

Supplementary Table 6a. : Total Community T0

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.934	-0.1481
Label	0.934	-0.1481

Supplementary Table 6b. : Total Community T1

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.878	-0.1528
pH	0.875	-0.1528
Label	0.876	-0.1528

Supplementary Table 6c. : Total Community T2

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.659	-0.04398
pH	0.625	-0.04398
Label	0.63	-0.04398

Supplementary Table 6d. : Total Community T3

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.463	-0
pH	0.478	-0
Label	0.494	-0

Chapter 2: Experiment 1

Supplementary Table 6e. : Total Community T4

Comparisons	ANOSIM p value (<0.05) Significance	R Statistic
Treatment	0.022*	0.3287
pH	0.915	-0.1875
Label	0.023*	0.3287

Supplementary Table 7. Demonstrates OTU's that were shown to change significantly in terms of their relative abundance during the length of the study (across time), within the Total Community [DNA] dataset for the different experimental pH treatments. OTU's with a superscript([1]) indicate taxa that are unique only to that treatment. OTU's listed with a superscript(*) are simultaneously co-occurring within the Control Treatment.

Treatment	OTU Number	Taxonomic Level (Greatest Depth Assignment)	Taxonomic Classification
CONTROL	OTU750	*Phylum*	*Proteobacteria*
	OTU882	*Genus*	*Planctomicrobium*
	OTU339	*Genus*	*Gimesia*
	OTU181	*Order*	*Rhodobacterales*
	OTU184	*Order*	*Alteromonadales*
	OTU145	*Family*	*Saprospinaceae*
STEPWISE REDUCTION	OTU450[1]	*Genus[1]*	*Dasania[1]*
	OTU1374[1]	*Phylum[1]*	*Bacteroidetes[1]*
	OTU284[1]	*Phylum[1]*	*Firmicutes[1]*
	OTU339*	*Genus**	*Gimesia**
	OTU181*	*Order**	*Rhodobacterales**
RAPID SHIFT	OTU361[1]	*Genus[1]*	*Porphyrobacter[1]*
	OTU429[1]	*Genus[1]*	*Gimesia[1]*
	OTU524[1]	*Genus[1]*	*Nitrosomonas[1]*
	OTU59[1]	*Genus[1]*	*Aestuariibacter[1]*
	OTU45[1]	*Order[1]*	*Family VIII[1]*
	OTU339*	*Genus**	*Gimesia**
	OTU181*	*Order**	*Rhodobacterales**
	OTU184*	*Order**	*Alteromonadales**

Supplementary Table 8. Demonstrates OTU's that were shown to change significantly in terms of their relative abundance during the length of the study (across time), within the Active Community [RNA] dataset for the different experimental pH treatments. OTU's with a superscript([1]) indicate taxa that are unique only to that treatment. OTU's listed with a superscript(*) are simultaneously co-occurring within the Control Treatment.

Treatment	OTU Number	Taxonomic Level (Greatest Depth Assignment)	Taxonomic Classification
CONTROL	OTU481	Order	Subdivision 3 Genera Incertae Sedis
	OTU1466	Phylum	Candidate Division WPS-2
	OTU524	Genus	Nitrosomonas
	OTU362	Order	Family XIII
	OTU181	Order	Rhodobacterales
	OTU248	Genus	Nitrosomonas
	OTU178	Genus	Gimesia
	OTU45	Order	Family VIII
STEPWISE REDUCTION	OTU1193[1]	Order[1]	Desulfobacterales[1]
	OTU99[1]	Order[1]	Oceanospirillales[1]
	OTU450[1]	Genus[1]	Dasania[1]
	OTU152	Phylum	Proteobacteria
	OTU182	Phylum	Planctomycetes
	OTU524*	Genus*	Nitrosomonas*
	OTU181*	Order*	Rhodobacterales*
	OTU481*	Order*	Subdivision 3 Genera Incertae Sedis*
RAPID SHIFT	OTU361[1]	Genus[1]	Porphyrobacter[1]
	OTU429[1]	Genus[1]	Gimesia[1]
	OTU59[1]	Genus[1]	Aestuariibacter[1]
	OTU160[1]	Genus[1]	Fluviicola[1]
	OTU152	Phylum	Proteobacteria
	OTU182	Phylum	Planctomycetes
	OTU248*	Genus*	Nitrosomonas*
	OTU362*	Order*	Family XIII*
	OTU524*	Genus*	Nitrosomonas*
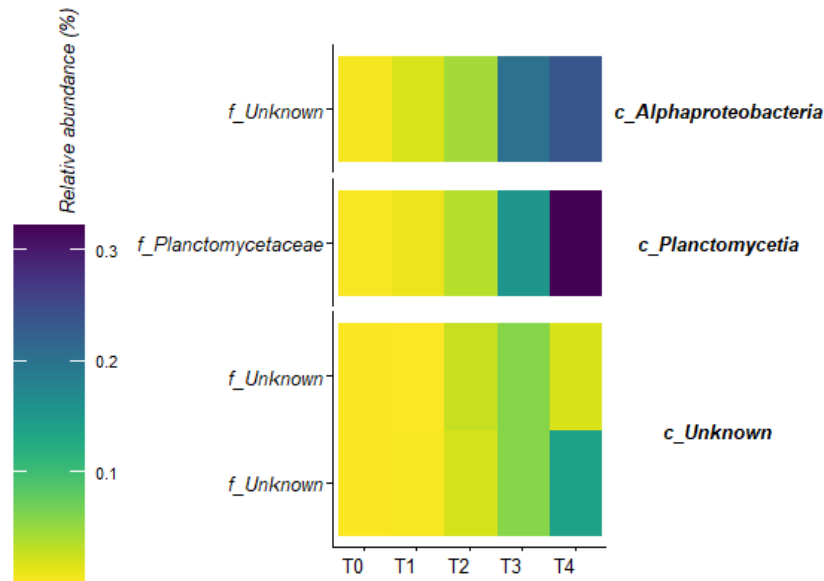
Supplementary Figure 5. Heatmap of the rare, responding sensitive taxa previously identified using the ANCOM statistical analysis for the Active [RNA] Community Control (C) mapped back against the total analysed OTU's (1,577 OTU's) to display the relative abundance (%) of the responding taxa within the whole microbial community, and how the abundances change relative to time (T0-T4).
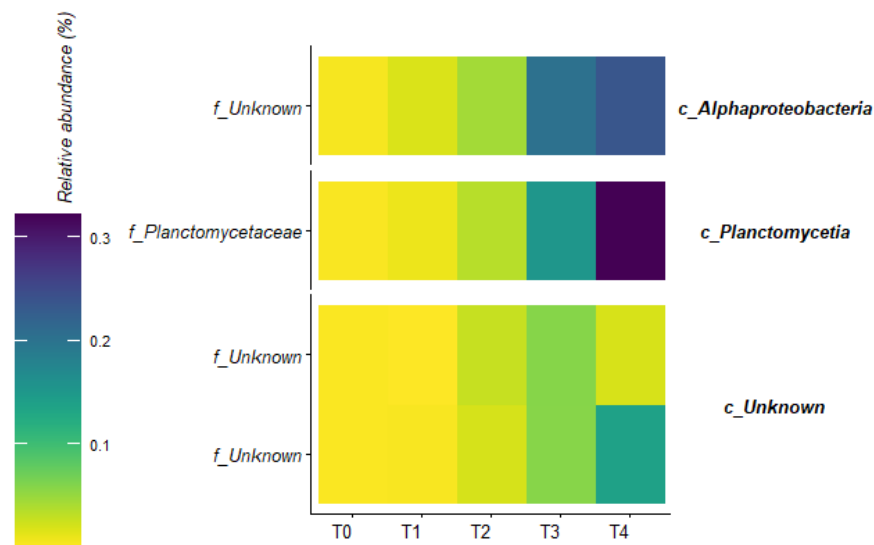
Supplementary Figure 6. Heatmap of the rare, responding sensitive taxa previously identified using the ANCOM statistical analysis for the Total [DNA] Community Control (C) mapped back against the total analysed OTU's (1,577 OTU's) to display the relative abundance (%) of the responding taxa within the whole microbial community, and how the abundances change relative to time (T0-T4).

Supplementary Table 9. ANCOM generated OTU's detected as responding significantly in terms of relative abundance within each sampling time point between the different pH treatments for both Active and Total Communities. Taxa are classified down to the Genus Level based on an 80% confidence cut-off value. No species could be identified at that confidence threshold. OTU's in green are from the Active [RNA] community dataset. OTU's in blue are from the Total [DNA] community dataset. Taxa highlighted in yellow denote OTU's of interest.

Time point	OTU Number	Kingdom	Phylum	Class	Order	Family	Genus
T0	OTU701	*Bacteria*	*Proteobacteria*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
	OTU369	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Alteromonadales*	*Alteromonadaceae*	*Alteromonas*
	OTU586	*Bacteria*	*Bacteroidetes*	*Sphingobacteriia*	*Sphingobacteriales*	*Rhodothermaceae*	*Rubricoccus*
	OTU522	*Archaea*	*Crenarchaeota*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
	OTU665	*Bacteria*	*Acidobacteria*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
	OTU586	*Bacteria*	*Bacteroidetes*	*Sphingobacteriia*	*Sphingobacteriales*	*Rhodothermaceae*	*Rubricoccus*
	OTU522	*Archaea*	*Crenarchaeota*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
T2	OTU240	*Bacteria*	*Proteobacteria*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
	OTU111	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Alteromonadales*	*Pseudoalteromonadaceae*	*Algicola*
	OTU1229	*Bacteria*	*Proteobacteria*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
	OTU39	*Bacteria*	*Proteobacteria*	*Unknown*	*Unknown*	*Unknown*	*Unknown*
T3	OTU1002	*Bacteria*	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Prolixibacteraceae*	*Mangrovibacterium*
	OTU99	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Oceanospirillales*	*Unknown*	*Unknown*
T4	OTU450	*Bacteria*	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadales incertae sedis*	*Dasania*
	OTU361	*Bacteria*	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Erythrobacteraceae*	*Porphyrobacter*
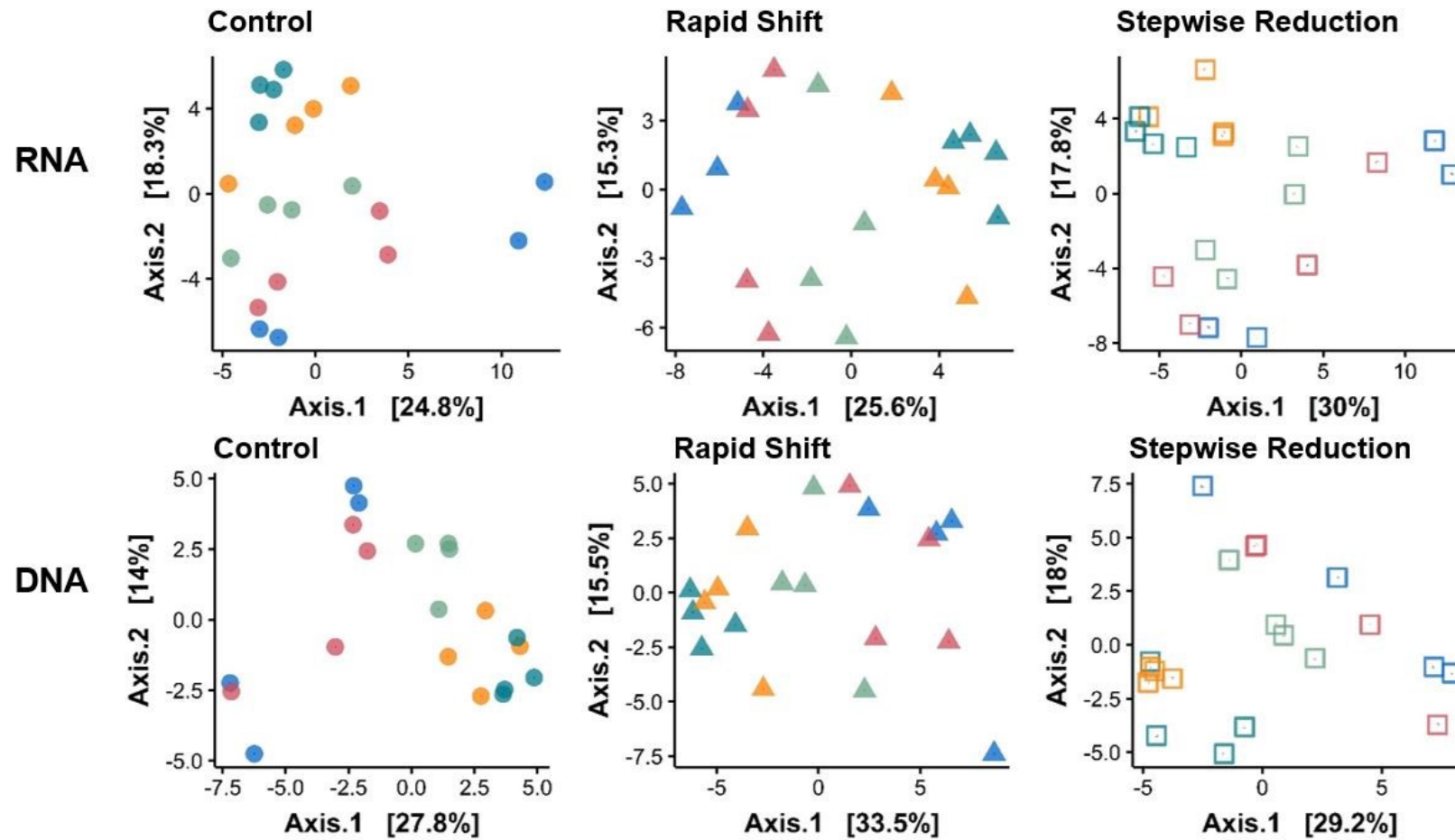
Supplementary Figure 7. Shows the PhILR Beta Diversity Metric Analyses for both the Active [RNA] (Top) and Total [DNA] (Bottom) communities for each of the experimental pH treatments (Baseline: Open Circle; Control: Closed Circle; Rapid Shift (RaS): Triangle; Stepwise Reduction (StR): Open Square). A: All Active Community Samples across time (T0-T4: 34 Days) between treatments (B; C; RaS; StR). B. Active Community: Control. C. Active Community: RaS. D. Active Community: StR. A: All Total Community Samples across time (T0-T4: 34 Days) between treatments (B; C; RaS; StR). B. Total Community: Control. C. Total Community: RaS. D. Total Community: StR.
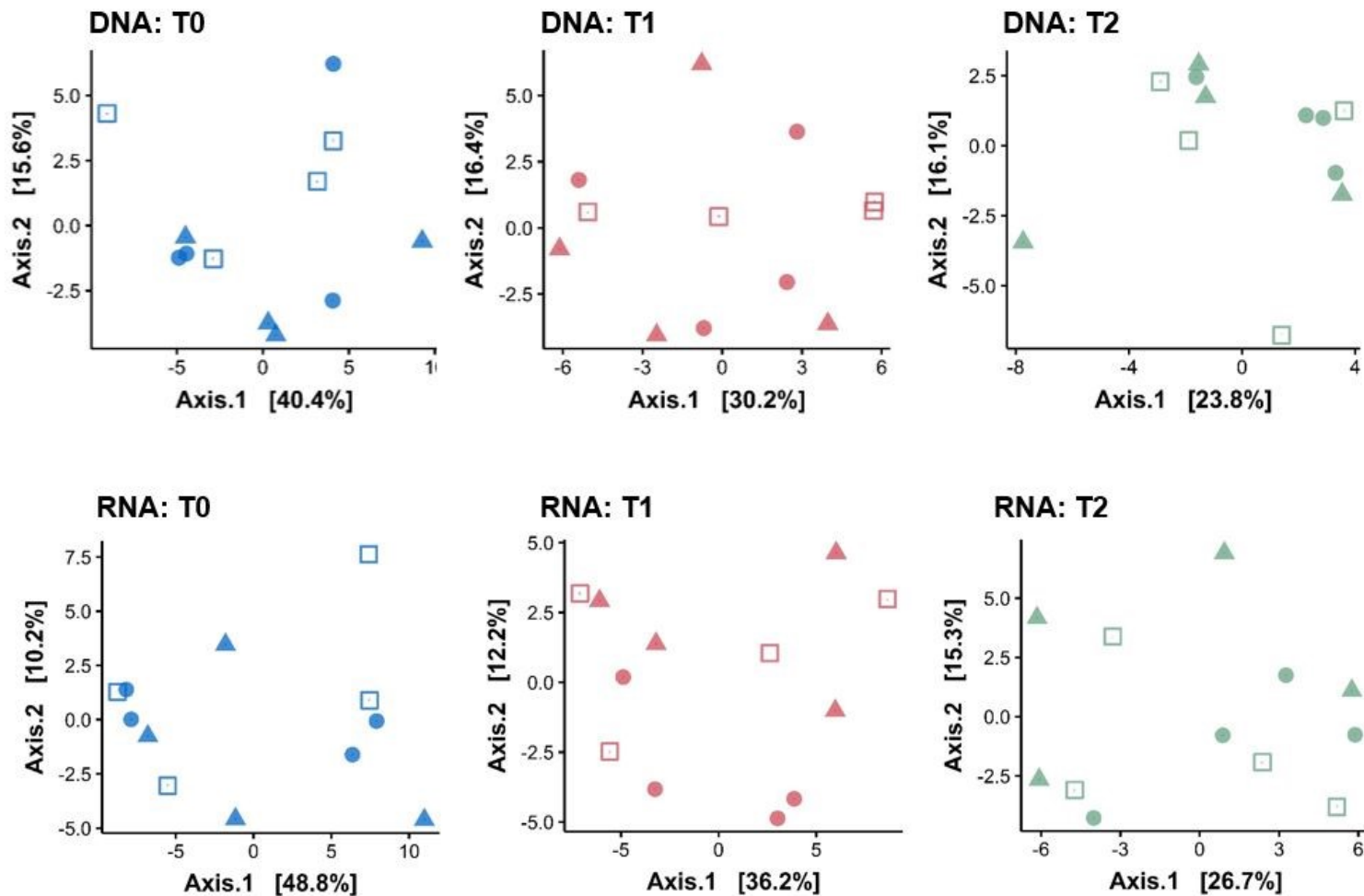
Supplementary Figure 8. Shows the Total [DNA] (Top) and Active [RNA] (Bottom) Community ordinations in terms of PhILR Beta Diversity Metric Analyses for each of the different experimental pH treatments (Baseline: Open Circle; Control: Closed Circle; Rapid Shift (RaS): Triangle; Stepwise Reduction (StR): Open Square) for the first (T0: 0 Days) second (T2: 4 Days) and Third (T3: 8 Days) sampling timepoints.
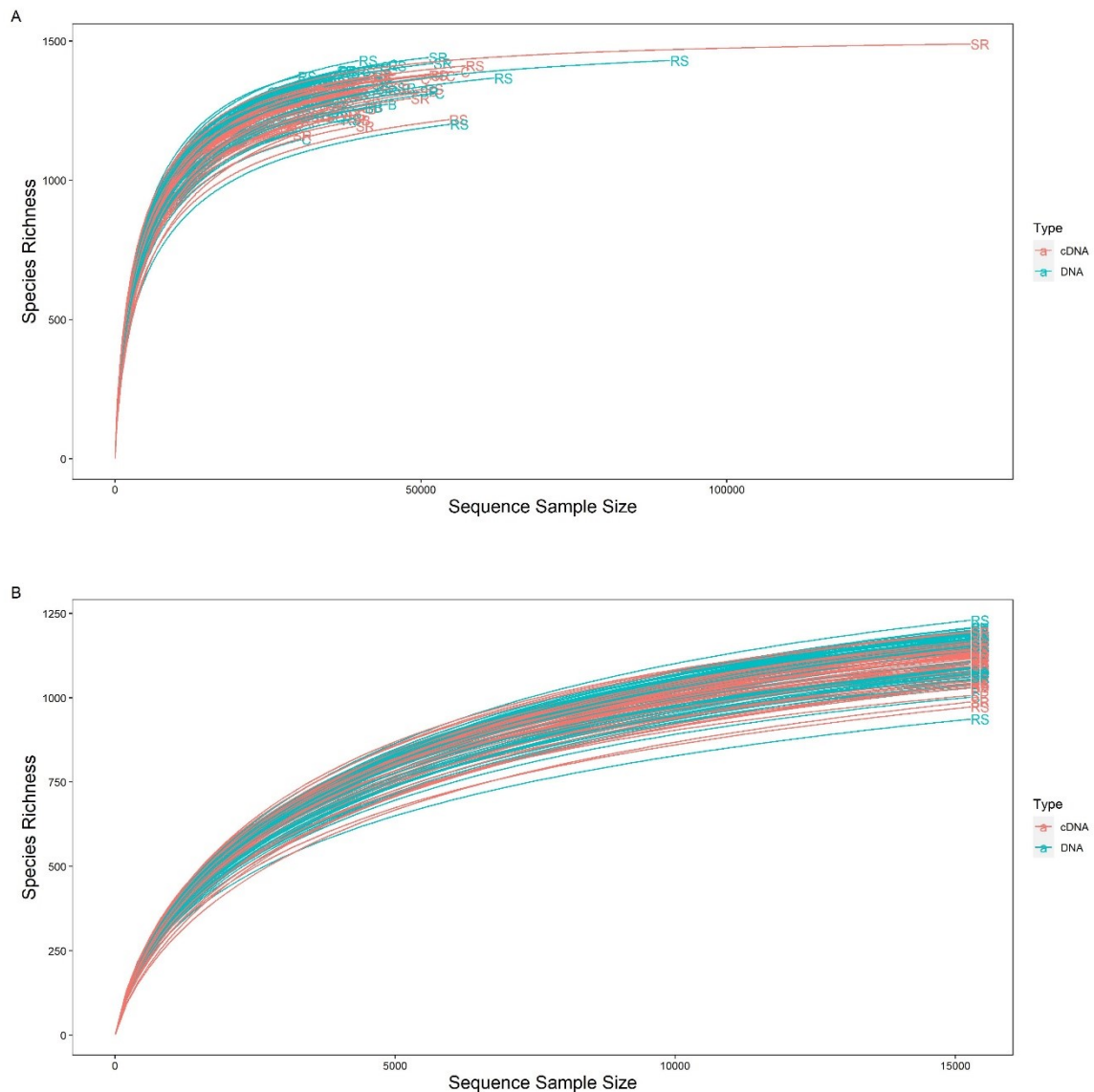
Supplementary Figure 9a. A. A rarefaction curve generated using the ggplot2 package in R to visualize that samples have been sequenced to full completion or are well represented in terms of the number of unique sequence variants of varying diversity and initial size. This QC step is used to verify how well each sample represents the total diversity by calculating species richness per each sample (N=65) across both nucleic acid types (cDNA (Red) and DNA (Blue)). B. Is an example of the rarefied library sampled without replacement (draws from a subset from the observed sequences)).
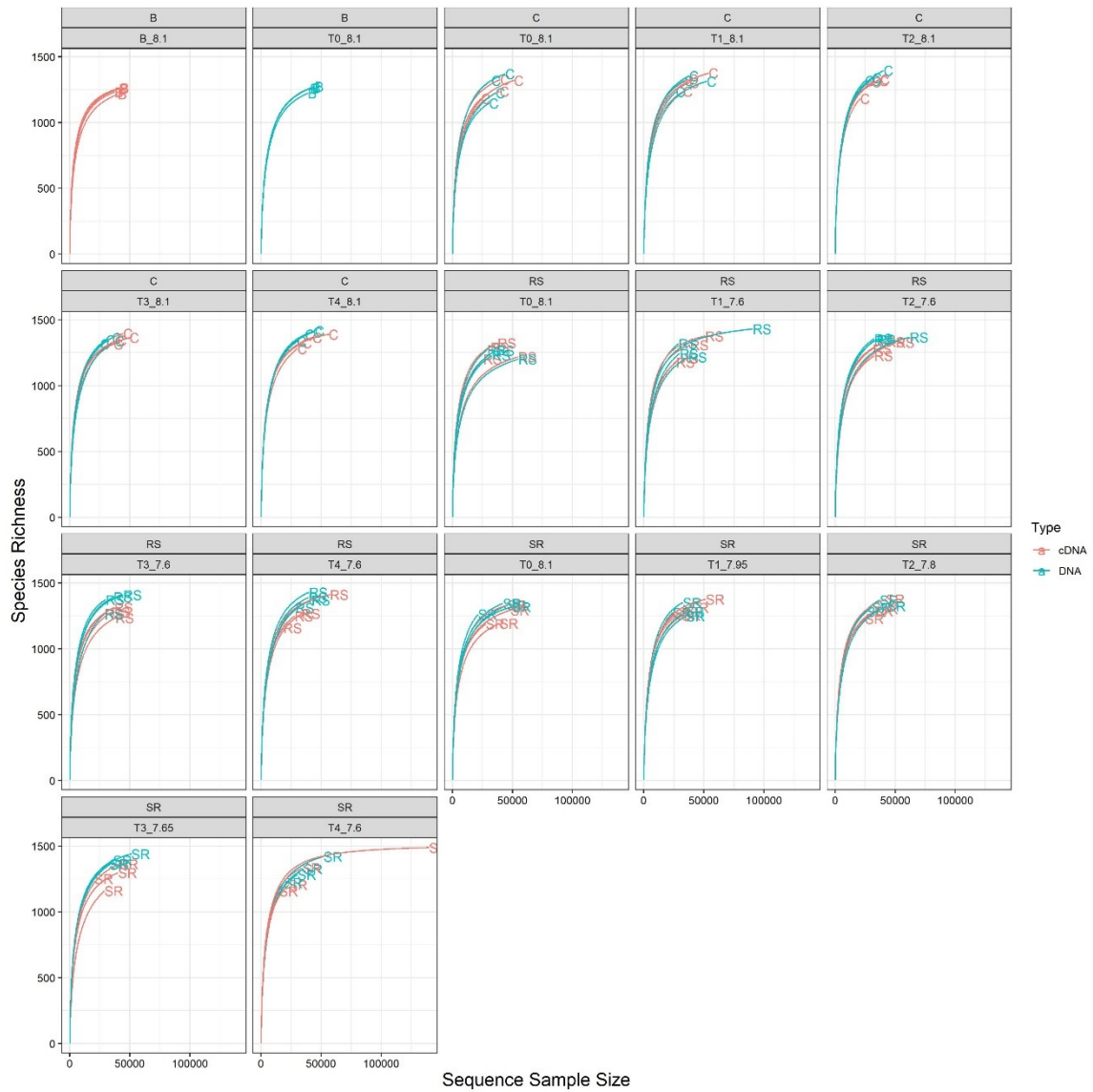
Supplementary Figure 9b. Expands upon supplementary figure 9a. A. showing a breakdown of each non rarefied sample separated by pH treatment for both cDNA and DNA.
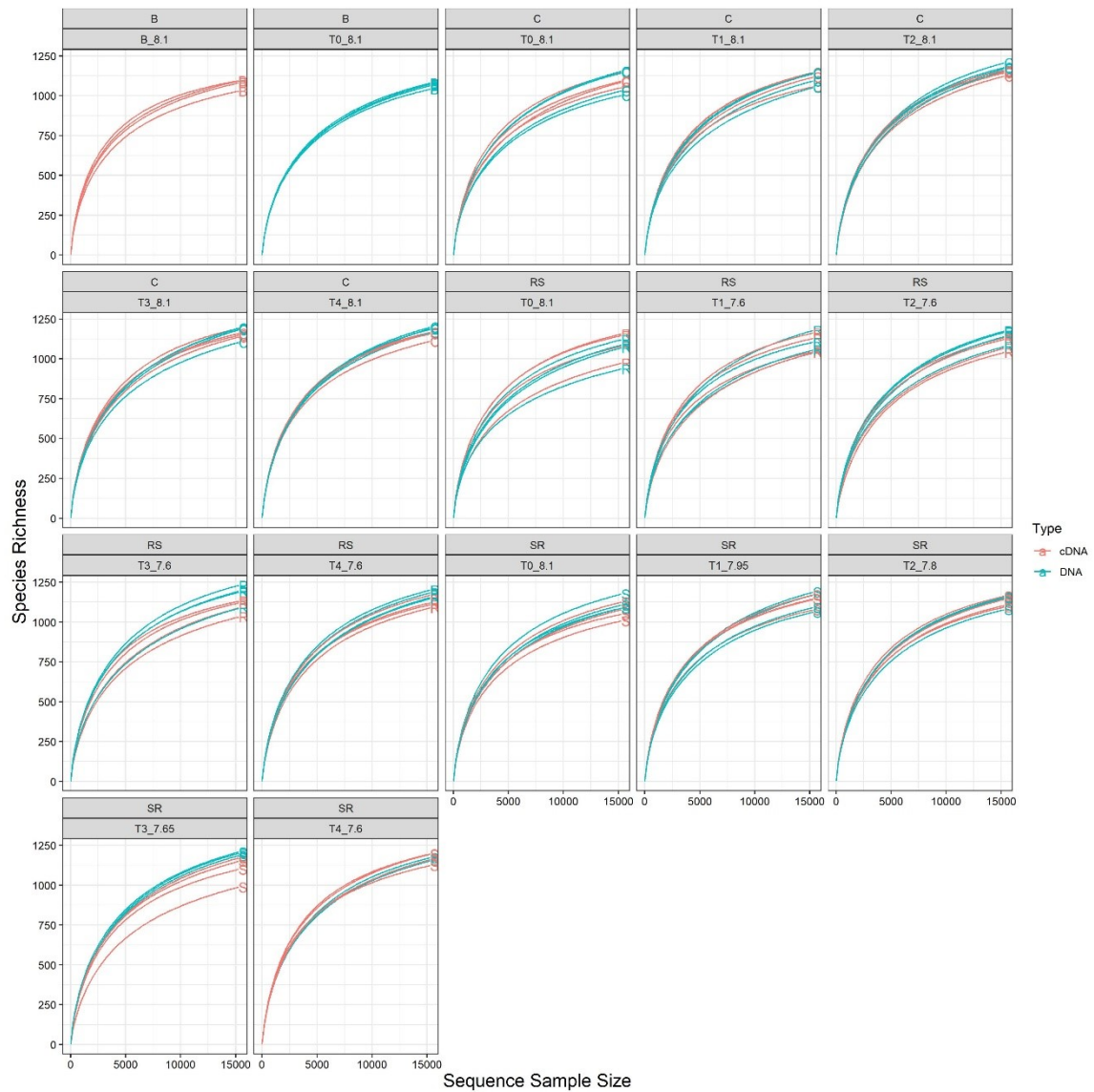
Supplementary Figure 9c. Demonstrates the same "by treatment" separation as shown in supplementary figure 9b. but for the Rarefied library for both cDNA and DNA types.

Chapter 2: Experiment 1

Chapter 3: The Effects of Ocean Acidification on Benthic Subtidal Microbial Communities from the Hauraki Gulf, New Zealand.

Shelly Brandt[1], Alexis Marshall[1], Kay Vopel[2], Bonnie Laverock[2], Conrad Pilditch[1], Adam Hartland[1], Charles Lee[1], and Craig Cary[1]

[1]The University of Waikato, Thermophile Research Unit
[2]The Auckland University of Technology

Abstract

Measuring the effects of Ocean Acidification (OA) on benthic coastal marine habitats remains a challenging scientific undertaking as these systems are intrinsically complex and inherently variable across different spatial and temporal scales. Increased coastal monitoring indicates that the impact of OA on these environments is not likely to be uniform. This heterogeneity creates complexity when predicting ecosystem-wide or organismal level response to changes in the oceanic climate. Subtidal zones act as the intermediary between the intertidal and open ocean ecosystem. These zones express greater dynamic potential reflected in their physical and chemical processes than open ocean environments, but with notably less activity than a standard intertidal system. Previous studies theorize that microbial communities are physiologically forged by the environments from which they originate. Therefore, microorganisms from variable and active systems are likely to demonstrate greater biological tolerance to change than those endemic to a comparatively less dynamic region. However, this microbial "tolerance" may be a result of the bacteria modulating to natural turnover, in anticipation of the system cycling back to normal conditions. In extension, the community is preset to adjust to predictable environmental change despite its apparent dynamic quality. Here we postulate that microbial communities from subtidal benthic sediments will exhibit high levels of compositional plasticity and resistance to the effects of increased pCO_2 and low pH exposure, though markedly less than communities from intertidal sediments (Chapter 2.). Community-wide analysis of bacterial communities from nearshore subtidal sediments from Man O' War Bay, Waiheke Island held under 4 different pH treatments (8.1 (Control), 7.9, 7.7, 7.5) showed that the subtidal community remained structurally and compositionally (16S rRNA gene) unchanged to the impact of any lowering of pH. Subtle differences were detected in the alpha diversity matrices, which displayed a notable

decrease in species richness and relative abundances over time and shown to correlate strongly with a reduction in pH. Beta Diversity analyses demonstrated significant differences between pH treatments after 21 days *in the mesocosm*. Compositional analyses (ANCOM) at the OTU level detected a small subset of low tolerant bacteria (*Alteromonadaceae, Oceanospirillales*) from the class *Gammaproteobacteria* that were identified as re-occurring taxa between this and the previous manipulation study (Chapter 2.). Here we demonstrate the general robustness of subtidal sediment microbial communities to different acidified seawater conditions based on IPCC projections for 2100, while simultaneously reinforcing the measured sensitivity of specific sediment bacterial individuals to low pH regimes.

Introduction

Ocean Acidification

Human activity remains the predominant driver behind the accelerated rate of Ocean Acidification (OA), where anthropogenically-induced stress on the marine environment acts as a catalyst for near-shore ecological degradation (Doney & Schimel, 2007; Lüthi et al., 2008). Thirty-six billion metric tons of carbon dioxide (CO_2) are released into the atmosphere per annum, where an estimated one-third of that is absorbed directly into the surface of the ocean (P. E. Land et al., 2015). The ocean, as a natural carbon sink, uptakes atmospheric CO_2 resulting in a decrease in seawater pH, with a subsequent reduction in carbonate ion concentration (Feely et al., 2004; Orr et al., 2005; Sabine et al., 2004). Global seawater pH has experienced a 0.1 decrease since the start of the pre-industrial era from 8.2 to 8.1, with a further predicted 0.3-0.4 pH reduction by the year 2100 (Brewer, 1997; Haugan & Drange, 1996; Stocker et al., 2013). Reducing the buffering efficiency of the oceans would likely have deleterious effects on marine ecosystem processes (Land et al., 2015; Sabine et al., 2004). In open-ocean systems, various taxa have been identified as increasingly vulnerable to the effects of OA, such as coccolithophores, pelagic mollusks, and foraminifera (Riebesell & Zondervan, 2000; Zondervan et al., 2001). In coastal environments, the most urgent concern is the effect of OA on calcifying organisms such as corals, bivalves, and calcifying algae (Doney et al., 2009; Gattuso et al., 1998; Hoegh-Guldberg et al., 2007). The effects of OA on seawater chemistry are well documented; however, there is a distinct gap in knowledge when it comes to understanding the impacts of OA on coastal benthic ecosystems and their co-associated organisms, particularly at the microbial level (Gattuso et al., 2013).

Marine microbial communities are at the heart of the oceanic ecosystem providing an essential service for the coastal environment through the transformational recycling of nutrients needed to drive the collective global marine biosphere (Säwström et al., 2016; Whitman et al., 1998). These complex microbiologically driven biogeochemical processes are responsible for converting organic material within shallow or near-shore coastal zones and supports ecosystem primary productivity (Horner-Devine et al., 2004; Lozupone & Knight, 2007; Säwström et al., 2016; Torsvik et al., 2002). An estimated 80% of the nitrogen required for performing crucial photosynthetic processes is derived from the physical regeneration of organic nutrients by the diverse and abundant microbial communities living within the

sediment (Dale & Prego, 2002). Estimations of marine subsurface habitats demonstrate that these systems harbor the majority of all global prokaryotic biomass, with a density of approximately 3.6×10^{19} cells per volume/cm^3 within the top 0-10 cm of coastal marine sediments (Whitman et al., 1998; Wilms et al., 2006).

Coastal systems are characterized by variability in carbonate chemistry and large oscillatory pH patterns, which can span from 0.3-0.5 pH units within a diel, or diurnal cycle, that are often equivalent to or greater than the predicted end-of-the-century global ocean pH values (~0.3) (Aufdenkampe et al., 2011; Caldeira & Wickett, 2005; Duarte et al., 2013; Hendriks et al., 2015; Hofmann et al., 2011; Mercado & Gordillo, 2011). Additionally, ecosystem metabolism affects coastal seawater pH through the removal of CO_2 via primary production (during the day) in a net autotrophic ecosystem and the release of CO_2 (at night) through respiration (i.e., metabolic signal) (Duarte, Hendriks, et al., 2013). The amplitude of this impact is determined by both residence time and by autotrophic productivity (Hendriks et al., 2015). Based on the extreme multi-scale dynamics of coastal ecosystem seawater pH, it is likely that organisms develop mechanisms that allow for the maintenance of physiological homeostatic conditions that are driven by selective evolutionary processes (Philippot et al., 2021). Further emphasizing the complex variability of pH in the coastal marine ecosystem, demonstrating that these systems do not follow the same pH trends observed in a more stable open-ocean system (Duarte, Hendriks, et al., 2013).

Across the coastal marine ecosystem gradient, the intertidal regions have been shown to have the highest levels of primary productivity, remineralization, and sedimentation rates compared to both subtidal and open-ocean systems; where beyond the tidal zone, steep chemical gradients can be observed with subsequently shallow subsurface sediment oxygen penetration depths (OPD) (Hondt et al., 2004; Parkes et al., 2005; Poremba, Tillmann, & Hesse, 1999; Dittman, 1999; Rasmussen & Jorgensen, 1992). The subtidal region (i.e., sublittoral zone) operates as the intermediary between the intertidal zone and the open ocean. Where subtidal ecosystems, unlike their intertidal counterparts, are exempt from near-shore tidal cycling, where they remain permanently covered by water, maintaining near-constant temperature, water pressure, and UV exposure (Blanchet et al., 2008; Ysebaert et al., 2003; Ysebaert & Herman, 2002). The subtidal zone also exemplifies differences in geomorphology, sediment stratification, substrate composition, porosity, and benthic

macrofaunal communities compared to intertidal or open ocean environments, which top-down influence biogeochemical processes and benthic microbial community structuring (Schiel, 1988). Sediment type is also a strong determining factor for microbial diversity and composition due to the distinct differences sediment biogeochemical processes (Anderson, Ford, Feary, & Honeywill, 2004). Recent studies have demonstrated apparent differences between various intertidal versus subtidal marine taxa across different trophic levels, indicating ecological and environmental specificity (Mandic, Ramon, Gracey, & Richards, 2014; McArley, Hickey, Wallace, Kunzmann, & Herbert, 2019; Sandoval-Gil et al., 2015).

The benthic sediments are intrinsically heterogeneous across large spatial scales (i.e., cm to km), where variation is influenced by differences in biotic and abiotic factors such as salinity, temperature, organic matter concentration, sediment composition, and pH, all of which govern changes in microbial community composition and structure (Köster, Dahlke, & Meyer-Reil, 2005; Lozupone & Knight, 2007; Wilde & Plante, 2002; Zinger et al., 2011). This heterogeneity can also be expressed on smaller spatial scales (i.e., mm to cm), where microbial composition is more likely to be governed by oxygen penetration depth (OPD) within the sediment and the bioavailability of nutrients and organic matter (Köpke et al., 2005; Köster, Wardenga, & Blume, 2008). Additional factors such as the physical manipulation and or mixing of the sediment by indigenous benthic macrofauna (i.e., bioturbation) can also have a pronounced effect on benthic microbial community dynamics (Papaspyrou et al., 2005; Papaspyrou et al., 2006). Spatial heterogeneity or *patchiness* also translates to vertical sediment stratification caused by the formation of oxic microniches, extending to depths of up to 20 cm along the inside of burrows formed by bioturbating zoobenthos (Reise, 2002). The resulting stratificational layering within the sediment at depth is therefore governed by anaerobic microbial processes operating along redox gradients (Reise, 2002).

In our previous study (Chapter 2.), we demonstrated the inherent structural and compositional resistance of microbial communities from intertidal benthic sediments, where the central community remained effectively resistant to high CO_2 exposure. We postulated that microbial communities from dynamic and variable intertidal systems would exhibit community wide compositional plasticity and robustness in the face of environmental change, given their complex origin. We determined that only <0.6% of the microbial community significantly responded to sustained low pH. Of the intolerant responsive community

members identified, several were shown to belong to important clades of *Gammaproteobacteria* (*Alteromonadaceae, Oceanospirillales*), which are known to serve a specific and crucial role within their respective ecological microbiomes (Jaekel et al., 2015; McCarren et al., 2010; Obi et al., 2016). This could suggest that from a microbial-biogeochemical perspective, the sedimentary ecosystem and its resident bacterial consortia may still be sensitive to decreasing pH than previously predicted. In this same study we also identified that physically disturbing (i.e., sieving, homogenizing, restructuring) the sediment had a greater impact on microbial community composition and structure than lowering pH to IPCC 2100 predicted levels.

Building on Chapter 2, in this study, we treated the sediment microbial community from a low carbonate subtidal (intertidal and open ocean intermediary) oceanic zone to 4 IPCC (Intergovernmental Panel on Climate Change) prediction scenarios for water column pH (Stocker et al., 2013). The experiment was maintained in the dark to eliminate any direct impact from dominating photosynthetic activity. We hypothesized that microbial communities from an undisturbed subtidal system with inherently low levels of carbonate will have lower sediment alkalinity and thus exhibit lower buffering capacity to increased [H+] and that the resident microbial consortia will also demonstrate significantly less compositional and structural tolerance to low pH exposure when compared to more dynamic intertidal systems. The use of a controlled complex mesocosm approach provides an ideal opportunity to decouple environmental sediment buffering capacity from microbial community resistance to decreasing and sustained low pH.

Chapter 3: Experiment 2

Methods and Materials

Field Sampling and Site Selection

Sediment cores were SCUBA diver collected at a depth of 18 m using a modified universal gravity core system (core barrel dimensions: 30 cm x 10 cm) from Man O' War Bay, Waiheke Island, located in the Hauraki Gulf, on the North Island of New Zealand (S 36° 47' 38", E 175° 10' 14") (**Figure 1**.). This subtidal region is comprised of well-characterized fine-grained silty sediment, with naturally low levels of infaunation, carbon, and calcium carbonate ratios (Vopel et al., 2018; Vopel, Laverock, Cary, & Pilditch, 2021). A total of 48 *intact* sediment cores were collected over a 2-day sampling period in early austral summer and immediately transferred to the Auckland University of Technology (AUT) Marine Research Facility.

Sediment cores were randomly allocated across four separate mesocosm chambers and left to acclimatize for a period of one week (T_{-7} to T_0) at a universal system pH of 8.10 based on the regional pH metrics as well as the current global IPCC pH standard. Each mesocosm chamber was filled with synthetic seawater (Artificial Sea Water (ASW)) using the chemical formula for Instant Ocean Aquarium Salt based on the Manufacturer's prescribed instructions. The water was then processed using reverse osmosis filtration and ion exchange before being continuously circulated from the overhead mixing barrels and jet distributed into the mesocosm chambers below. Cores that were found to be heavily dominated by bioturbating macrofaunal organisms (i.e., *Echinocardium*) were discontinued from the experiment where they were sacrificed and sieved to enumerate the presenting macrofauna and then subsequently replaced with additional sediment core samples, with decidedly fewer burrowing taxa. The pH was monitored and regulated using CO_2 gas exchange aeration where the influx of CO_2 was determined by a computer-operated software program that controlled the solenoid valve on the CO_2 canisters methodically opened or closed the valve to reach or maintain a desired system pH (Vopel et al., 2018; Vopel, Laverock, et al., 2021). Following the 7-day acclimatization period, the pH within each of the four mesocosm chambers was incrementally reduced by -0.03 pH units per day B_7 to T_0) to achieve the following mesocosm treatment pH values Chamber One (RE1 Control: 8.10 pH); Chamber Two (RE2: 7.70 pH); Chamber Three (RE3: 7.90 pH); and Chamber Four (RE4: 7.50 pH) (**Figure 2**.) (**Table 1.**). Once the final treatment pH value was achieved, the pH within that chamber was then stabilized and remained at that constant value for the remainder of the study, which was completed

over a 35-day period. Temperature, pH, water column nutrients, Dissolved Inorganic Carbon (DIC), and Total Alkalinity (TA) measurements were collected once a week for the duration of the experiment for the mesocosm tank seawater. Salinity and conductivity measurements were also made daily from the water column which allowed for micro-adjustments to be made to account for the effects of evaporation. For molecular analysis, sediments samples were collected at three different sampling timepoints from the sediment surface at the sediment water interface at depths of 1 cm. The molecular sampling scheme is described as follows: At day(s) T_0 (Baseline) replicate sediment cores (n=2 per treatment) were sampled. At $T_{15,}$ replicate sediment cores (n=3 per treatment) were sampled. At $T_{30,}$ replicate sediment cores (n=5 per treatment) were sampled for a total of 40 sediment core samples for microbial analysis (**Figure 3. and Supp. Table 1.**).

Chemistry Analyses and System Monitoring

The Mesocosm system set-up is as described in (Vopel et al., 2018). In brief, 560 L of synthetic seawater (Instant Ocean® Sea Salt: Instant Ocean Spectrum Brands, VA, USA, based on manufactures instructions: salinity 35, pH 8.2–8.4, alkalinity 4.4–4.5 mEq L^{-1}, calcium 0.455–0.475 g L^{-1}, magnesium 1.36–1.42 g L^{-1}, potassium 0.39–0.41 g L^{-1}) were continuously circulated throughout each of the flow-through chambers at a controlled 18°C. The experiment was maintained in the dark for the length of the study period to control for dominating photosynthetic microbial taxa. Seawater carbonate chemistry was determined by sampling seawater samples (1-L) from each of the flow-through chambers every 4-6 days throughout the experiment to analyze dissolved inorganic carbon (DIC)(coulometry) and total alkalinity (TA) (potentiometric titration) as described by the SOP's 2 and 3a procedures by (Dickson et al., 2007). The $CO_2Sys.BAS$ program was used to compute seawater pCO_2 and pH (seawater scale: mol kg-SW^{-1}) (Lewis and Wallace, 1998). The injection of CO_{2-} enriched air (5% CO_2, 21% O_2 in N_2) into each chamber was controlled automatically using the CapCtr Software Program (Loligo Systems Aps), which activated a solenoid valve to moderate the input CO_2 gas. A SenTix HWD electrode attached to a 3310pH meter (WTW) was used to continuously monitor seawater pH within the chamber and relay the data to the laptop running the software program to regulate the valve and subsequent gas induction.

Sediment Sampling and Nucleic Acid Preservation

Chapter 3: Experiment 2

Below the sediment water interface, the top <1 mm oxygenated surficial layer of the sediment within each core was carefully subsampled (20x 1x1 mm micro-profiled cores pooled per each collected sample) for molecular analysis using a modified sterile transfer pipette to profile and collect the surficial oxic sediment layer. Pooling sub samples per core was done to account for in core heterogeneity. Sediment samples were allocated to a 15 ml falcon tube containing MoBio Lifeguard^{TM} RNA Preservation Agent using a 1:1 ratio of sediment to Lifeguard (©Qiagen 2013-20), transported to the University of Waikato Thermophile Research Unit, and stored at -20°C prior to nucleic acid extraction. A total of 40 sediment samples were collected for molecular analysis across three sampling timepoints (T0-T2) for each of the Treatments and Control (RE1: Control (8.1 pH); RE2: 7.70 pH; RE3: 7.90 pH; RE4: 7.50 pH) (**Figure 3**.) (**Table 1**.).

Physicochemical Sediment Analyses and Flow System Chemistry

An intact sediment core was collected from each of the four pH treatments at each sampling timepoint over the course of the experiment (12 sediment cores total for physicochemical analysis) and sectioned to depths of 0-1 cm and 1-5 cm using a well-fitted plunger device to delicately extrude the core from the bottom up to then be sectioned. Homogenized sediment subsamples were collected from each of the depth sections and analysed for grain size, pigments (Chlorophyll a, Phaeopigments), porosity, pore water extraction, and organic content based on the previously described protocol by (Drylie et al., 2019). Sediment was homogenized across the different depth profiles for each section, except for porosity which was measured using an intact sediment core collected using a modified syringe corer to profile the original sampled core. For porewater extraction, a 50 ml volume of sediment was centrifuged at 3,000 RPM for 10 minutes to separate off the porewater, which was collected from the surface of the sediment with a 10 ml syringe. The resulting extracted porewater was then pressure filtered through a Whatman GF/C filter (25 mm dia) with a Swinnex filter holder and stored frozen (-20°C) until inorganic nutrient analyses could be conducted. A minimum volume of 5 ml of porewater was acquired from each sample, enough to allow for duplicate analyses. For porosity and organic matter content, a syringe corer was used to measure the exact volume of sediment to determine the wet weight, oven-dry weight (60°C to constant), and Ash Free Dry Weight (AFDW) (combusted at 550°C for 4 hours). For the 0-1 cm depth profile, a volume of 10.62 cm^3 was used for analysis (2 x1 cm

depth cores of 2.6 cm dia =10.62 cm^3), and for the 1-5 cm depth profile, a volume of 21.24 cm^3 (1 x 4 cm depth core of 2.6 mm dia =21.24 cm^3) was used for analysis. Grain size samples were analyzed using a Mastersizer (Specs: Malvern Mastersizer 2000.) to process the raw undigested and nonfrozen sediment samples. Data were analysed using a two-tailed distribution paired *t*-test assuming equal sample variance (homoscedastic) to compare variations between sediment core physical and chemical properties between the different pH treatments.

Flow chamber system analyses were monitored in the dark (to maintain the established environmental conditions) over the course of the experiment for the assessment of pH, pCO$_2$, temperature, salinity, alkalinity, Dissolved Inorganic Carbon (DIC), and denitrification rates across the different treatment mesocosm systems as previously described by (Vopel et al., 2018; Vopel, Laverock, et al., 2021). In brief, CO$_2$-enriched air (5% CO$_2$, 21% O$_2$ in N$_2$) influx per each chamber was controlled automatically using the CapCtr Software Program (Loligo Systems Aps). All flow chambers were initially set at a standard pH of 8.10. Each system was continuously circulated with a 560 L volume (per flow chamber) of artificial seawater mixture (Instant Ocean, Spectrum Brands, Blacksburg, VA, USA). Natural advective processes were maintained using air jet flow to mimic turbidity. The system was maintained in total darkness for the length of the study to remove photosynthetic processes. The natural CO$_2$ degassing caused by the departure from the seawater air equilibrium offset of the pH by 0.02 units between the mixing barrel and mesocosm flow-through chamber. This variation was algorithmically adjusted for and continuously calibrated as needed.

Seawater pH was frequently monitored and controlled for using the automatic injection of CO$_2$-enriched air (5% CO$_2$, 21% O$_2$ in nitrogen) to carbonate the seawater and reduce pH by 0.03 units per day. The subsequent deposition and rate of CO$_2$ gas input were automatically regulated using the CapCtr Software Program (Loligo Systems Aps) and SenTix HWD electrode connected to a calibrated pH 3310-meter electrode (WTW) with an adjoined solenoid valve (Vopel et al., 2018). Salinity and temperature readings were taken every 2 days throughout the study. Seawater temperature was maintained at an average 14.6^{o}C. Salinity was measured using a conductivity meter (Knick Portamess® Conductivity Meter) and maintained through the addition of ultrapure H$_2$0 to regulate salinity at a constant 33.6 ppt based on filed data readings. A 1 L volume of seawater was periodically collected over the

course of the experiment from each mesocosm to measure Dissolved Inorganic Carbon (DIC) using coulometry and total alkalinity (TA) using potentiometric titration. Analyses were based on the SOP's (2 & 3a) described by Dickson et al., 2007 and Pierrot's adaptation of the CO_2Sys.BAS program previously established by Lewis & Wallace, 1998 for seawater pCO$_2$ and pH (seawater scale, mol kg-SW^{-1}) computation. The HSO$_4^-$ dissociation constant was also based on the methodology by Dickson, 1990, and the corresponding K_1 and K_2 values for carbonic acid measurements were based on Mehrbach et al., 1973 and then refitted based on Dickson & Millero, 1987. Total alkalinity measurements were regularly taken to monitor system carbonation levels using an AS-Alk 2 Total Alkalinity Titrator (Hoppe-Jones et al., 2010) (Apollo SciTech Inc., Georgia, USA) at Plymouth Marine Laboratory.

Figure 1. Describes the sediment core sampling dive site (D), in Man O' War Bay (C), off the coast of Waiheke Island (B), located on the North Island of New Zealand (A).
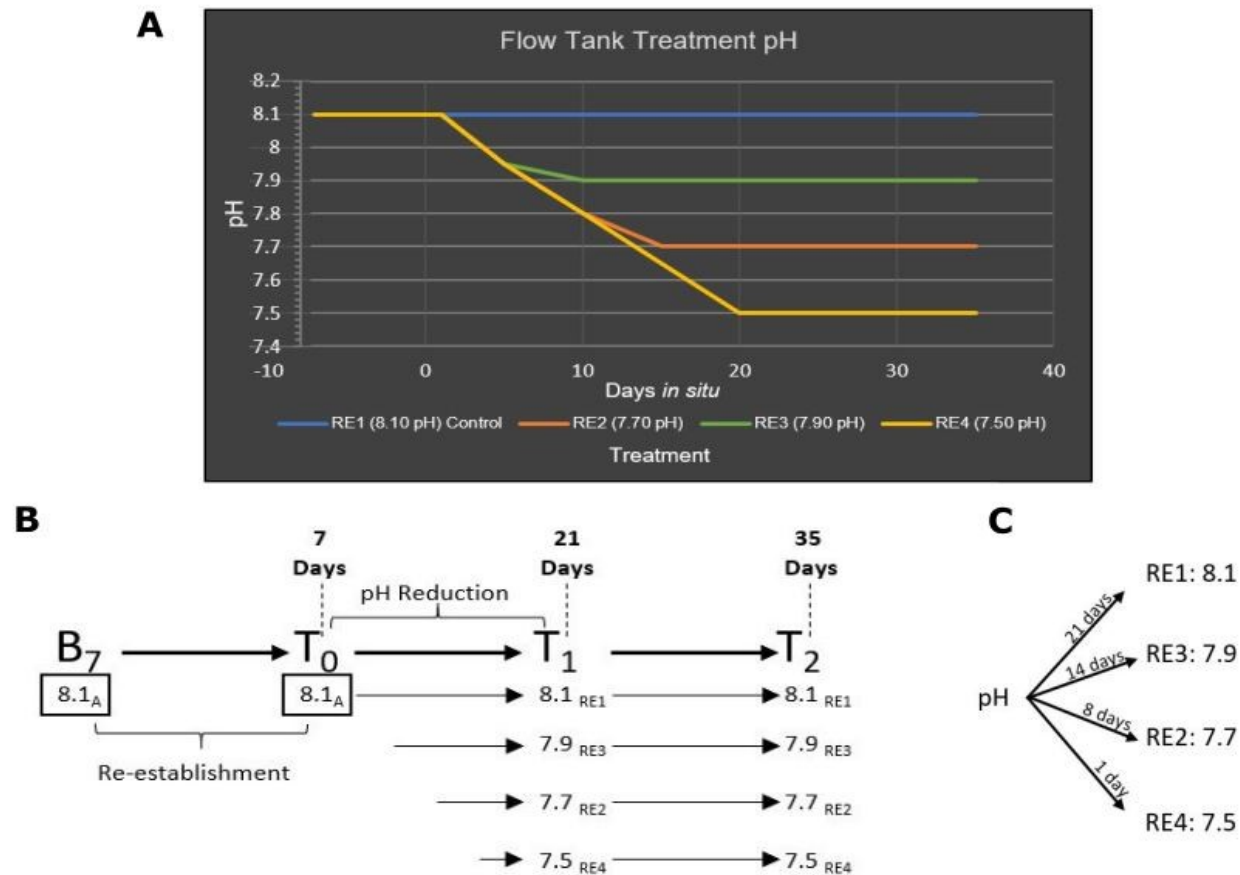
Figure 2. An explanation of the experimental design approach. A. demonstrates the gradual reduction in flow tank seawater pH over the course of the experiment for each of the treatment mesocosm chambers. B. shows the sampling days (T0, T1, and T2) and how the pH was reduced from Baseline (B$_7$) after the 7-day re-establishment period at pH 8.1, where T0 shows all treatments maintained at a controlled pH of 8.1. After T0, the pH is reduced by 0.03 pH units per day until stabilizing at their designated pH values. C. signifies the amount of time that each treatment was at their desired pH value when they were sampled at T0 for the first time (example: RE3: 7.7 is at a pH of 7.7 for 8 days prior to being sampled at 21 days (T0)).

Table 1. Demonstrates the four different pH treatments across time and incremental reductions in system pH in accordance with time.

Time (Days) *In Mesocosm*	RE1 (pH) Control 8.10	RE2 (pH) 7.70	RE3 (pH) 7.90	RE4(pH) 7.50
7 Day Incubation	8.10	8.10	8.10	8.10
1 (T0)	8.10	8.10	8.10	8.10
5	8.10	7.95	7.95	7.95
10	8.10	7.80	7.90	7.80
15	8.10	7.70	7.90	7.65
20 (T1$_{+1 day}$)	8.10	7.70	7.90	7.50
25	8.10	7.70	7.90	7.50
30	8.10	7.70	7.90	7.50
35 (T2)	8.10	7.70	7.90	7.50
Final 35	8.10	7.70	7.90	7.50
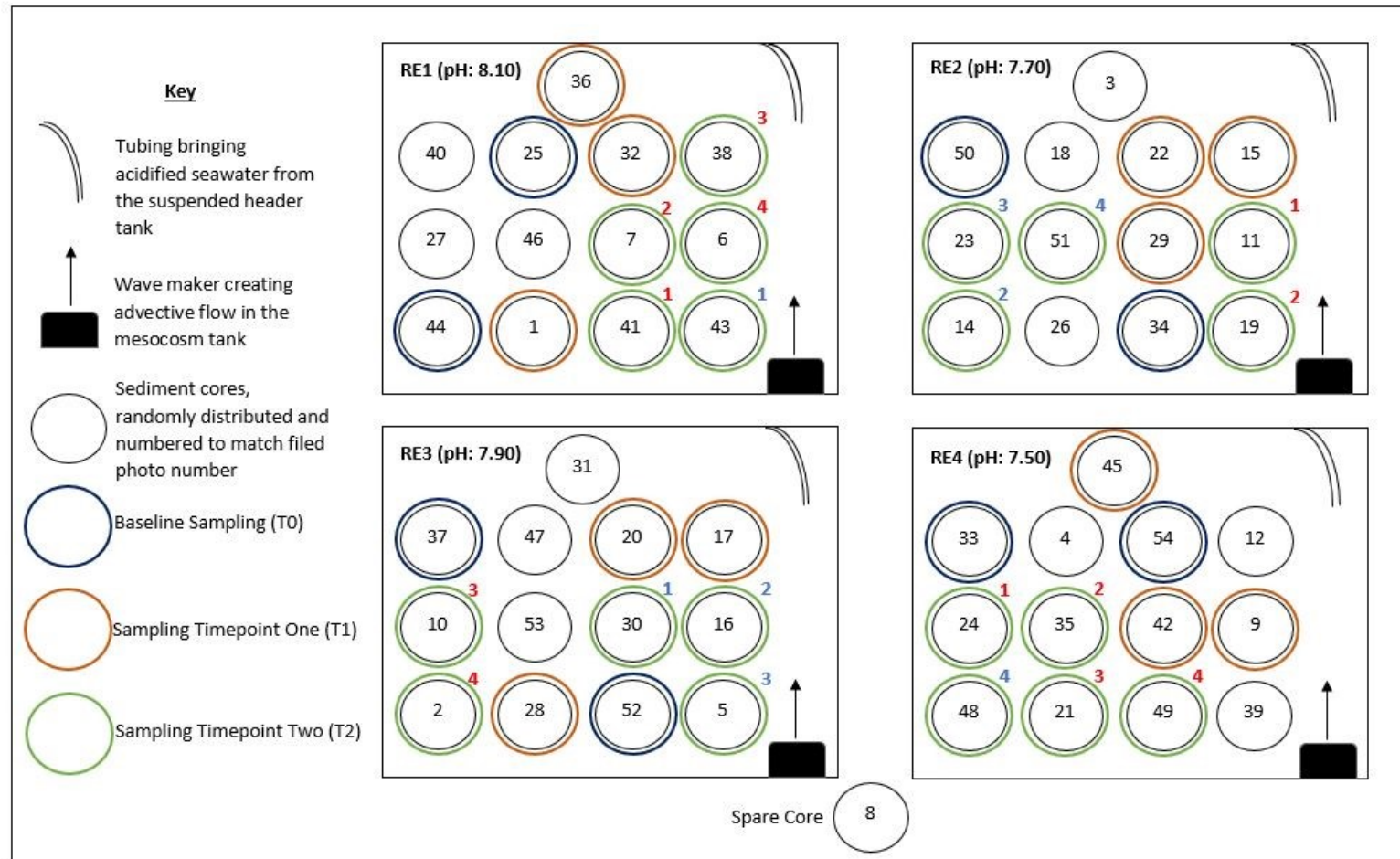
Figure 3. Describes the mesocosm system set up and the orientation of the different cores. Cores encircled in dark blue demonstrates the initial baseline sampling measurement at T0 (0$_{-7}$ Days *in mesocosm*) (2 cores sampled for molecular analysis). Cores encircled in orange denote sampling time point T1 (21$_{-7}$ Days *in mesocosm*) (3 cores sampled for molecular analysis). Cores encircled in green indicate sampling time point T2 (35$_{-7}$ Days *in mesocosm*) (5 cores sampled for molecular analysis). Cores with a proximal red or blue number were used for nutrient/O$_2$ flux measurements, denitrification, micro-profiling, and molecular sampling.

Chapter 3: Experiment 2

Nucleic Acid Extraction from Subtidal Sediment

Prior to DNA and RNA co-extraction, frozen sediment samples were defrosted on ice and centrifuged at 2,500 RPM for 5 minutes to separate off excess pore water and additional Lifeguard RNA Preservation agent. The supernatant was decanted, and 3.0 grams (wet weight) of the remaining subtidal sediment were weighed out for nucleic acid extraction. The MoBio RNA Powersoil Total RNA Isolation Kit and the DNA Elution Accessory Kit were used for the extraction of nucleic acids as per the Manufacturer's instructions (Qiagen Inc.: Germantown, MD, USA). The Quant-it ^{TM} PicoGreen High Sensitivity RNA and HS DNA Assay Kits were used for the quantification of Total RNA and Total DNA (Invitrogen, Thermo Fisher Scientific, Basingstoke, UK). The Nanodrop^{TM} 1000 Spectrophotometer (Thermo Fisher Scientific) was used to assess the quality and purity of both the Total RNA and Total DNA based on the acquired 260:280 ratio values. The Turbo DNA-Free^{TM} Kit (Thermo Fisher Scientific) was used to remove residual DNA contamination from the RNA prior to cDNA synthesis. The SuperScript® IV First-Strand Synthesis System (Thermo Fisher Scientific) was used for the synthesis of cDNA from single-stranded RNA using random hexamers based on the Manufacturer's instructions. Extracted nucleic acids were stored at -20°C [DNA] and -80°C [RNA] until further downstream processing.

PCR Amplification and Ion Torrent PGM Sequencing

The V4 region of the 16S rRNA gene was amplified using PCR (Polymerase Chain Reaction) using Earth Microbiome Ion Torrent Fusion Primers as described by Parada et al., 2016 and Quince et al., 2011. A 5 ng concentration of reverse-transcribed cDNA or total DNA were amplified in triplicate (each individual run in triplicate and pooled back together following PCR amplification) for each sample using a PCR reaction volume of 25 µl/per 1 reaction for the following master mix volumes and concentrations H_2O (Adjusted to 25 µl Total); 0.8 µl BSA (Bovine Serum Albumin) (0.4 mg/ml)(Promega Corporations, USA); 3 µl of DNTP's (2 mM) (Invitrogen Ltd. New Zealand); 3 µl of 10X PCR Buffer (Invitrogen Ltd. New Zealand); 3 µl of M_gCl_2 (50 mM); 0.12 µl of Taq Polymerase Enzyme (0.12 µl) (Invitrogen Ltd. New Zealand); 0.5 µl of Forward Primer (10 mM) and 0.5 µl of Reverse Primer (10 mM) (Integrated DNA Technologies Inc.); 2 µl of respective cDNA or DNA (5ng/µl). Thermocycler conditions incorporated an initial hot start incubation at 94°C for 3 minutes followed by 30 cycles of 94°C for 45 seconds, 50°C for 1 min and 72°C for 1.5 minutes, and a final extension

step of 10 minutes at 72 °C with an infinite hold at 4 °C. PCR amplicon products were pooled together and visually assessed on an agarose gel (1% TAE, SYBER safe) (Invitrogen).

The SequelPrep^{TM} Normalization Kit in a 96 well plate format was used to normalize PCR products. Products were then pooled in a 1.5 ml microcentrifuge tube and re-quantified using the Quant-it ^{TM} PicoGreen High Sensitivity RNA and DNA Assay Kits and stored at 4°C prior to sequencing. The Ion PGM^{TM} IA 500 Kit (Thermo Fisher Scientific, Massachusetts, USA) was used to examine sequence amplification quality and then sequenced on the Ion Torrent PGM^{TM} System using with an Ion 318v2 chip (Thermo Fisher Scientific, Massachusetts, USA) at The University of Waikato Sequencing Facility, Hamilton, New Zealand. Raw sequence trimming (FASTQ Format) and quality filtering were completed using the Mothur bioinformatic software (Version 1.40.5)(Schloss et al., 2009) to remove unusually short reads (<275bp) and long reads (>345bp), and homopolymers (>6). The USEARCH program (Version 10.0.240 (Linux 64-bit) was used for quality filtering pipelines (Edgar, 2010). High-quality sequence reads were recovered post-QC and clustered into OTU's (Operational Taxonomic Units) using a 97% similarity threshold. Quality filtering and sequence trimming were based on the previously described post sequence methodology described by (Schloss & Westcott, 2011;)(Power et al., 2018) (Power et al., 2018; Schloss & Westcott, 2011)

A 0.005% cut-off was applied to the raw Operational Taxonomic Unit (OTU) counts table to remove poorly represented OTU's within the dataset (Bokulich et al., 2013). The approach resulted in the removal of any OTU from the read count matrix with cumulatively less than 102 reads across all samples. Pre-filtering of the OTU matrix shifted the minimum read count from 2 to 98 reads. The total number of reads pre-filtering was 2,069,193, with a post-filtering read count of 1,805,485. The original OTU count pre-filtering was 17,280 with a post-filtering OTU count of 2,319, where a total of 87% of the count data is contained within 13% of the OTU's. The minimum read count threshold was set at 10,000 reads. Based on this metric, no samples were removed from the dataset, where the minimum read count detected was 10,954 reads. To reduce the risk of false discovery, the OTU matrix was subsampled without replacement to the minimum read count. The requirement for rarefication is based on a final read count variation score that is calculated using the difference between the highest (58,197 reads) and lowest (10,954 reads) read counts. This method produced a read count variation score of 5.31, indicating that the final read counts varied by 5.3x. The risk of

false discovery is considered highest when the read count variation is 10x or close to 10x. Given that the moderate read count variation score is <6, the requirement for rarefying the data is not compulsory. A comparison of the rarefied versus non-rarefied preliminary data was used to inform a conservative decision. At length, it was decided that the need to rarefy in this context was not necessary. All reported analyses are based on the outputs of the "non-rarefied" dataset. Justifications and descriptions for non-rarefication and identification of outlier are described in full in the supplementary data (**Supp. Figure 1**.) along with information regarding the additional treatment and transformative correction of the dataset where a batch effect was identified between sequencing sample runs.

Bioinformatic Analyses

Data was taxonomically classified post-sequencing using an RDP classifier database (Michigan State University Ribosomal Database Project) for the examination of raw 16S rRNA sequences in Fasta file format (Wang et al., 2007). Reassignment outputs with a confidence value of less than 80% were reclassified as "Unknown" taxa. Statistical analysis of the resulting sequence data was achieved using R (Version 4.0.3) (R Core Team 2020-10-10) and the corresponding packages and dependencies for compositional analysis: Phyloseq (McMurdie & Holmes, 2013); ggplot2 (Villanueva & Chen, 2019); vegan (Oksanen et al., 2008); viridis (Garnier, 2017); tidyverse (Wickham et al., 2019); adespatial (Jombart et al., 2020); ggpubr(Alboukadel Kassambara & Kassambara, 2020). Alpha philr (Washburne et al., 2018); ape (Paradis et al., 2004); CoDaSeq (Fernandes et al., 2018). Alpha diversity indices (Shannon, Observed, Chao1) were run using Phyloseq for examining changes in species richness, diversity (OTU Count), and evenness within the different pH exposed communities(Brose & Martinez, 2004; Chao, 1984; Shannon, 1948). ANOVA (Analysis of Variance) statistics were used to test for statistical differences in diversity and abundance between treatments within a timepoint and across time, followed by a Tukey HSD (Honestly Significant Difference) test for post-hoc significance verification to avoid the reporting of erroneous Type I errors (R Version 4.0.3). Only ANOVA produced significance values that also demonstrated a significant post hoc *p* adjusted value (Tukey HSD) were reported.

Beta diversity indices were achieved using PhILR (Phylogenetic Isometric Log Ratio Transform) statistical ordinations (Silverman et al., 2017) using a phylogenetic tree output to transform the data into an unconstrained coordinate system. ANOSIM analyses tested

similarity between samples from different pH treatments within a time point and over time using a ranked dissimilarity matrix. ANCOM (Analysis of Composition of Microbiomes) statistics were used to identify compositional changes within the microbial community in terms of taxonomic relative abundance (%) occurring at the OTU level within the different pH treatments within a time point and across time. ANCOM was useful for addressing the underlying structure of the microbial community without making distributional assumptions when comparing different community compositions (Mandal et al., 2015). Compared to a standard t-test, this approach has been shown to reduce the false discovery rate (FDR) while simultaneously improving statistical power (Mandal et al., 2015). Here we apply it for the identification of compositional variances between the four different pH treatments within a sampling time point and across the study period.

Results

Flow System Chemistry

System seawater temperature was maintained at a constant 18°C throughout the experiment. Seawater salinity measurements collected every 4-6 days showed an average salinity of 33.5 (RE1: 8.1: 33.7±0.4; RE2: 7.7: 33.6 ±0.4; RE3: 7.9: 33.5 ±0.3; RE4: 7.5: 33.5±0.4). The seawater salinity with the RE2:7.7 flow chamber was shown to be significantly different than the seawater salinity in the RE4:7.5 flow chamber (*t*-test: *p<0.05*) (**Supp. Tables 3. and 4.**). The dissolved inorganic carbon (DIC: µmol/kg) seawater concentrations were shown to increase overtime in each of the pH Treatment flow chambers, where the highest averaged DIC concentrations (overtime) were observed within the two lowest pH Treatments (RE2: 7.7: average: 4225.3 µmol/kg; RE4: 7.5: 4442.7 µmol/kg) (**Supp. Tables 3. and 4.**) (**Supp. Figure 3.**). Differences in DIC were detected between the Control and the two lowest pH Treatments (RE2: 7.7; RE4:7.5) (*t*-test: p<*0.001*), and between each of the pH Treatments (RE2:7.7/RE3:7.9 & RE3/RE4:7.5: *t*-test: p<*0.05*) (RE2/RE4: *t*-test: *p<0.001*) (**Supp. Table 4.**) Total alkalinity (µmol/kg) was shown to decrease over time for each of the pH Treatments (RE1: 8.1: -866 µmol/kg; RE2: 7.7: -899 µmol/kg; RE3: 7.9: -205 µmol/kg; RE4: 7.5 -1402 µmol/kg) (**Supp. Tables 3. and 4.**) (**Supp. Figure 4.**). Though the low pH treatments RE2 (7.7 pH) and RE4 (7.5 pH) demonstrated the greatest decrease in alkalinity from the first sampling measurement (September 25, 2017) to the final sampling measurement (November 13th, 2017) forty-seven days later, the Control Treatment showed the fastest rate in reduction. This was thought to be a result of technical difficulties when taking alkalinity readings for the Control tank, where the output results were more than likely confounded by introduced external error. From this we take extreme caution when inferring any meaningful results from the Control alkalinity data given this unforeseen complication. Subsequently, significant differences were detected in seawater alkalinity between the Control and the two lowest pH Treatments (RE2:7.7/RE4:7.5) (*t*-test: p<*0.05*). Differences were also observed between each of the other pH Treatments (RE2/RE3 and RE3/RE4: *t*-test: p<*0.05*) (RE2/RE4: t-test: p<*0.001*) (**Supp. Table 4.**).

Chapter 3: Experiment 2

Physicochemical Sediment Properties

No significant differences were identified between the different pH treatments for dry weight (g), total organic carbon content (g), porosity (porosity units (pu)) or grain size (63 μm) for sediment depth profiles 0-1 cm and 1-5 cm. The average dry weight between all treatments was greater in sediment samples taken from depths of 1-5 cm (Dry weight: 0-1 cm: 4.70 g; 1-5 cm: 8.75 g). Organic content was also greater in the deeper layers of the sediment (Organic content: 0-1 cm: 0.41 g; 1-5 cm: 0.75 g). As anticipated, the average porosity was greater in the surficial layers of the sediment (0-1 cm) (Porosity: 0-1 cm: 1.02 pu; 1-5 cm: 0.80 pu). The average sediment grain size was shown to be greater at depth rather than at the surface (Grain size: 0-1 cm: 3.11 μm (63); 1-5 cm: 3.40 μm) (**Supp. Tables 2. and 5.**).

Subtidal Sediment Taxonomic Diversity

Following the 0.005% cull, all unknown (i.e., unclassifiable taxa) at the level of order and all cyanobacteria/chloroplasts at the level of class were selectively filtered out from the dataset. A total of 2,234 OTU's were taxonomically identified and maintained from the original culled dataset (2,319 OTU's), where post taxonomic cull removed just 4.6% of the total OTU's, preserving 95% of the read count data. The subtidal sediments were dominated by bacteria (2,150 OTU's) and archaea (85 OTU's). At the phylum level, the community was dominated by Proteobacteria (DNA: 50.7%; RNA: 42.9%), Unclassified (DNA: 21.1%; RNA: 21.5%), Bacteroidetes (DNA: 13.6%; RNA: 18.9%), Acidobacteria (DNA: 4.25%; RNA: 2.65%), Cyanobacteria (DNA: 1.91%; RNA: 1.58%), Planctomycetes (DNA: 1.80%; RNA: 3.06%), and Verrucomicrobia (DNA: 1.61%; RNA: 2.20%) (**Supp. Figure 2.**).

Differences in Community Structure Between pH Treatments

Alpha diversity metric analyses used to assess changes in structure, species richness and evenness (i.e., distribution and relative abundances) between the three pH treatments (7.9, 7.7 and 7.5), and the experimental Control (8.1) identified that at each sampling time point (T0-T2). No impact of pH was observed on species richness in the Active [RNA] or Total [DNA] community between Treatments at T0 (0-7 Days *in mesocosm*) or T1 (21 days *in mesocosm*), where only the Active community demonstrated no effect of pH at the final sampling timepoint (T2: 35 days *in mesocosm*). In the Total community on day 35 (T2), an

impact of pH on species richness was detected between the Control (8.1: RE1) and pH treatment 7.7 (RE2), between pH treatment 7.7 (RE2) and pH treatment 7.5 (RE4) (ANOVA: *p<0.05*/F score: 5.416), and between the Control (8.1: RE1) and pH treatment 7.9 (RE3) (ANOVA: *p<0.05*/F score: 4.079) (**Supp. Figure 5.**). Species richness (Observed) and the proportion of species abundance (frequency) of the Active [RNA] and Total [DNA] community increased over time from the first sampling time point (T0: 0_{-7} Day *in mesocosm*) to the final sampling time point on day 35 (T2) (**Figure 4. and Figure 5.**). Interestingly, in the Active [RNA] community, species richness was shown to increase in the Control (8.1: RE1) between time points T0 and T1 (21 Days) and between T0 and T2 (35 Days) (ANOVA: *p<0.01*/F score: 18.38) (**Figure 4 A.**). No significant differences were detected within the experimental pH treatments 7.7 (RE2), 7.9 (RE3), and 7.5 (RE4) over time (**Figure 4. B-D**.). In the Total [DNA] community, the Control (8.1: RE1) was shown to change at each sampling time point (Control (8.1: RE1) (T0 & T1; T0 & T2; T1 & T2) (ANOVA: *p<0.01*/F Score: 16.19 (T0-T2; T1-T2); ANOVA: *p<0.01*/F score: 20.3 (T0-T1)) (**Figure 5.A**.). Treatment 7.9 (RE3) was shown to be significantly different between the first (T0) and the last sampling time points (T2: 35 Days) (ANOVA: *p<0.05*/F score: 5.717) (**Figure 5.B**.). Treatment 7.7 (RE2) and Treatment 7.5 (RE4) also displayed significant differences between the first (T0) and final (T2: 35 Days) sampling time points (RE2: ANOVA: *p<0.05*/F score: 13.19 (T0-T1; T0-T2); RE4: ANOVA: *p≤0.05*/F score: 7.985 (T0-T2)) (**Figure 5. D**.).
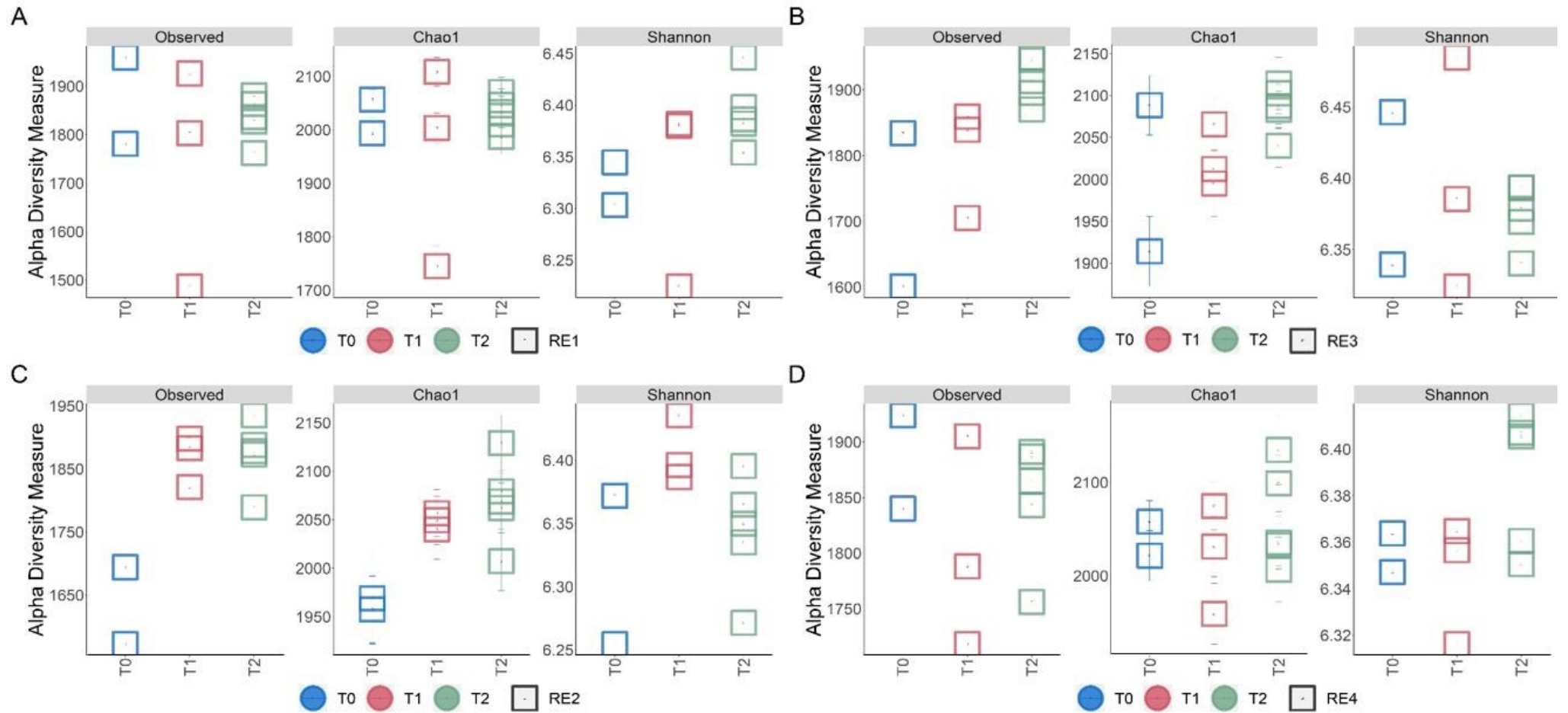
Figure 4. Active [RNA] community alpha diversity metric analyses between the different pH treatments (A: RE1: 8.1 pH; B: RE3: 7.9 pH; C: RE2: 7.7 pH; D: RE4: 7.5 pH) over time (T0-T2).
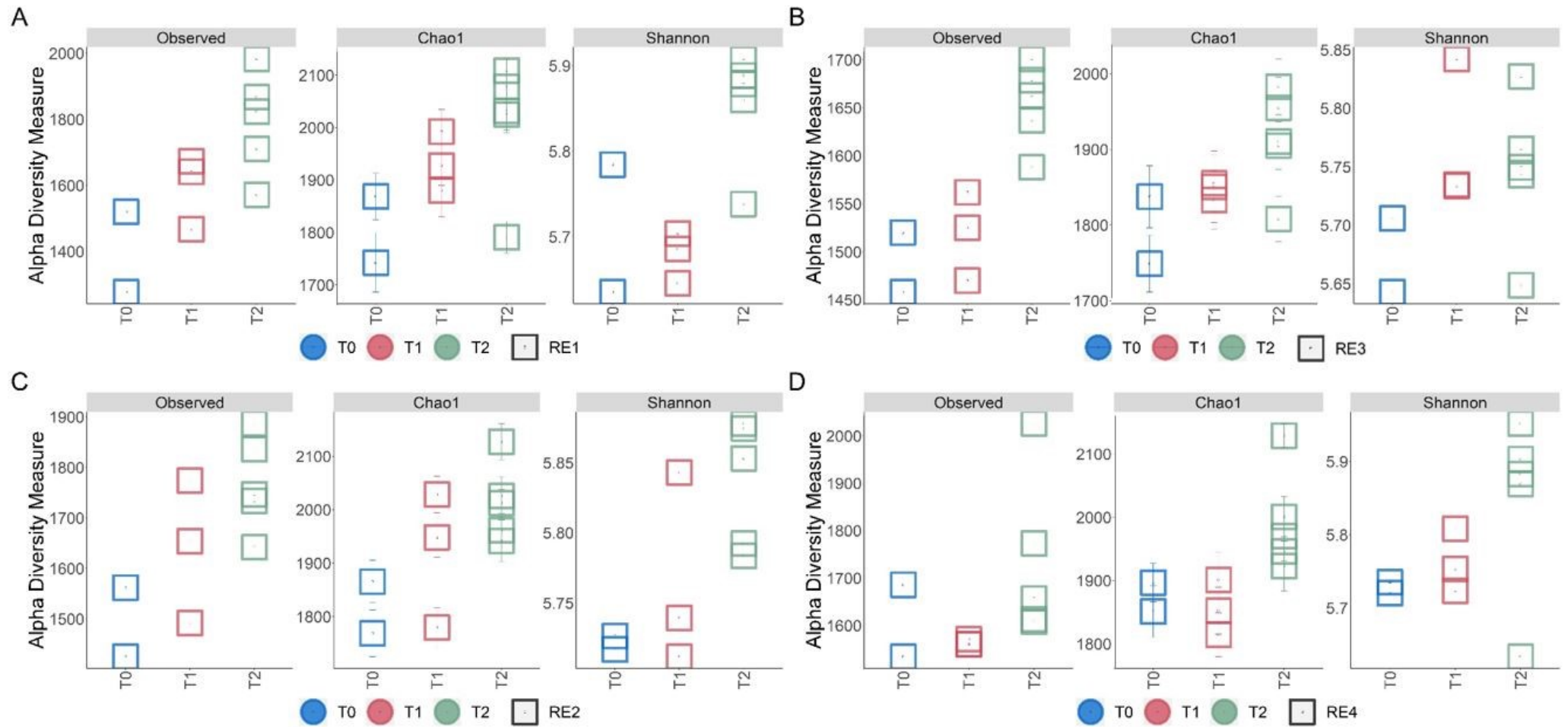
Figure 5. Total [DNA] community alpha diversity metric analyses between the different pH treatments (A: RE1: 8.1 pH; B: RE3: 7.9 pH; C: RE2: 7.7 pH; D: RE4: 7.5 pH) over time (T0-T2).

Variances in Community Composition and Relative Abundances

Beta diversity metric analyses calculated using phylogenetic tree branch distances were used to explain differences in community composition between the Control (8.1) and the different experimental pH treatments (7.9, 7.7 and 7.5) in terms of relative abundance. Results showed that the community structure changed with time, not by pH Treatment. No effect of pH was detected on the structure of the microbial community between the four different pH Treatments (RE1: 8.1; RE3: 7.9; RE2: 7.7; RE4: 7.5) at T0 (0_{-7} Days *in mesocosm*), at T1 (21 Days *in mesocosm*) or at T2 (35 Days *in mesocosm*) for both the Total [DNA] and Active [RNA] Communities (**Figure 6. A. and 6. B.**). Over time the community could be observed shifting between T0 (0_{-7} Days *in mesocosm*) and T1 (21 Days *in mesocosm*), and between T0 and T2 (35 Days *in mesocosm*), for both the Total [DNA]: (ANOSIM: *p<0.001/R= 0.7476*) and Active [RNA]: (ANOSIM: *p<0.001/R= 0.5509*) communities (**Figure 7. And Supp. Figure 6**.). Plotted PhILR ordinations with the primary axis as *time* effectively explained an estimated 20% of the detected variation in both the Total and Active communities (**Figure 6. A. and 6. B.**). Variation increased markedly over time across all Treatments, with the highest detectable variation at the final time point T2. The Control (RE1: 8.1 pH) was shown to display the least amount of variation compared to the pH Treatments. In contrast, all pH Treatments (RE3: 7.9; RE2: 7.7; RE4: 7.5) expressed much higher levels of variation, which was evident following the first pH amendment. Interestingly, there was increased variation in RE3 (7.9 pH) and RE4 (7.5 pH) than either the Control (8.1 pH) or the second-lowest pH Treatment RE2 (7.7 pH), where the effect of pH was shown to have a destabilizing impact on the community structure. Given the confounding effects of *time* on the ordination and distribution of the data, the PhILR ordinations were repeated using the second and third (2:3) axes. The approach showed that 6-8% of the variation observed between samples could be explained by treatment pH as the first axis (**Figure 6. C. and 6. D.)**. The Control still clustered tightly together despite the outlier, with the least amount of variation detected. The lowest pH Treatments (RE2: 7.7; RE4: 7.5) demonstrated the highest levels of variation, shown to increase from T0 to the final sampling time point (T2) for both the Total [DNA] and Active [RNA] communities. Results of both the PhILR ordinations with *time* as the primary axis and with *pH* as the primary axis maintain that the effect of time exerted more significantly greater impact on species diversity and evenness than that of pH treatment.
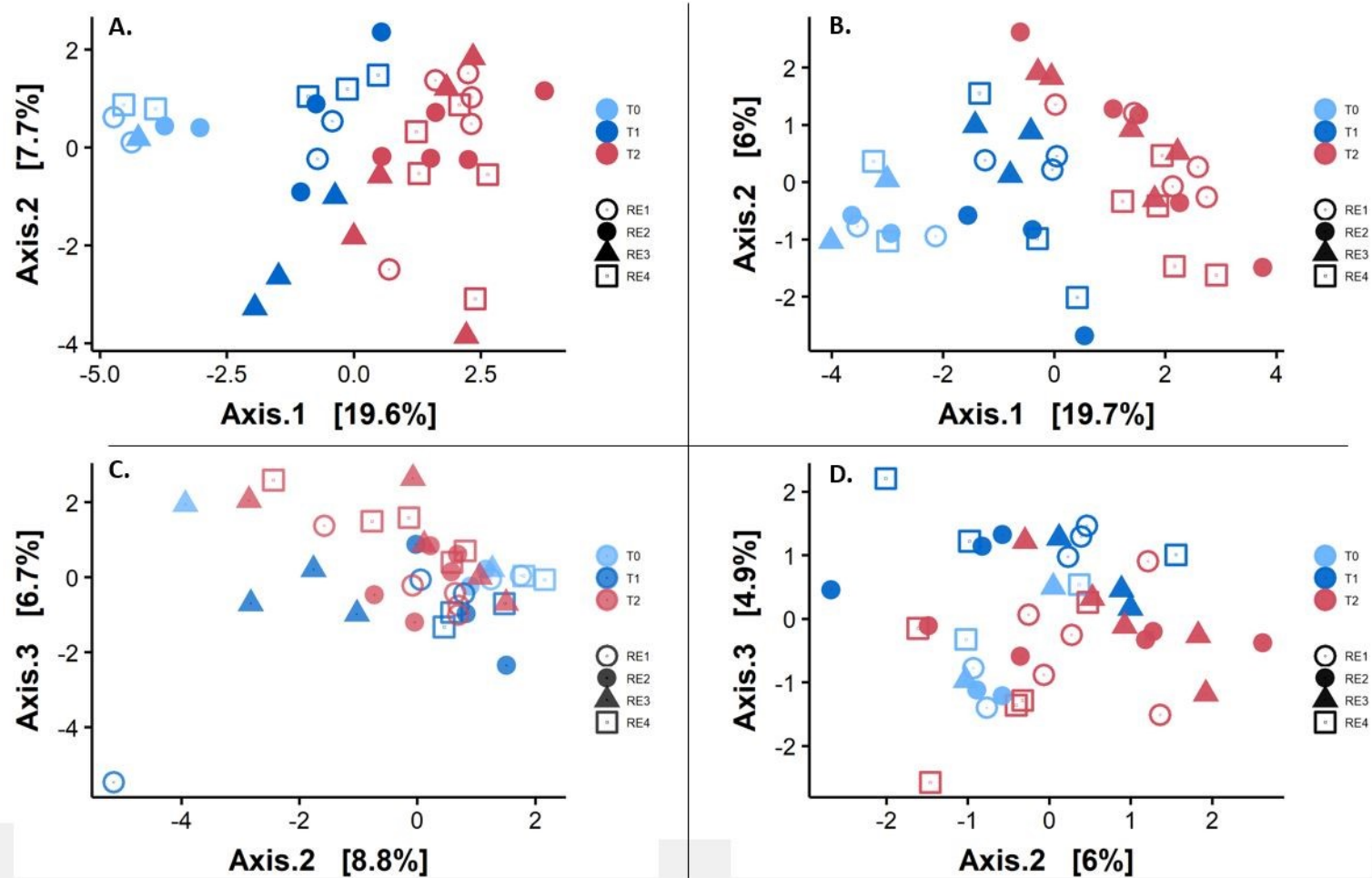
Figure 6. A. Shows the distribution of the Active [RNA] and Total [DNA] (B.) community using the PhILR ordination with time as the primary axis. An estimated 20% (RNA and DNA) of the inter-sample variation was explained within the first axis (RE1: 8.1 pH; RE2: 7.7 pH; RE3: 7.9 pH; RE4: 7.5 pH) (T0: 0-7 days; T1: 21 days; T2: 35 days). The same ordination was repeated for the Active [RNA] (C.) and Total [DNA] (D.) communities now using pH as the primary axis to determine treatment response, where 6-8% (DNA; RNA) of the variation can be explained with in the first axis (RE1: 8.1 pH; RE2: 7.7 pH; RE3: 7.9 pH; RE4: 7.5 pH) (T0: 0-7 days; T1: 21 days; T2: 35 days).
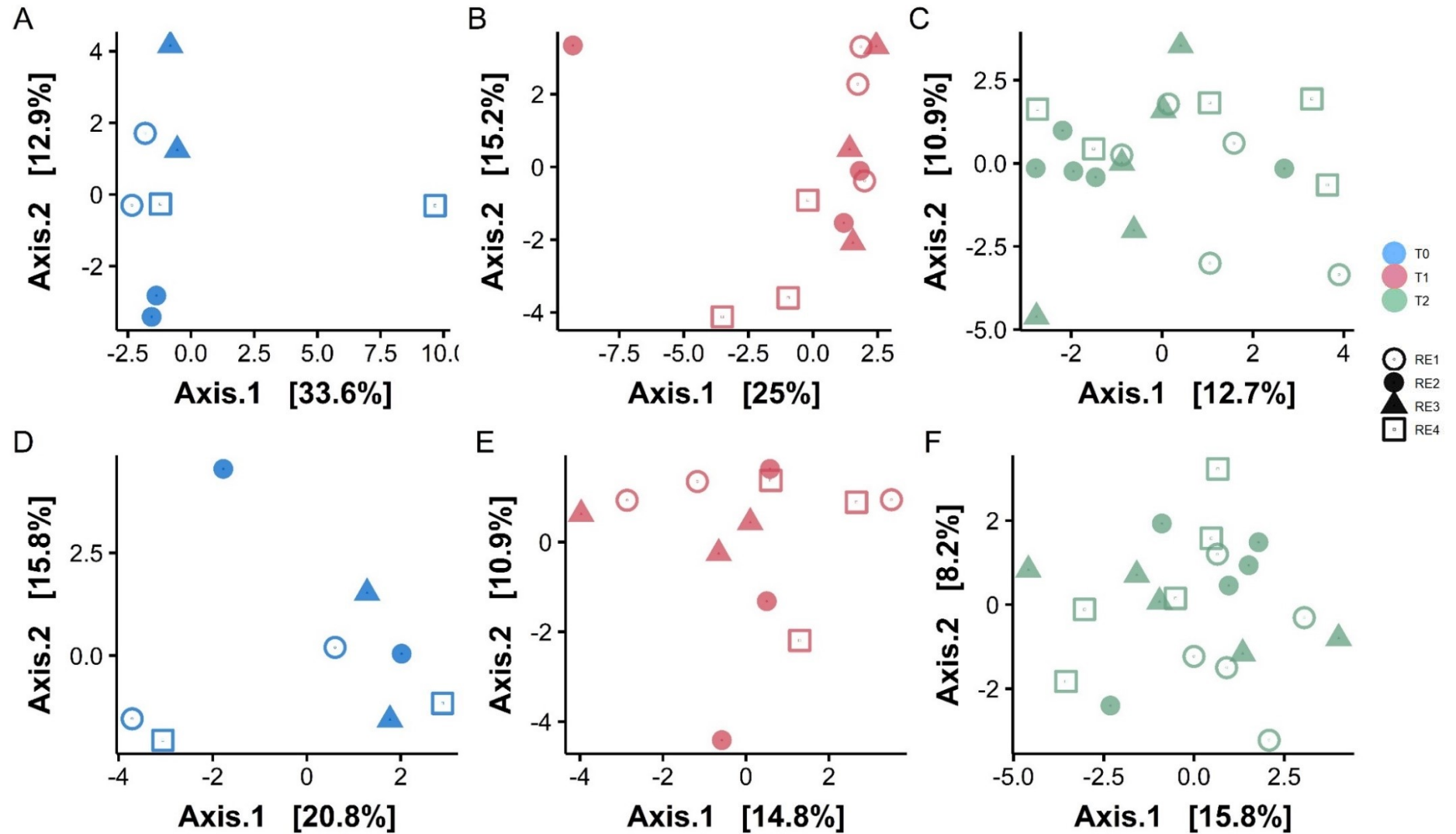
Figure 7. Distribution of the Active [RNA] community (A-C) and Total [DNA] community (D-F) by pH treatment (RE1: 8.1 = Open Circles; RE3: 7.9= Triangles; RE2: 7.7= Solid Circles; RE2: 7.5= Open Squares) at each sampling time point (T0: Blue; T1: Red; T2: Green) where *time* is the primary axis.

ANCOM

Analysis of composition of microbiomes (ANCOM) was used to statistically account for the compositional constraints introduced in microbial ecological data by detecting differentially abundant taxonomic individuals within the sediment community, while simultaneously maintaining high statistical power (Mandal et al., 2015). ANCOM is traditionally applied to mainly compositional data (i.e., 16S rDNA: Total DNA), though here we also use it to distinguish differences in the *relative activity* of taxa expressed through 16S rRNA (Active cDNA) under varying low pH treatments. Examination of the relative activity of particular taxa under different pH conditions provides greater depth and sensitivity than standard compositional analyses for interpreting microbial response, where the community may not be shown to respond significantly in structure of composition, but the relative activity of individual OTU's is responding to the disturbance. Analysis of the Active [RNA] community identified there were no significant differences in taxonomic relative abundance (%) of the 16S rRNA (i.e., relative activity), in any of the four experimental pH treatments over the course of the experiment (across time). Between each treatment, no significant changes were detected at T0 (0_{-7} Days *in mesocosm*). After 21_{-7} days *in the mesocosm* (T1), the relative activity of *Oceanospirillales* (OTU571) increased in both the RE2 (pH 7.7) and RE4 (pH 7.5) treatment communities, with a subsequent reduction in the RE3 (pH 7.9) treatment when compared to the Control. By the final timepoint (T2: 35 Days *in mesocosm*), the relative activity of three taxa: *Myxococcales* (OTU981), *Cytophagia* (OTU435), and *Porticoccus* (OTU427), were shown to respond significantly between the different pH treatments. *Myxococcales* (OTU981) increased in the RE3 (pH 7.9), RE2 (pH 7.7), and RE4 (pH 7.5) treatments relative to the Control (**Figure 8.**) (**Supp. Table 6**.).

As anticipated, the Active [RNA] community identified more responding sensitive taxa than in the Total [DNA] community. Though compared to the overall community (2,234 OTU's) less than <0.2% of OTU's were shown to change in in relative abundance under low pH conditions for both Active and Total communities. Across time, for the Total [DNA] community, the relative abundance of *Haliea* (OTU15802) was shown to change significantly between the different pH treatments. At T0 (0 Days *in mesocosm*), no significant changes in taxonomic abundance were detected. After 21 days *in mesocosm* (T1), two OTU's (OTU6921; OTU824) showed significant differences in their relative abundances between the different

pH treatments. *Porphyrobacter* (OTU6921) decreased for each of the experimental treatments (RE2, RE3 & RE4) relative to the Control. *Chromatiales* (OTU824) showed an increase in relative abundance for RE4 (pH 7.5) and a decrease in abundance for RE2 (pH 7.7), where RE3 (pH 7.9) remained unchanged compared to the Control. After 35 days in the mesocosm (T2), *Porticoccus* (OTU427) was shown to decrease for all experimental treatments (RE2, RE3 & RE4) relative to the Control (**Figure 9.**).

A comparative analysis was conducted on overlapping sentinel taxa that were detected between this and the previous experiment (Chapter 2.) as responding to low pH conditions. This was done to determine if a pattern exists between the two manipulation studies that identifies an aspect of the microbial community that is responding in the same way to changes in seawater pH; and to verify whether those responding taxa not only belong to the same genera of bacteria, but possibly even identify as the same species. A comparison of the OTU sequences (FASTA file) generated post sequencing was nucleotide blasted in NCBI (National Center for Biotechnology Information) to determine sequence overlap (%) between four taxa identified from this (Chapter 3.) and the previous experiment (Chapter 2.). *Oceanospirillales* was consistently observed as one of the most responsive taxa between both experiments, NCBI sequence blast of (OTU99) (Experiment 1 Chapter 2.) and OTU571 (Experiment 2 Chapter 3.) detected a 76.4% sequence overlap between the two OTU's (**Supp. Figure 7**.). *Porphyrobacter* was also shown to respond significantly between both experiments, where the NCBI blast between OTU361 (Experiment 1. Chapter 2.) OTU6921 (Experiment 2. Chapter 3.) resulted in a 73% sequence overlap between the two OTU's (**Supp. Figure 8**.). Given the <80% confidence of sequence similarity between the OTU's of interest (*Oceanospirillales* and *Porphyrobacter*) it was determined that these bacteria originate from the same genera of bacteria but are not taxonomically identified as the same species.
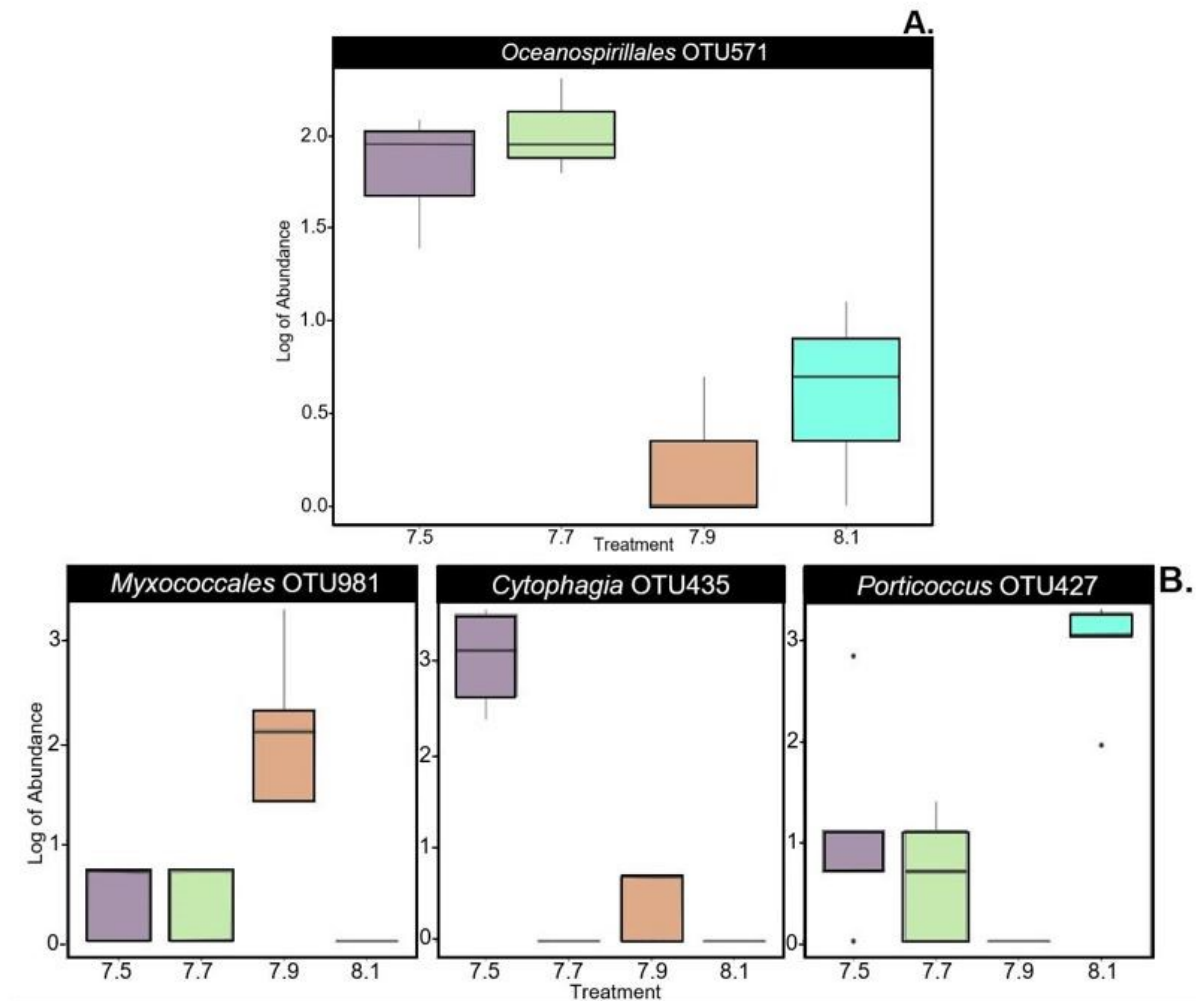
Figure 8. Demonstrates the taxonomic relative abundance of OTU's on a logarithmic scale (y-axis: Log of (relative) abundance); x-axis: pH Treatment) that were detected as significantly responding between the different pH treatments at A. Time point T1 (21 Days *in mesocosm*) and B. Time point T2 (35 Days *in mesocosm*) for the Active [RNA] community.

187
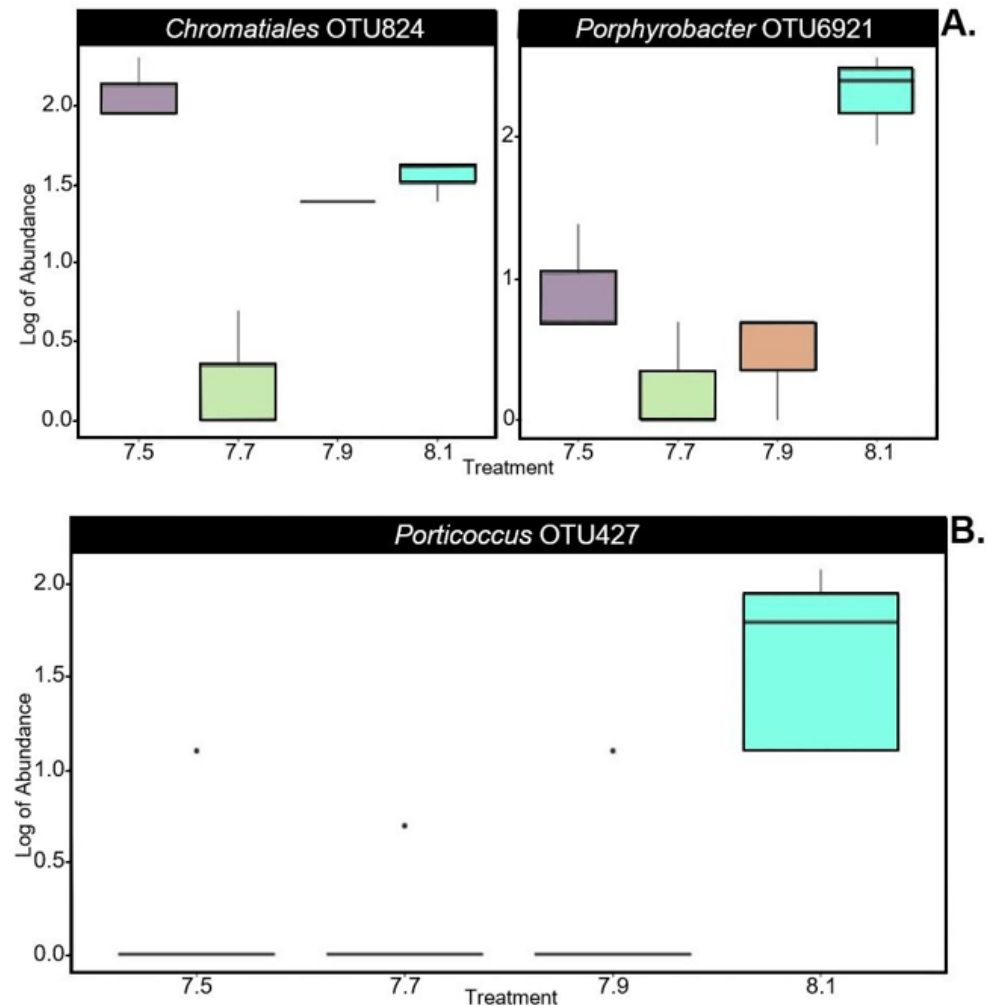
Figure 9. Demonstrates the taxonomic relative abundance of OTU's on a logarithmic scale (y-axis: Log of (relative) abundance); x-axis: pH Treatment) that were detected as significantly responding between the different pH treatments at A. Time point T1 (21 Days *in mesocosm*) and B. Time point T2 (35 Days *in mesocosm*) for the Total [DNA] community.

Discussion

The subtidal region (i.e., sublittoral zone) is the interface between the dynamic high intensity intertidal ecosystem and the more constant and stable open ocean environment (Christiansen et al., 2000; Dyer et al., 2000; Peters, 1997; RZalston & Stacey, 2005; Stevens, 2003). It operates as the buffer zone between these two highly contrasting oceanic regions (Desjardins et al., 2012). It has been previously suggested that the organisms from these different systems would also observe and reflect the same gradient-like properties representative of each unique ocean region (Kolda et al., 2019; O'Malley, 2008). Given that subtidal regions are not as dynamic as intertidal systems (research previously (Chapter 2)), we hypothesize that the resident bacteria from a more stable (i.e., less dynamic) environment would express reduced tolerance compared to organisms from more dynamic habitats. In this study, we show that microbial communities from subtidal, low carbonate benthic sedimentary environments appear to exhibit exceptional structural plasticity and compositional resistant to changes in overlying water column pH, though comparatively less than their intertidal coastal ecosystem counterparts.

We identified that the core microbial community was both structurally and compositionally resistant to the effects of the varying OA pH treatments over time. This finding is consistent with our previous observations (Chapter 2.), where the intertidal benthic community remained unperturbed by prolonged exposure to low pH across highly contrasting pH treatments with the added implementation of physical sedimentary disturbances. The results from this study corroborate our previous work and support similar experimental findings (Edmonds et al., 2009; Kitidis et al., 2011a; Oliver et al., 2014) that sediment microbial communities under the conditions tested are highly resistant to lower pH. However, previous research has also shown that microbial communities from marine sediments and biofilms exposed to high CO_2 environments do demonstrate clear structural community shifts or subsequent reductions in recovery to low pH (Borrero-Santiago et a., 2017; Currie et al., 2017; Hassenruck et al., 2016; Witt et al., 2011), where the stability of the environment appears to dictate the extent of the impact.

The general observation that the whole community was unaltered in relation to sustained low pH environments, brings to question whether we are detecting *environmental resilience* or *microbiological resistance* and how we might decouple these two parameters.

Chapter 3: Experiment 2

The stability of a system (i.e., tolerance) to environmental change could be attributed to the environment's inherent ability to buffer the effects of increased pCO_2, and not necessarily because of biological plasticity. Past studies have demonstrated that benthic coastal sediments have a greater affinity for pCO_2 uptake (i.e., higher alkalinity) than seawater (Carman & Rahm, 1997; Chatterjee et al., 2011; Lukawska-Matuszewska & Kielczewska, 2016), supporting the assumption that the environment (e.g., the sediments) is buffering the effects of increased pCO_2 from the microbial communities.

In this study the composition of the subtidal sediment was determined to have naturally low concentrations of carbon and calcium carbonate ($CaCO_3$). This would suggest that the sediment would exhibit considerably less buffering capacity for pCO_2 than sediments with higher levels of carbon and $CaCO_3$ as an important carbonate reservoir providing critical potential buffering capacity against low pH (Andersson et al., 2006; Rassmann et al., 2018). Alkalinity was measurably higher under the lowest pH treatments (7.7 and 7.5 pH), where higher CO_2 concentrations will have an inverse effect on pH at a given alkalinity. This can also translate to an increase in alkalinity depending on the equilibrium of aqueous CO_2 concentrations in the seawater at different atmospheric CO_2 concentrations, and the collective equilibrium of TA (total alkalinity) concentrations in a continuously acidified environment, as was simulated in this experiment (Andersson et al., 2007). Here we demonstrate that the subtidal sediment microbial community can withstand this reduction in alkalinity over time, though it cannot be determined whether this is attributed to the sediment composition and chemistry or to the physiological durability of the bacteria.

Responsive taxa

Despite the sedimentary composition and the absence of phototrophic organisms, we observe only nominal changes in community structure in response to reductions in overlying seawater pH. Importantly, this response to change in pH was only captured in the Total [DNA] community and not in the Active [RNA] community, and only within one sampling time point at T1: 21 days *in situ*. Though significant, this variation could be a result of organic matter turnover, where both living and non-living bacterial cells are accounted for in community composition and taxonomic abundance. This is in contrast to the Active community (only living cells) which showed no significant differences (De Vrieze et al., 2018). As in our previous study ANCOM analysis identified a small subset of OTU's (<0.2% of the Total 2,234 OTU's) in

the community whose relative abundances were shown to increase significantly in response to reductions in pH. Of these sensitive or low tolerant taxa, several were consistent with previously identified low tolerant nitrifying organisms (*Order: Oceanospirillales; Genus: Porphyrobacter*) observed in Chapter 2. and in other studies (Ng & Chi, 2020, Currie et al., 2017). This suggests that a genus of bacteria from the subtidal and intertidal benthic microbial community respond consistently to sustained low pH across different marine benthic systems. In contrast, other studies such as Monier et al., 2014 and Lamim & Procópio, 2021 have demonstrated as great as a 10-fold reduction in *Oceanospirillales* under high pCO_2 conditions.

The impact of removing dominating photosynthetic taxa

In this study we also assessed the effects of dark (total light deprivation) carbon fixation on the microbial community structure across the four different pH treatments. Photosynthetic marine organisms drive primary productivity within the coastal environment by supplying more than 90% of the ocean-produced TOC (Total Organic Carbon) by fixing CO_2. Cyanobacteria are responsible for contributing an estimated 50% of the fixed carbon back into the system (Burns et al., 2005; Partensky et al., 1999). Many phototrophic organisms have developed physiological inorganic carbon concentrating mechanisms (CCM) to adapt and overcome limited CO_2 environments through the uptake of HCO_3^- (Bicarbonate Ions), allowing for continual growth maintenance under varying DIC conditions (Badger et al., 2006). Increasing pCO_2 has been identified as a strong contributor to changes in cyanobacterial cell division, subsequently altering C: N or N:P ratios (Barcelos e Ramos, Biswas, Schulz, LaRoche, & Riebesell, 2007; Levitan et al., 2007; Liu et al., 2010; Lomas et al., 2012). However, these studies showed contrasting responses to increased pCO_2, where some reported decreased cell growth and others showed acclimatization to low pH. The inconclusiveness of these studies implies that primary productivity is regulated and manipulated by more than one environmental factor (i.e., seawater temperature, changes in seawater chemistry (pH), introduction of nutrients) and the delicate interaction of these factors may have either beneficial or detrimental impacts on photosynthetic organisms(Eggers et al., 2014; Kroeker et al., 2013). Our study sought to decouple some these ecological variables and their impacts on microbial community composition and structure under low pH conditions through controlled environmental settings and the selective removal of dominating photosynthetic

organisms (e.g., cyanobacteria). We identified that even in the absence of phototrophic bacteria, who may or may not possess the physiological potential to adjust to high pCO_2 conditions, the collective core microbial community remained structurally sound and compositionally resistant to reduced pH. This suggests that the plasticity of benthic marine microbial organisms across species and whole communities is inherently more complex in its resistance than previously anticipated across varying spatiotemporal scales (Joint et al., 2011; Kitidis et al., 2011).

Mesocosm Effect

We attest that the impact of treatment will change over time. Even given the experimental design approach used in this study, which could influence the length of time at which a flow chamber community would sit at a certain pH relative to other treatments, the community still demonstrated high levels of resistance. Where the effect of the pH treatment had only a very small role in determining microbial community structure. This could suggest that the response of benthic microbial taxa to low pH environments is not universal or uniform across the community in terms of impact and could therefore be more species-specific than previously anticipated, as was demonstrated in other marine organisms (Price et al., 2011). This could imply that the action of natural selectivity amongst different subtidal benthic bacteria varies from organism to organism and may be further complicated by additional factors (e.g., exposure time) (Collins et al., 2014). We also identified that "time" (i.e., temporal effect) as an external factor, played an important role in influencing the sediment microbial community structure and composition, as demonstrated in both the alpha and beta diversity matrices.

Conclusion

This study examined the response of subtidal benthic microbial communities to a changing ocean climate by identifying differences between sensitivity, tolerance, and inferred *compositional* resilience. These metrics provide an important framework for measuring and quantifying microbial compositional changes and understanding and investigating their response to deviations in the functioning of the natural environment. This study sought to investigate the response of subtidal sediment microbial communities to the effects of OA using a dual pronged experimental approach to survey the diversity of the 16S rDNA and the relative activity of the 16S rRNA (cDNA) microbial communities under varying low pH regimes. The most sensitive measure of microbial response to disturbance is reflected in the activity of the community. We demonstrate that a strictly compositional survey of the microbial community using 16S rDNA alone would have produced no measurable differences to the community under OA conditions over time (i.e., temporally). Conversely, the metabolically functional fraction of the community displayed only minor responses to shifts in seawater pH.

Active community analyses using cDNA alone provides only a small fraction of the picture, where subtle biological changes are not perceived on the molecular [RNA] transcriptional level, even when the outward compositional profile indicates no change. We encourage the use of metatranscriptomic analyses for the in-depth assessment of microbial response to the effects of ocean acidification for a more 3-dimensional depiction of what is happening to the marine system at a microbial level and what this means for the future assessment of coastal monitoring and OA bottom-up predictive surveys. Previous studies have also emphasized the ecological impact of both short-term extreme low pH exposure and slow and continuous low pH exposure on the marine ecosystem, as both methods of disturbance will likely result in a whole new series of ecological and biological challenges. Our understanding from this and the previous study (Chapter 2.) provide supportive evidence that the hidden majority of sentinel or bio-indicative bacteria may be slowly degrading under even short-term or sustained pH exposure in both intertidal and subtidal coastal marine environments (Gattuso et al., 1999; Gutowska et al., 2008; Iglesias-Rodriguez et al., 2008). We also attest to the challenges and complexities in attempting to represent a realistic and natural environment in a mesocosm system, which comes with all its inherent limitations. Here we address one such limitation, where the use of a staggered reduction approach to

lowering the pH across all four pH treatments overtime may have introduced undesirably complexity and variation into the study, given that the separate treatments were maintained at their final pH thresholds for differing periods of time. However, we argue that it is increasingly difficult to ordinate the design of the experiment in such a way to allow for this uniformity in establishment time for each of the treatments. One of the only avenues available would be to rapidly reduce the pH to reach the desired concentration within each system over a very short window of time. As established in the previous experiment (Chapter 2.), the application of an extreme shift in pH results in its own series of complications and should not be used as a primary option. Therefore, despite the inherent shortcomings of using the staggered reduction approach, we substantiate that this was the most effective experimental strategy available to us. Despite these and other known limitations, mesocosm studies are still considered to be one of the most effective methods for analyzing environmental impacts and decoupling species interactions. The lack of light used in our experiment and subsequent selective removal of photosynthetic diazotrophic cyanobacteria under dark conditions may significantly affect the functionality of the sediment community, where the absence of nitrogen fixers that are not replaced through functional redundancy resulting in the loss and inability to maintain the nitrification process in the marine ecosystem (Hutchins & Fu, 2017; Kitidis et al., 2017). By removing these dominating photosynthetic processes and forcing the microbial community to restructure under acidified seawater conditions is likely to have far reaching consequences over time. Future microbial ecological manipulation studies should work to maintain more natural and realistic diel cycling patterns for a more accurate representation of microbial resistance to the effects of OA. These futuristic studies should also seek to incorporate more ecological variables into their analysis to understand the complex interconnectivity of these micro-scale relationships and what this means in terms of their ability to respond and adapt to a high pCO_2 oceanic environment.

Finally, we also emphasize the importance of examining other regional impacts on coastal marine environments, where the proximity of coastal and nearshore environments to terrestrial input from land caused by human-related activity poses a significant additional acidifying problem (eutrophication-induced-acidification) (Körtzinger, Hedges, & Quay, 2001). The combined effect of both disturbances on seawater pH is likely to have a more pronounced impact on nearshore environments than any other marine system alone

(Melzner et al., 2013; Provoost et al., 2010; Sunda & Cai, 2012). Given that these processes due not work in isolation, understanding how the microbial communities in coastal habitats will respond to a "dual" disturbance is critical for assessing the sensitivity, stability, and resistance of the benthic environment. This also provides a unique opportunity to examine the application of various mitigation strategies on stochastic coastal acidifying events (i.e., eutrophication), where the use of $CaCO_3$ as a natural system buffer may help to reduce to projected impacts of low pH on the residential microbial taxa. More analyses are required to identify and measure the role of microbial communities in benthic sediments in response to the effects of global ocean acidification and coastal eutrophication.

References

Anderson, M. J., Ford, R. B., Feary, D. A., & Honeywill, C. (2004). Quantitative measures of sedimentation in an estuarine system and its relationship with intertidal soft-sediment infauna. *Marine Ecology Progress Series*, *272*, 33–48. https://doi.org/10.3354/meps272033

Andersson, A. J., & MacKenzie, F. T. (2012). Revisiting four scientific debates in ocean acidification research. *Biogeosciences*, *9*(3), 893–905. https://doi.org/10.5194/bg-9-893-2012

Andersson, Andreas J., Bates, N. R., & Mackenzie, F. T. (2007). Dissolution of carbonate sediments under rising pCO2 and ocean acidification: Observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, *13*(3), 237–264. https://doi.org/10.1007/s10498-007-9018-8

Aufdenkampe, A. K., Mayorga, E., Raymond, P. A., Melack, J. M., Doney, S. C., Alin, S. R., … Yoo, K. (2011). Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, *9*(1), 53–60.

Badger, M. R., Price, G. D., Long, B. M., & Woodger, F. J. (2006). The environmental plasticity and ecological genomics of the cyanobacterial CO2 concentrating mechanism. *Journal of Experimental Botany*, *57*(2), 249–265.

Barcelos e Ramos, J., Biswas, H., Schulz, K. G., LaRoche, J., & Riebesell, U. (2007). Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer Trichodesmium. *Global Biogeochemical Cycles*, *21*(2).

Blanchet, H., Lavesque, N., Ruellet, T., Dauvin, J. C., Sauriau, P. G., Desroy, N., … de Montaudouin, X. (2008). Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats-Implications for the implementation of the European Water Framework Directive. *Ecological Indicators*, *8*(4), 360–372. https://doi.org/10.1016/j.ecolind.2007.04.003

Borrero-Santiago, A. R., Bautista-Chamizo, E., DelValls, T., & Riba, I. (2017). A possible CO2 leakage event: Can the marine microbial community be recovered? *Marine Pollution Bulletin*, *117*(1–2), 380–385. https://doi.org/10.1016/j.marpolbul.2017.02.027

Brewer, P. G. (1997). Ocean chemistry of the fossil fuel CO2 signal: The haline signal of "business as usual." *Geophysical Research Letters*, *24*(11), 1367–1369.

Brose, U., & D. Martinez, N. (2004). Estimating the richness of species with variable mobility. *Oikos*, *105*(2), 292–300.

Burns, R. A., MacDonald, C. D., McGinn, P. J., & Campbell, D. A. (2005). INORGANIC CARBON REPLETION DISRUPTS PHOTOSYNTHETIC ACCLIMATION TO LOW TEMPERATURE IN THE CYANOBACTERIUM SYNECHOCOCCUS ELONGATUS 1. *Journal of Phycology*, *41*(2), 322–334.

Caldeira, K., & Wickett, M. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research C: Oceans*, *110*(9), 1–12. https://doi.org/10.1029/2004JC002671

Carman, R., & Rahm, L. (1997). Early diagenesis and chemical characteristics of interstitial water and sediments in the deep deposition bottoms of the Baltic proper. *Journal of Sea Research*, *37*(1–2), 25–47.

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 265–270.

Chatterjee, S., Dickens, G. R., Bhatnagar, G., Chapman, W. G., Dugan, B., Snyder, G. T., & Hirasaki, G. J. (2011). Pore water sulfate, alkalinity, and carbon isotope profiles in shallow sediment above marine gas hydrate systems: A numerical modeling perspective. *Journal of Geophysical Research: Solid Earth*, *116*(B9).

Christiansen, T., Wiberg, P. L., & Milligan, T. G. (2000). Flow and sediment transport on a tidal salt marsh surface. *Estuarine, Coastal and Shelf Science*, *50*(3), 315–331. https://doi.org/10.1006/ecss.2000.0548

Collins, S., Rost, B., & Rynearson, T. A. (2014). Evolutionary potential of marine phytoplankton under ocean acidification. *Evolutionary Applications*, *7*(1), 140–155.

Currie, A. R., Tait, K., Parry, H., de Francisco-Mora, B., Hicks, N., Mark Osborn, A., … Stahl, H. (2017). Marine microbial gene abundance and community composition in response to ocean acidification and elevated temperature in two contrasting coastal marine sediments. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.01599

Dale, A. W., & Prego, R. (2002). Physico-biogeochemical controls on benthic pelagic coupling of nutrient fluxes and recycling in a coastal upwelling system. *Marine Ecology Progress Series*, *235*, 15–28.

De Vrieze, J., Pinto, A. J., Sloan, W. T., & Ijaz, U. Z. (2018). The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. *Microbiome*, *6*(1), 63. https://doi.org/10.1186/s40168-018-0449-9

Desjardins, P. R., Buatois, L. A., & Mángano, M. G. (2012). Tidal Flats and Subtidal Sand Bodies. In *Developments in Sedimentology* (Vol. 64). https://doi.org/10.1016/B978-0-444-53813-0.00018-6

Dickson, A., Sabine, C., & Christian, J. (2007). Guide to Best Practices for Ocean C02 Measurments. In *North Pacific Marine Science Organization* (Vol. 3). Retrieved from www.pices.int

Dickson, A G, & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(10), 1733–1743.

Dickson, Andrew G. (1990). Standard potential of the reaction: AgCl (s)+ 12H2 (g)= Ag (s)+ HCl (aq), and and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, *22*(2), 113–127.

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: The other CO2 problem. *Annual Review of Marine Science*, *1*, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834

Doney, S. C., & Schimel, D. S. (2007). *Carbon and Climate System Coupling on Timescales*

from the Precambrian to the Anthropocene *. 32(1), 31–66. https://doi.org/10.1146/annurev.energy.32.041706.124700

Drylie, T. P., Needham, H. R., Lohrer, A. M., Hartland, A., & Pilditch, C. A. (2019). Calcium carbonate alters the functional response of coastal sediments to eutrophication-induced acidification. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-48549-8

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., … McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts*, *36*(2), 221–236.

Dyer, K. R., Christie, M. C., Feates, N., Fennessy, M. J., Pejrup, M., & van der Lee, W. (2000). An Investigation into Processes Influencing the Morphodynamics of an Intertidal Mudflat, the Dollard Estuary, The Netherlands: I. Hydrodynamics and Suspended Sediment. *Estuarine, Coastal and Shelf Science*, *50*(5), 607–625. https://doi.org/https://doi.org/10.1006/ecss.1999.0596

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edmonds, J. W., Weston, N. B., Joye, S. B., Mou, X., & Moran, M. A. (2009). Microbial community response to seawater amendment in low-salinity tidal sediments. *Microbial Ecology*, *58*(3), 558–568. https://doi.org/10.1007/s00248-009-9556-2

Eggers, S. L., Lewandowska, A. M., Barcelos e Ramos, J., Blanco-Ameijeiras, S., Gallo, F., & Matthiessen, B. (2014). Community composition has greater impact on the functioning of marine phytoplankton communities than ocean acidification. *Global Change Biology*, *20*(3), 713–723.

FEELY, R. A., SABINE, C. L., LEE, K., BERELSON, W., KLEYPAS, J., FABRY, V. J., & MILLERO, F. J. (2004). Impact of anthropogenic CO2 on the CaCO3 system in the oceans. *Science*, *305*(5682), 362–366. https://doi.org/10.1126/science.1097329

Fernandes, A. D., Vu, M. T. H. Q., Edward, L. M., Macklaim, J. M., & Gloor, G. B. (2018). A reproducible effect size is more useful than an irreproducible hypothesis test to analyze high throughput sequencing datasets. *ArXiv*, 1–15.

Garnier, S. (2017). viridis: Default Color Maps from "matplotlib." *R Package Version 0.5.1*, 1–6. Retrieved from https://cran.r-project.org/package=viridis

Gattuso, J.-P., Allemand, D., & Frankignoulle, M. (1999). Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. *American Zoologist*, *39*(1), 160–183.

Gattuso, J. P., Frankignoulle, M., Bourge, I., Romaine, S., & Buddemeier, R. W. (1998). Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change*, *18*(1–2), 37–46. https://doi.org/10.1016/S0921-8181(98)00035-6

Gattuso, Jean Pierre, Mach, K. J., & Morgan, G. (2013). Ocean acidification and its impacts: An expert survey. *Climatic Change*, *117*(4), 725–738. https://doi.org/10.1007/s10584-012-0591-5

Gutowska, M. A., Pörtner, H. O., & Melzner, F. (2008). Growth and calcification in the cephalopod Sepia officinalis under elevated seawater pCO2. *Marine Ecology Progress Series*, *373*, 303–309.

Hassenruck, C., Fink, A., Lichtschlag, A., Tegetmeyer, H. E., de Beer, D., & Ramette, A. (2016). Quantification of the effects of ocean acidification on sediment microbial communities in the environment: the importance of ecosystem approaches. *Fems Microbiology Ecology*, *92*(5). https://doi.org/ARTN fiw027 10.1093/femsec/fiw027

Haugan, P. M., & Drange, H. (1996). Effects of CO2 on the ocean environment. *Energy Conversion and Management*, *37*(6–8), 1019–1022.

Hendriks, I. E., Duarte, C. M., Olsen, Y. S., Steckbauer, A., Ramajo, L., Moore, T. S., … McCulloch, M. (2015). Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems. *Estuarine, Coastal and Shelf Science*, *152*, A1–A8. https://doi.org/10.1016/j.ecss.2014.07.019

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., … Hatziolos, M. E. (2007). Coral reefs under rapid climate change and ocean acidification. *Science (New York, N.Y.)*, *318*(5857), 1737–1742. https://doi.org/10.1126/science.1152509

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., … Takeshita, Y. (2011). High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PloS One*, *6*(12), e28983.

Hondt, S. D., Jørgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., … Acosta, S. (2004). *Esearch rticle*. (December), 2216–2222.

Hoppe-Jones, C., Oldham, G., & Drewes, J. E. (2010). Attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems. *Water Research*, *44*(15), 4643–4659. https://doi.org/10.1016/j.watres.2010.06.022

Horner-Devine, M. C., Lage, M., Hughes, J. B., & Bohannan, B. J. M. (2004). A taxa–area relationship for bacteria. *Nature*, *432*(7018), 750.

Hutchins, D. A., & Fu, F. (2017). Microorganisms and ocean global change. *Nature Microbiology*. https://doi.org/10.1038/nmicrobiol.2017.58

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., … von Dassow, P. (2008). Phytoplankton calcification in a high-CO2 world. *Science*, *320*(5874), 336–340.

Jaekel, U., Zedelius, J., Wilkes, H., & Musat, F. (2015). Anaerobic degradation of cyclohexane by sulfate-reducing bacteria from hydrocarbon-contaminated marine sediments. *Frontiers in Microbiology*, *6*, 116.

Joint, I., Doney, S. C., & Karl, D. M. (2011). Will ocean acidification affect marine microbes. *ISME Journal*. https://doi.org/10.1038/ismej.2010.79

Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2020). *Package ' adespatial .'* https://doi.org/10.1890/11-1183.1>.Maintainer

Kassambara, A., & Kassambara, M. A. (2020). *Package 'ggpubr.'*

Kitidis, V, Tait, K., Nunes, J., Brown, I., Woodward, E. M. S., Harris, C., … Kröger, S. (2017). Seasonal benthic nitrogen cycling in a temperate shelf sea: the Celtic Sea. *Biogeochemistry*, *135*(1), 103–119.

Kitidis, Vassilis, Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., … Widdicombe, S. (2011a). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters*. https://doi.org/10.1029/2011GL049095

Kitidis, Vassilis, Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., … Widdicombe, S. (2011b). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters*, *38*(21), n/a-n/a. https://doi.org/10.1029/2011GL049095

Kolda, A., Petrić, I., Mucko, M., Gottstein, S., Žutinić, P., Goreta, G., … Udovič, M. G. (2019). How environment selects: Resilience and survival of microbial mat community within intermittent karst spring Krčić (Croatia). *Ecohydrology*, *12*(2), e2063.

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., & Sass, H. (2005). Microbial diversity in coastal subsurface sediments: A cultivation approach using various electron acceptors and substrate gradients. *Applied and Environmental Microbiology*, *71*(12), 7819–7830. https://doi.org/10.1128/AEM.71.12.7819-7830.2005

Körtzinger, A., Hedges, J. I., & Quay, P. D. (2001). Redfield ratios revisited: Removing the biasing effect of anthropogenic CO2. *Limnology and Oceanography*, *46*(4), 964–970.

Köster, M., Dahlke, S., & Meyer-Reil, L. A. (2005). Microbial colonization and activity in relation to organic carbon in sediments of hypertrophic coastal waters (Nordrügensche Bodden, Southern Baltic Sea). *Aquatic Microbial Ecology*, *39*(1), 69–83. https://doi.org/10.3354/ame039069

Köster, M., Wardenga, R., & Blume, M. (2008). Microscale investigations of microbial communities in coastal surficial sediments. *Marine Ecology*, *29*(1), 89–105. https://doi.org/10.1111/j.1439-0485.2007.00219.x

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., … Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*(6), 1884–1896. https://doi.org/10.1111/gcb.12179

Lamim, V. B., & Procópio, L. (2021). Influence of Acidification and Warming of Seawater on Biofouling by Bacteria Grown over API 5L Steel. *Indian Journal of Microbiology*, *61*(2), 151–159. https://doi.org/10.1007/s12088-021-00925-7

Land, P. E., Shutler, J. D., Findlay, H. S., Girard-Ardhuin, F., Sabia, R., Reul, N., … Bhadury, P. (2015). Salinity from space unlocks satellite-based assessment of ocean acidification. *Environmental Science and Technology*. https://doi.org/10.1021/es504849s

Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., … BERMAN-FRANK, I. (2007). Elevated CO2 enhances nitrogen fixation and growth in the marine cyanobacterium Trichodesmium. *Global Change Biology*, *13*(2), 531–538.

Lewis, E. R., & Wallace, D. W. R. (1998). *Program developed for CO2 system calculations*. Environmental System Science Data Infrastructure for a Virtual Ecosystem.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J. P. (2010). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*. https://doi.org/10.3354/ame01446

Lomas, M. W., Hopkinson, B. M., Ryan, J. L. L. D. E., Shi, D. L., Xu, Y., & Morel, F. M. M. (2012). Effect of ocean acidification on cyanobacteria in the subtropical North Atlantic. *Aquatic Microbial Ecology*, *66*(3), 211–222.

Lozupone, C. A., & Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences*, *104*(27), 11436–11440.

Lukawska-Matuszewska, K., & Kielczewska, J. (2016). Effects of near-bottom water oxygen concentration on biogeochemical cycling of C, N and S in sediments of the Gulf of Gdansk (southern Baltic). *Continental Shelf Research*, *117*, 30–42.

Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.-M., Siegenthaler, U., … Kawamura, K. (2008). High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature*, *453*(7193), 379.

Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease*, *26*(0). https://doi.org/10.3402/mehd.v26.27663

Mandic, M., Ramon, M. L., Gracey, A. Y., & Richards, J. G. (2014). Divergent transcriptional patterns are related to differences in hypoxia tolerance between the intertidal and the subtidal sculpins. *Molecular Ecology*, *23*(24), 6091–6103. https://doi.org/10.1111/mec.12991

McArley, T. J., Hickey, A. J. R., Wallace, L., Kunzmann, A., & Herbert, N. A. (2019). Intertidal triplefin fishes have a lower critical oxygen tension (P crit), higher maximal aerobic capacity, and higher tissue glycogen stores than their subtidal counterparts. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *189*(3–4), 399–411. https://doi.org/10.1007/s00360-019-01216-w

McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R., … DeLong, E. F. (2010). Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proceedings of the National Academy of Sciences*, *107*(38), 16420–16427.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure 1. *Limnology and Oceanography*, *18*(6), 897–907.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., … Körtzinger, A. (2013). Future ocean acidification will be amplified by hypoxia in coastal

habitats. *Marine Biology*, *160*(8), 1875–1888.

Mercado, J. M., & Gordillo, F. J. L. (2011). Inorganic carbon acquisition in algal communities: Are the laboratory data relevant to the natural ecosystemš. *Photosynthesis Research*, *109*(1–3), 257–267. https://doi.org/10.1007/s11120-011-9646-0

Monier, A., Findlay, H. S., Charvet, S., & Lovejoy, C. (2014). Late winter under ice pelagic microbial communities in the high Arctic Ocean and the impact of short-term exposure to elevated CO2 levels. *Frontiers in Microbiology*, *5*(SEP), 1–12. https://doi.org/10.3389/fmicb.2014.00490

Morse, J. W., Andersson, A. J., & Mackenzie, F. T. (2006). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO2 and "ocean acidification": Role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, *70*(23), 5814–5830.

O'Malley, M. A. (2008). 'Everything is everywhere: but the environment selects': ubiquitous distribution and ecological determinism in microbial biogeography. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, *39*(3), 314–325.

Obi, C. C., Adebusoye, S. A., Ugoji, E. O., Ilori, M. O., Amund, O. O., & Hickey, W. J. (2016). Microbial communities in sediments of Lagos Lagoon, Nigeria: elucidation of community structure and potential impacts of contamination by municipal and industrial wastes. *Frontiers in Microbiology*, *7*, 1213.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P. M., … & Wagner, H. (2008). The vegan package. *Community Ecology Package*, (May 2014), 190. Retrieved from https://bcrc.bio.umass.edu/biometry/images/8/85/Vegan.pdf

Oliver, A. E., Newbold, L. K., Whiteley, A. S., & van der Gast, C. J. (2014). Marine bacterial communities are resistant to elevated carbon dioxide levels. *Environmental Microbiology Reports*, *6*(6), 574–582. https://doi.org/10.1111/1758-2229.12159

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., … Yool, A. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, *437*(7059), 681–686. https://doi.org/10.1038/nature04095

Papaspyrou, S., Gregersen, T., Cox, R. P., Thessalou-Legaki, M., & Kristensen, E. (2005). Sediment properties and bacterial community in burrows of the ghost shrimp Pestarella tyrrhena (Decapoda: Thalassinidea). *Aquatic Microbial Ecology*, *38*(2), 181–190. https://doi.org/10.3354/ame038181

Papaspyrou, S., Gregersen, T., Kristensen, E., Christensen, B., & Cox, R. P. (2006). Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes (Nereis diversicolor and N. virens). *Marine Biology*, *148*(3), 541–550. https://doi.org/10.1007/s00227-005-0105-3

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., … Fry, J. C. (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature*, *436*(7049), 390–394. https://doi.org/10.1038/nature03796

Partensky, F., Hess, W. R., & Vaulot, D. (1999). Prochlorococcus, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews*, *63*(1), 106–127.

Peters, H. (1997). Observations of Stratified Turbulent Mixing in an Estuary: Neap-to-spring Variations During High River Flow. *Estuarine, Coastal and Shelf Science*, *45*(1), 69–88. https://doi.org/10.1006/ecss.1996.0180

Philippot, L., Griffiths, B. S., & Langenheder, S. (2021). Microbial Community Resilience across Ecosystems and Multiple Disturbances. *Microbiology and Molecular Biology Reviews*, *85*(2), 1–24. https://doi.org/10.1128/mmbr.00026-20

Poremba, K., Tillmann, U., & Hesse, K. J. (1999). Tidal impact on planktonic primary and bacterial production in the German Wadden Sea. *Helgoland Marine Research*, *53*(1), 19–27. https://doi.org/10.1007/PL00012133

Power, J. F., Carere, C. R., Lee, C. K., Wakerley, G. L. J., Evans, D. W., Button, M., … Stott, M. B. (2018). Microbial biogeography of 925 geothermal springs in New Zealand. *Nature Communications*, *9*(1), 2876. https://doi.org/10.1038/s41467-018-05020-y

Price, N. N., Hamilton, S. L., Tootell, J. S., & Smith, J. E. (2011). Species-specific consequences of ocean acidification for the calcareous tropical green algae Halimeda. *Marine Ecology Progress Series*, *440*, 67–78.

Provoost, P., van Heuven, S., Soetaert, K., Laane, R., & Middelburg, J. J. (2010). Long-term record of pH in the Dutch coastal zone: a major role for eutrophication-induced changes. *Biogeosciences Discussions*, *7*(3).

Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics*, *12*(1), 38. https://doi.org/10.1186/1471-2105-12-38

Ralston, D. K., & Stacey, M. T. (2005). Stratification and turbulence in subtidal channels through intertidal mudflats. *Journal of Geophysical Research C: Oceans*, *110*(8), 1–16. https://doi.org/10.1029/2004JC002650

Rasmussen, H., & Jorgensen, B. B. (1992). Microelectrode studies of seasonal oxygen uptake in a coastal sediment: role of molecular diffusion. *Marine Ecology Progress Series*, *81*(3), 289–303. https://doi.org/10.3354/meps081289

Rassmann, J., Lansard, B., Gazeau, F., Guidi-Guilvard, L., Pozzato, L., Alliouane, S., … Rabouille, C. (2018). Impact of ocean acidification on the biogeochemistry and meiofaunal assemblage of carbonate-rich sediments: Results from core incubations (Bay of Villefranche, NW Mediterranean Sea). *Marine Chemistry*, *203*(May 2017), 102–119. https://doi.org/10.1016/j.marchem.2018.05.006

Reise, K. (2002). Sediment mediated species interactions in coastal waters. *Journal of Sea Research*, *48*(2), 127–141. https://doi.org/10.1016/S1385-1101(02)00150-8

Riebesell, U., & Zondervan, I. (2000). *Rie2000a*. *407*(September), 2–5.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., … Rios, A. F. (2004). The Oceanic Sink for Anthropogenic CO<sub>2</sub&gt. *Science*, *305*(5682), 367. Retrieved from http://science.sciencemag.org/content/305/5682/367.abstract

Sandoval-Gil, J. M., Barrote, I., Silva, J., Olivé, I., Costa, M. M., Ruiz, J. M., … Santos, R. (2015). Plant-water relations of intertidal and subtidal seagrasses. *Marine Ecology*, *36*(4), 1294–1310. https://doi.org/10.1111/maec.12230

Säwström, C., Hyndes, G. A., Eyre, B. D., Huggett, M. J., Fraser, M. W., Lavery, P. S., … Laverock, B. (2016). Coastal connectivity and spatial subsidy from a microbial perspective. *Ecology and Evolution*. https://doi.org/10.1002/ece3.2408

Schiel, D. R. (1988). Algal interactions on shallow subtidal reefs in northern New Zealand: A review. *New Zealand Journal of Marine and Freshwater Research*, *22*(3), 481–489. https://doi.org/10.1080/00288330.1988.9516317

Schloss, P. D., & Westcott, S. L. (2011). Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.02810-10

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*(3), 379–423.

Stevens, C. L. (2003). Turbulence in an estuarine embayment: Observations from Beatrix Bay, New Zealand. *Journal of Geophysical Research: Oceans*, *108*(2), 1–15. https://doi.org/10.1029/2001jc001221

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., … Midgley, P. M. (2013). Climate change 2013: the physical science basis. Intergovernmental panel on climate change, working group I contribution to the IPCC fifth assessment report (AR5). *New York*.

Sunda, W. G., & Cai, W. J. (2012). Eutrophication induced CO2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric P CO2. *Environmental Science and Technology*, *46*(19), 10651–10659. https://doi.org/10.1021/es300626f

Torsvik, V., xd, vre, xe, s, L., & Thingstad, T. F. (2002). Prokaryotic Diversity: Magnitude, Dynamics, and Controlling Factors. *Science*, *296*(5570), 1064–1066. Retrieved from http://www.jstor.org.ezproxy.waikato.ac.nz/stable/3076691

Villanueva, R. A. M., & Chen, Z. J. (2019). ggplot2: Elegant Graphics for Data Analysis (2nd

ed.). *Measurement: Interdisciplinary Research and Perspectives*, *17*(3), 160–167. https://doi.org/10.1080/15366367.2019.1565254

Vopel, K., Del-Río, C., & Pilditch, C. A. (2018). Effects of CO2 enrichment on benthic primary production and inorganic nitrogen fluxes in two coastal sediments. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-017-19051-w

Vopel, K., Laverock, B., Cary, C., & Pilditch, C. A. (2021). Effects of warming and CO2 enrichment on O2 consumption, porewater oxygenation and pH of subtidal silt sediment. *Aquatic Sciences*, *83*(1). https://doi.org/10.1007/s00027-020-00765-5

Washburne, A. D., Morton, J. T., Sanders, J., McDonald, D., Zhu, Q., Oliverio, A. M., & Knight, R. (2018). Methods for phylogenetic analysis of microbiome. *Nature Microbiology*, *3*(6), 652–661. https://doi.org/10.1038/s41564-018-0156-0

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences*, *95*(12), 6578–6583. https://doi.org/10.1073/pnas.95.12.6578

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wilde, S. B., & Plante, C. J. (2002). Spatial heterogeneity of bacterial assemblages in marine sediments: The influence of deposit feeding by Balanoglossus aurantiacus. *Estuarine, Coastal and Shelf Science*, *55*(1), 97–107. https://doi.org/10.1006/ecss.2001.0889

Wilms, R., Köpke, B., Sass, H., Chang, T. S., Cypionka, H., & Engelen, B. (2006). Deep biosphere-related bacteria within the subsurface of tidal flat sediments. *Environmental Microbiology*, *8*(4), 709–719. https://doi.org/10.1111/j.1462-2920.2005.00949.x

Witt, V., Wild, C., Anthony, K. R. N., Diaz-Pulido, G., & Uthicke, S. (2011). Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef. *Environmental Microbiology*, *13*(11), 2976–2989. https://doi.org/10.1111/j.1462-2920.2011.02571.x

Ysebaert, T., Herman, P. M. J., Meire, P., Craeymeersch, J., Verbeek, H., & Heip, C. H. R. (2003). Large-scale spatial patterns in estuaries: Estuarine macrobenthic communities in the Schelde estuary, NW Europe. *Estuarine, Coastal and Shelf Science*, *57*(1–2), 335–355. https://doi.org/10.1016/S0272-7714(02)00359-1

Ysebaert, Tom, & Herman, P. M. J. (2002). Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Marine Ecology Progress Series*, *244*(Levin 1992), 105–124. https://doi.org/10.3354/meps244105

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., … Ramette, A. (2011). Global Patterns of Bacterial Beta-Diversity in Seafloor and Seawater Ecosystems. *PLoS ONE*, *6*(9), e24570. https://doi.org/10.1371/journal.pone.0024570

Zondervan, I., Zeebe, R. E., Rost, B., & Riebesell, U. (2001). Decreasing marine biogenic calcification: A negative feedback on rising atmospheric pCO2. *Global Biogeochemical*

Cycles, *15*(2), 507–516. https://doi.org/10.1029/2000GB001321

Supplementary Data

Data QC, Sequencing Batch Correction, and Identification of Community Outliers

Hierarchal clustering with a fixed subset "set seed" value (1234) of the rarefied (subsampled without replacement) versus nonrarefied data was used to determine which method was appropriate for our specific dataset. An OTU matrix rarefaction curve was also run prior to any rarefying analyses to ensure that samples were sequenced to full completion (**Supplementary Figure 1.**). All samples were sequenced to full depth. Here we report only the non-rarefied dataset analyses, which were determined to be the statistical best fit and the most effective approach. Observations of the data identified the presence of statistical outliers (RE1: 1; RE3: 37) within the Active [RNA] community dataset, which were removed from the analysis. A "batch-effect" was also identified in the dataset due to samples being run across different sequencing plates, as all samples could not be processed in one sequencing run given limited sample space availability. Preliminary analysis demonstrated that the data ordinated based on sequencing run not by treatment or any other meta factor. To address this issue, the Limma Package in R (Gibbons et al., 2018) with the function "removebatcheffect" was applied to the dataset post rarefication (subsampled without replacement) and OTU table transformation (log(x+1)). The Limma corrected dataset was then transformed back from log-space (i.e., exponential) prior to downstream processing (Gibbons et al., 2018). We acknowledge differences in pseudo count between our dataset and the data presented in Gibbons et al., 2018. However, this method appears to have systematically corrected the batch-effect. The rarefied and "Batch Corrected" dataset was identified as the "better fit" compared to the non-rarefied and non-batch-corrected dataset. All reported analyses and results were determined and reported using the rarefied and batch-corrected dataset with removed outliers.

Chapter 3: Experiment 2

Supplementary Table 1. Describes the different pH ammendments for each of the different flow through chambers (Treatments) over the course of the experiment. It also designates which cores were randomly sampled on which dates and the method of intercore profiling sample replication per each sampled core.

T0

Treatment	pH	Core Number	Date Sampled	Replicate
RE1	8.10	25	October 5th	4 Replicates
RE1	8.10	44	October 5th	4 Replicates
RE2	7.50	50	October 5th	4 Replicates
RE2	7.50	34	October 5th	4 Replicates
RE3	7.70	37	October 5th	4 Replicates
RE3	7.70	52	October 5th	4 Replicates
RE4	7.90	33	October 5th	4 Replicates
RE4	7.90	54	October 5th	4 Replicates

T1

Treatment	pH	Core Number	Date Sampled	Replicate
RE1	8.10	36	October 20th	4 Replicates
RE1	8.10	32	October 20th	1x(Pooled)
RE1	8.10	1	October 20th	1x(Pooled)
RE2	7.50	22	October 20th	4 Replicates
RE2	7.50	15	October 20th	1x(Pooled)
RE2	7.50	29	October 20th	1x(Pooled)
RE3	7.70	20	October 20th	4 Replicates
RE3	7.70	17	October 20th	1x(Pooled)
RE3	7.70	28	October 20th	1x(Pooled)
RE4	7.90	45	October 20th	4 Replicates
RE4	7.90	42	October 20th	1x(Pooled)
RE4	7.90	9	October 20th	1x(Pooled)

T2

Treatment	pH	Core Number	Date Sampled	Replicate
RE1	8.10	6	November 11th	4 Replicates
RE1	8.10	7	November 11th	4 Replicates
RE1	8.10	38	November 11th	1x (Pooled)
RE1	8.10	41	November 11th	1x(Pooled)
RE1	8.10	43	November 11th	1x(Pooled)
RE2	7.50	11	November 11th	4 Replicates
RE2	7.50	14	November 11th	4 Replicates
RE2	7.50	19	November 11th	1x(Pooled)
RE2	7.50	23	November 11th	1x(Pooled)
RE2	7.50	51	November 11th	1x(Pooled)
RE3	7.70	2	November 11th	4 Replicates
RE3	7.70	5	November 11th	4 Replicates
RE3	7.70	10	November 11th	1x(Pooled)
RE3	7.70	16	November 11th	1x(Pooled)
RE3	7.70	30	November 11th	1x(Pooled)
RE4	7.90	21	November 11th	4 Replicates
RE4	7.90	24	November 11th	4 Replicates
RE4	7.90	35	November 11th	1x(Pooled)
RE4	7.90	48	November 11th	1x(Pooled)
RE4	7.90	49	November 11th	1x(Pooled)
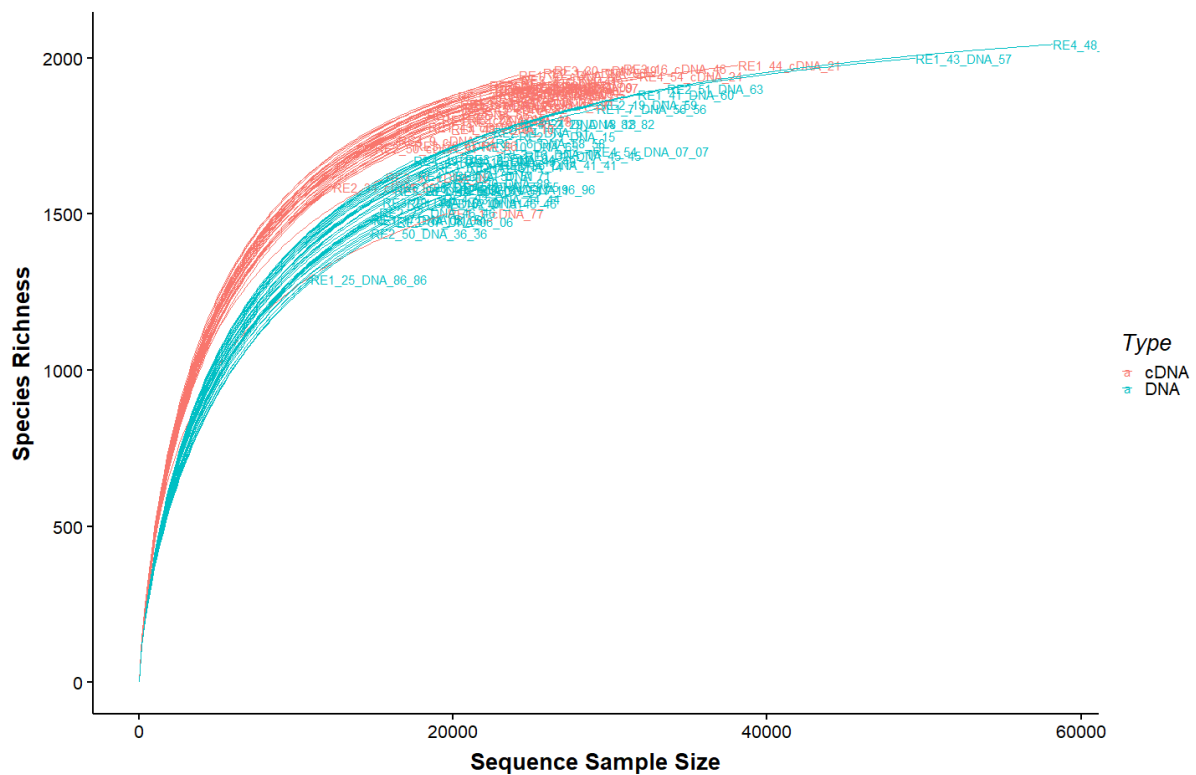
Supplementary Figure 1. A rarefaction curve generated using the ggplot2 package in R to visualize that all sediment samples have been sequenced to full completion and to verify how well each sample represents the total diversity by calculating species richness per each sample (N=48) across both nucleic acid types (cDNA/DNA). This plot demonstrates that all samples were sequenced to completion. Calculated differences between the minimum and maximum read counts determined that the need for rarefication based on the produced read count variation score was not high enough to justify rarefying the dataset for downstream analysis.
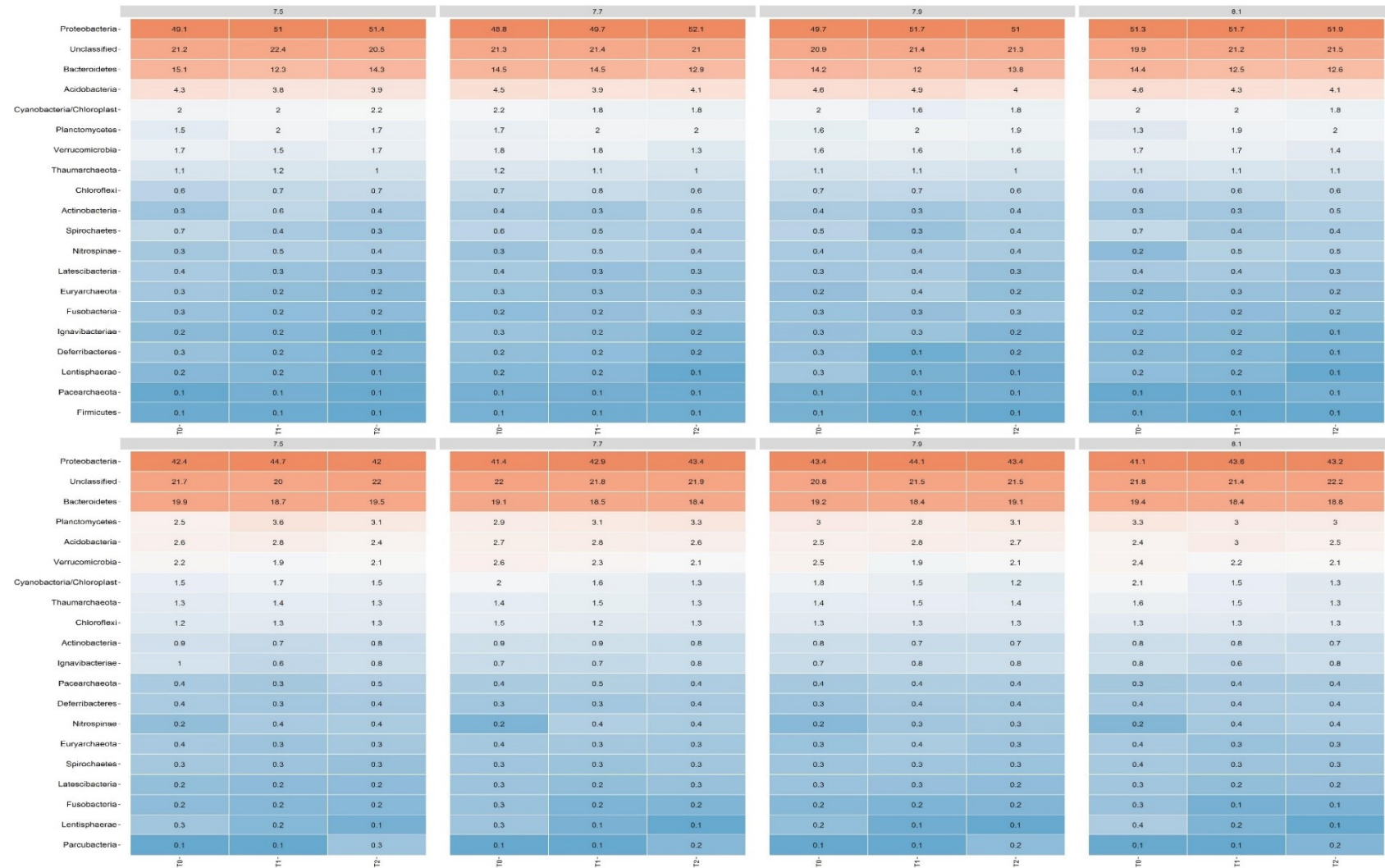
Supplementary Figure 2. A Heatmap of taxonomic abundance across each of the different pH Treatments occurring at Phylum level.

Chapter 3: Experiment 2

Supplementary Table 2. Analysis of the physicochemical subtidal sediment properties sampled from each treatment tank over the course of the experiment for wet weight (g), dry weight (g), organic content (g/%) and sediment porosity. Notes: determination of dry weight was done by maintaining the furnace temperature at 550C for 4 hours. All porewater samples had NO ultra pure water added before centrifugation. All porewater samples extracted from 50 mL of sediment. 0-1 cm = 2 × 0-1 cm cores of 2.6 cm diameter. 1-5 cm = 1 × 1-5 cm cores of 2.6 cm diameter (i.e., 4 cm deep × 2.6 cm diameter).

Experimental Treatment	Core #	Depth (cm)	Dish weight (g)	Wet weight + dish (g)	Dry weight + dish (g)	Furnace dry weight + dish (g)	Wet weight (g)	Dry weight (g)	Furnace dry weight (g)	Volume of sediment (cm3)	Organic content (g)	Organic content (%)	Porosity
RE1_27_8.1	27	0-1	0.8874	15.6435	5.3816	4.9762	14.7561	4.4942	4.0888	10.62	0.4054	9.020515	0.966281
RE1_27_8.1	27	1-5	0.8848	27.4552	9.6925	8.9201	26.5704	8.8077	8.0353	21.24	0.7724	8.769599	0.836285
RE1_40_8.1	40	0-1	0.8948	15.0255	5.3574	4.9638	14.1307	4.4626	4.069	10.62	0.3936	8.81997	0.910367
RE1_40_8.1	40	1-5	0.902	28.2134	9.8946	9.1326	27.3114	8.9926	8.2306	21.24	0.762	8.473634	0.862467
RE1_46_8.1	46	0-1	0.8883	17.4391	5.9852	5.5315	16.5508	5.0969	4.6432	10.62	0.4537	8.901489	1.078522
RE1_46_8.1	46	1-5	0.8972	27.0537	9.7971	9.0131	26.1565	8.8999	8.1159	21.24	0.784	8.809088	0.812458
RE2_18_7.7	18	0-1	0.8842	18.4883	5.7279	5.2672	17.6041	4.8437	4.383	10.62	0.4607	9.511324	1.201544
RE2_18_7.7	18	1-5	0.8938	24.6802	8.6062	7.9468	23.7864	7.7124	7.053	21.24	0.6594	8.549868	0.75678
RE2_26_7.7	26	0-1	0.8844	16.8434	5.679	5.2405	15.959	4.7946	4.3561	10.62	0.4385	9.145706	1.051262
RE2_26_7.7	26	1-5	0.8861	26.842	9.3467	8.5731	25.9559	8.4606	7.687	21.24	0.7736	9.14356	0.823696
RE2_3_7.7	3	0-1	0.8958	16.1714	5.8404	5.417	15.2756	4.9446	4.5212	10.62	0.4234	8.562877	0.972787
RE2_3_7.7	3	1-5	0.8952	25.9238	9.8021	9.0483	25.0286	8.9069	8.1531	21.24	0.7538	8.463102	0.759025
RE3_31_7.9	31	0-1	0.8963	17.4398	5.9685	5.5337	16.5435	5.0722	4.6374	10.62	0.4348	8.572217	1.08016
RE3_31_7.9	31	1-5	0.8942	27.3121	9.6938	8.9459	26.4179	8.7996	8.0517	21.24	0.7479	8.49925	0.829487
RE3_47_7.9	47	0-1	0.8873	15.2136	5.0226	4.6632	14.3263	4.1353	3.7759	10.62	0.3594	8.691026	0.959605
RE3_47_7.9	47	1-5	0.8874	29.532	10.687	9.8542	28.6446	9.7996	8.9668	21.24	0.8328	8.498306	0.887241
RE3_53_7.9	53	0-1	0.8978	17.5078	5.8482	5.4148	16.61	4.9504	4.517	10.62	0.4334	8.754848	1.097891
RE3_53_7.9	53	1-5	0.8894	26.1728	9.7525	9.0149	25.2834	8.8631	8.1255	21.24	0.7376	8.322145	0.773084
RE4_12_7.5	12	0-1	0.8875	15.4726	5.384	4.9888	14.5851	4.4965	4.1013	10.62	0.3952	8.789058	0.949962
RE4_12_7.5	12	1-5	0.8842	25.7538	9.177	8.4783	24.8696	8.2928	7.5941	21.24	0.6987	8.425381	0.780452
RE4_39_7.5	39	0-1	0.8847	14.532	5.0608	4.6866	13.6473	4.1761	3.8019	10.62	0.3742	8.960513	0.891827
RE4_39_7.5	39	1-5	0.8872	27.7958	10.3138	9.4865	26.9086	9.4266	8.5993	21.24	0.8273	8.776229	0.82307
RE4_4_7.5	4	0-1	0.8817	17.869	5.9251	5.4734	16.9873	5.0434	4.5917	10.62	0.4517	8.95626	1.124661
RE4_4_7.5	4	1-5	0.896	24.2952	9.0366	8.3672	23.3992	8.1406	7.4712	21.24	0.6694	8.222981	0.71839

Chapter 3: Experiment 2

Supplementary Table 3. Describes the raw data for the Salinity, DIC (Dissolved Organic Carbon: µmol/kg) and Alkalinity (µmol/kg) measurements collected from each experimental pH treatment tank over the course of the study.

	Salinity supplied	measured 16-24 April 2018	measured 17-24 April 2018
Sample: Date/Treatment	**Salinity:**	**DIC :**	**Alkalinity**
RE1: 8.1; RE2:7.7; RE3: 7.9; RE4: 7.5		umol/kg	umol/kg
250817-RE1	33.8	4239	4524
250817-RE1 repeat, 2x acid	33.8	4237	DIC was high, repeated measurment
250817-RE2	33.8	4430	4622
210817RE1	33.7	3630	3698
210817RE2	33.5	3614	3693
210817RE3	33.4	3856	4108
210817RE4	33.4	3770	3843
160817-RE1	33.3	3707	3788
160817-RE2	33.2	3663	3756
160817-RE3	33.2	3790	3882
160817-RE4	33.1	3865	3943
110917-RE1	34.0	3486	3957
110917-RE2	34.0	4554	4619
110917-RE3	33.6	3012	3386
110917-RE4	33.7	4750	4912
300817-RE1	33.9	4177	4501
300817-RE2	33.8	4555	4625
300817-RE3	33.2	3109	3184
300817-RE4	33.7	4788	4937
050917-RE1	33.7	3350	3484
050917-RE2	33.8	4536	4593
050917-RE3	33.8	2897	3021
050917-RE4	33.5	4737	4896
250817-RE3	33.8	3415	3488
250817-RE4	33.6	4746	4935

Supplementary Table 4. Demonstrates significant differences (*t*-test) in system seawater Total Alkalinity (TA), dissolved inorganic carbon (DIC), and salinity measurements from each of the mesocosm flow chambers per each treatment over time.

Treatments for Comparison (t-Test) p≤0.05	Alkalinity (µmol/kg)	DIC (Dissolved Inorganic Carbon) (µmol/kg)	Salinity
RE1/RE2	0.02*	0.002*	0.76
RE1/RE3	0.30	0.56	1
RE1/RE4	0.012	0.001*	0.18
RE2/RE3	0.03*	0.03*	0.76
RE2/RE4	0.007*	0.001*	0.04*
RE3/RE4	0.021*	0.02*	0.07
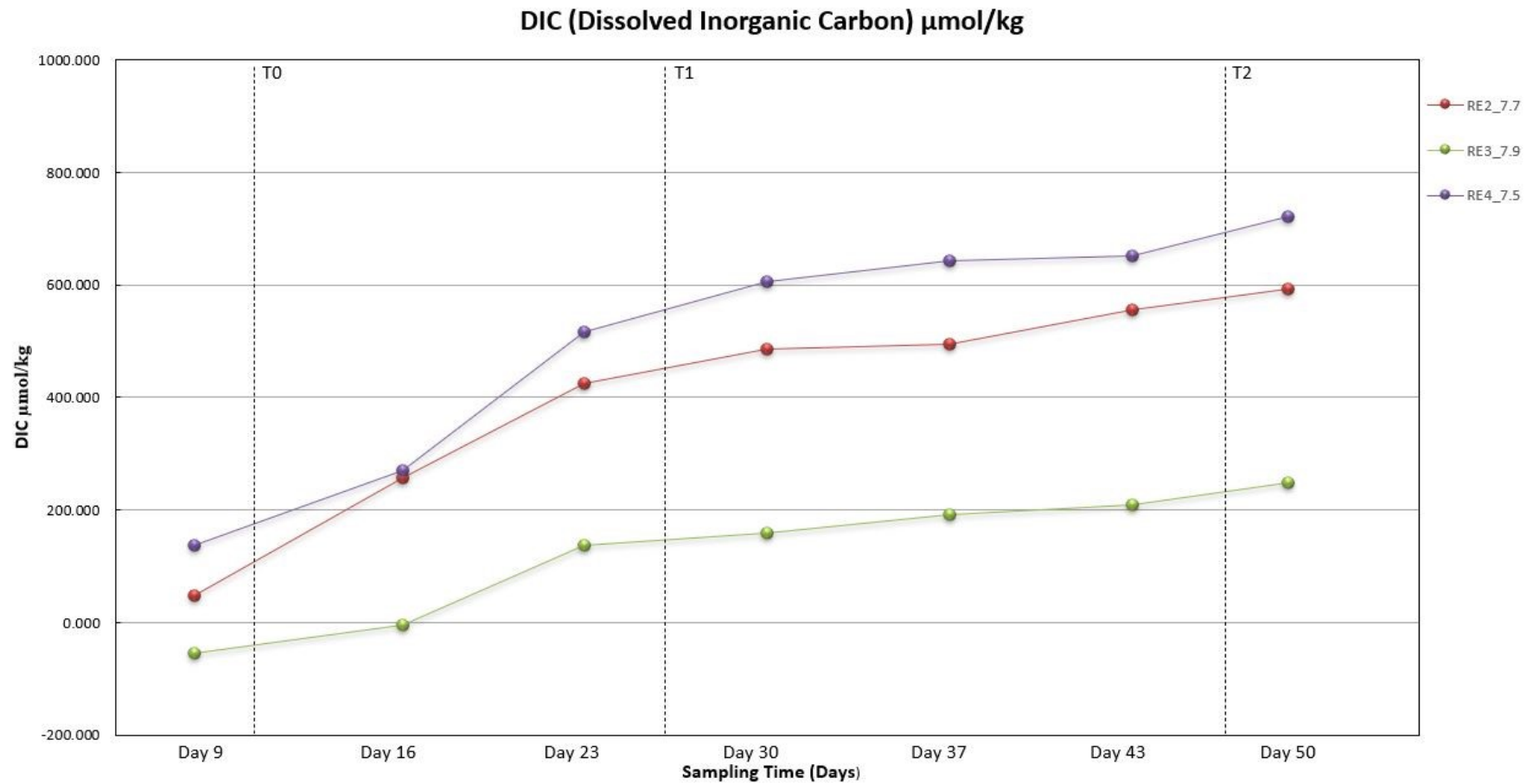
Supplementary Figure 3. Shows the seawater dissolved inorganic carbon (DIC) (µmol/kg) measurements for each of the pH Treatments over the course of the experiment averaged against the Control (RE1:8.1). DIC shows a linear increase overtime for each treatment. The two lowest pH Treatments are shown to have higher DIC concentrations.
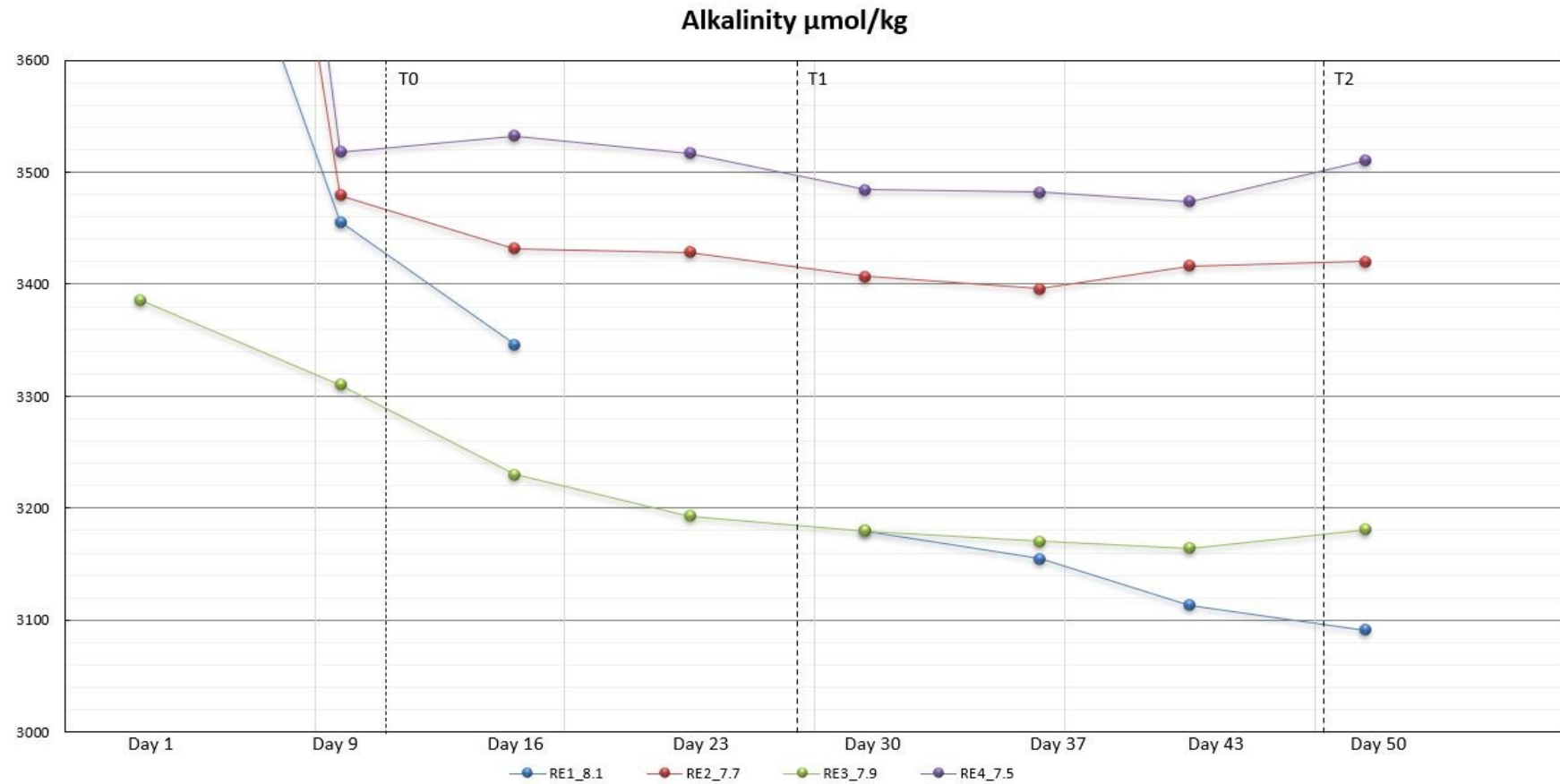
Alkalinity μmol/kg

Supplementary Figure 4. Shows the seawater Total Alkalinity (μmol/kg) measurements for each of the pH Treatments over the course of the experiment. Technical complications resulted in the inability to detect accurate readings for the Control (RE1: 8.1 pH) treatments at two sampling points (12/10/17; 17/10/17). Alkalinity is shown to decrease over time for each Treatment where TA is highest in the two lowest pH Treatments.

Chapter 3: Experiment 2

Supplementary Table 5. Shows the calculated averages and standard deviations for the different physicochemical sediment properties represented by the different pH treatments. Sediment samples were collected at depth intervals of 0-1 cm and 1-5 cm and measured for dry weight (g), organic content (g), porosity (pu) and sediment grain size using the 63 μm fractionation value.

Depth (cm)	Dry Weight (g)	Organic Content (g)	Porosity (pu)	Grain Size (63 μm)
RE1: 8.1 pH				
0-1 cm	4.70±0.3	0.417±0.02	0.98±0.07	3.07±0.04
1-5 cm	8.90±0.07	0.772±0.008	0.83±0.02	3.40±0.12
RE2: 7.7 pH				
0-1 cm	4.86±0.06	0.52±0.10	1.07±0.10	3.13±0.07
1-5 cm	8.36±0.50	0.65±0.16	0.78±0.03	3.51±0.43
RE3: 7.9 pH				
0-1 cm	4.71±0.41	0.40±0.03	1.04±0.06	3.07±0.12
1-5 cm	9.15±0.45	0.77±0.04	0.82±0.04	3.34±0.20
RE4: 7.5 pH				
0-1 cm	4.60±0.36	0.40±0.03	1.00±0.10	3.18±0.05
1-5 cm	8.62±0.60	0.73±0.07	0.77±0.04	3.30±0.05
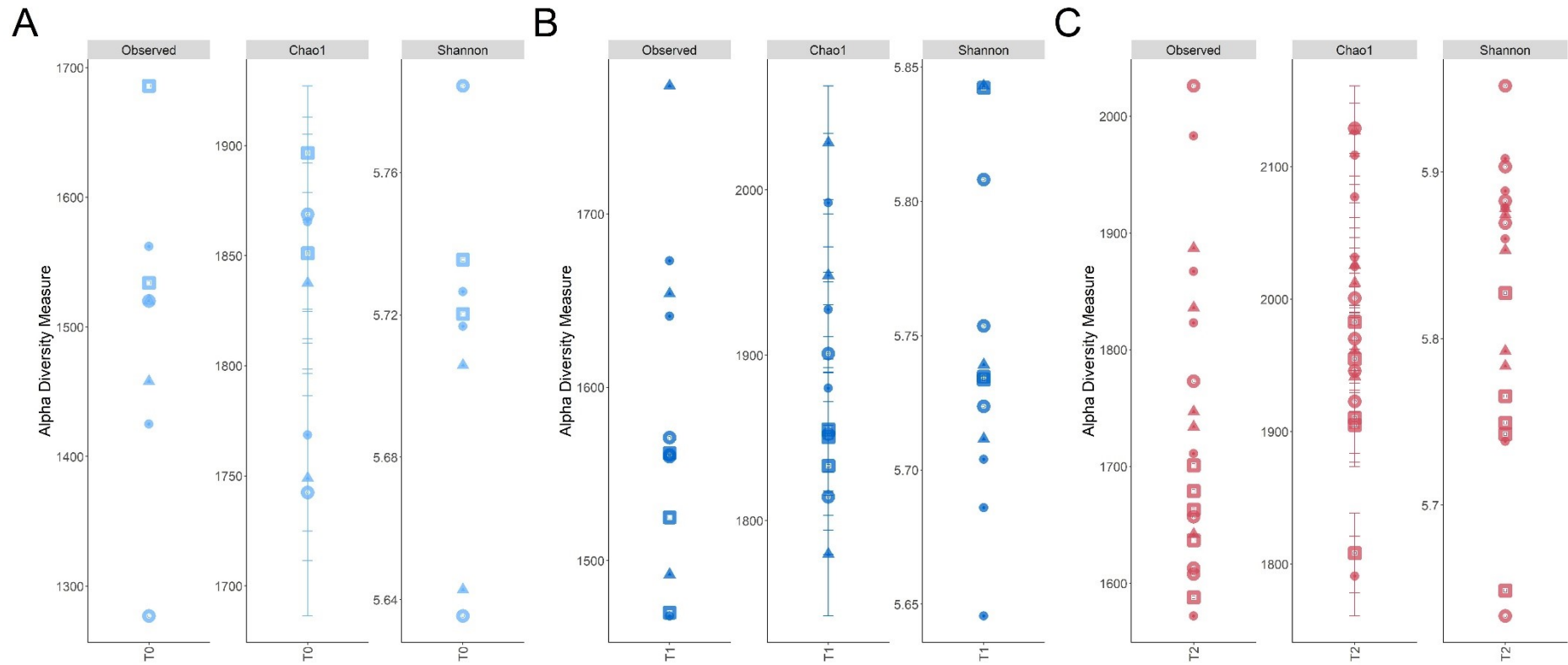
Supplementary Figure 5. Total [DNA] community alpha diversity metric analyses between the different pH treatments within an individual sampling time point (A: T0: 0$_{-7}$ days; B: T1: 21 days; C: T2: 35 days) axis (RE1: 8.1: Open Circle; RE2: 7.7: Closed Circle; RE3: 7.9: Triangle; RE4: 7.5: Square).
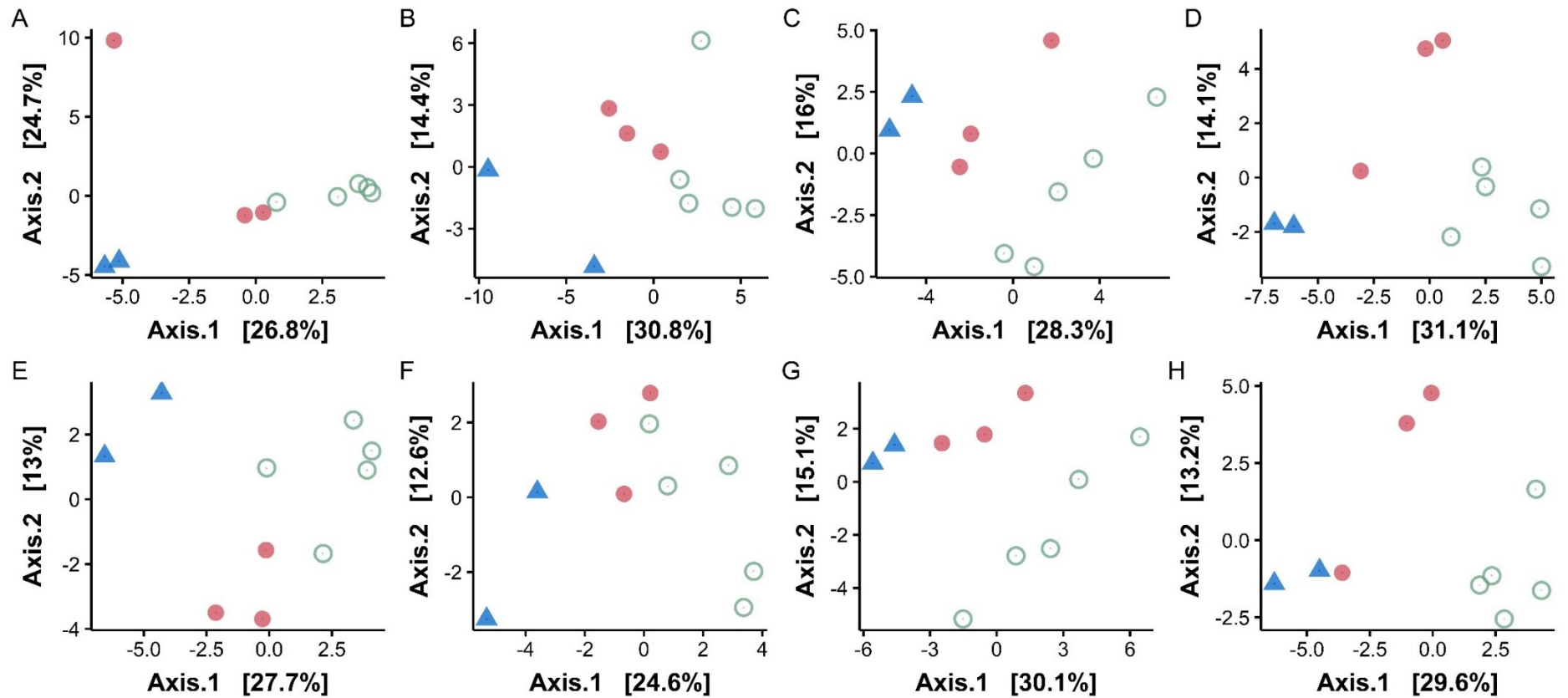
Supplementary Figure 6. Distribution of the Active [RNA] community (A-D) and Total [DNA] community (E-H) by pH treatment A & E= RE1: 8.1; B & F = RE3: 7.9; C & G= RE2: 7.7; and D & H = RE2: 7.5 ordinated by sampling time point T0: Blue Triangles; T1: Red Circles; T2: Green Circles) where *time* is the primary axis.

Sequence ID: **Query_12437** Length: **250** Number of Matches: **1**

Range 1: 1 to 250 Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
178 bits(196)	1e-49	194/254(76%)	8/254(3%)	Plus/Plus

```
Query    1   TACGGAGGGTGCAAGCGTTATCCGGAATTACTGGGTTTAAAGGGTGCGTAGGCGGCGGTT   60
             |||| |||||||||||||||| |||| ||||||||   ||||| | |||||||||| |||
Sbjct    1   TACGGGGGGTGCAAGCGTTATTCGGATTTACTGGGCGTAAAGCGCGCGTAGGCGGCCGTT   60

Query   61   CAAGTCAGGAGTGAAAGTTTGCGGCATAACCGCAGAATTGCTTTTGATACTGGATT--GC   118
             ||||||| ||||||| ||||   |||| |||| | ||| ||| |||||||||   |  ||
Sbjct   61   TAAGTCAGATGTGAAAGCCCGGGGCTCAACCCCGGAAGTGCATTTGATACT--ATTCGGC   118

Query  119   TTGAATTAGGTTGAGGTTAGCGGAAT--GTGGCATGTAGCGGTGAAATGCATAGATATGC   176
             |||| |   ||  |||| ||  ||||     ||||| ||||||||| | |||||||||
Sbjct  119   TTGAGTATGGGAGAGGGAAGTGGAATTCCTGG--TGTAGAGGTGAAATTCGTAGATATCA   176

Query  177   CATAGAACACCGATTGCGAAGGCAGCTAACTGGACCTGGATTGACGCTGAGGCACGAAAG   236
             |||||||| ||||| | |||||||||  || ||||||| | |||||||||||||| |||| |
Sbjct  177   GGAGGAACACCGGTGGCGAAGGCGACTTCCTGGACCAATACTGACGCTGAGGCGCGAAGG   236

Query  237   CGTGGGGAGCGAAC   250
             |||||||||| |||
Sbjct  237   CGTGGGGAGCAAAC   250
```

Supplementary Figure 7. NCBI nucleotide sequence comparisons for *Oceanospirillales* detected between Experiment 2. (OTU99) and Experiment 3. (OTU571) demonstrating only a 76% sequence overlap between the two OTU sequences.

Sequence ID: **Query_50141** Length: **250** Number of Matches: **1**

Range 1: 1 to 250 Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
130 bits(143)	7e-35	183/252(73%)	4/252(1%)	Plus/Plus

```
Query    1   TACGGAGGGTGCAAGCGTTGTTCGGAATCACTGGGCGTAAAGCGCTCGTAGGTGGT-ATG   59
             |||||||||||||||||||| | |||| ||| |||| ||||| | ||  || ||||  |||
Sbjct    1   TACGGAGGGTGCAAGCGTTATCCGGATTCATTGGGTTTAAAGGGTGCGCAGGTGGGGATG   60

Query   60   TGAAGTCTGGTGTGAAAGCCCTCGGCTCAACCGAGGAAGTGCGCTGGAAACCAGCATA-C   118
             | |||||  ||| ||||   |  | ||||||| || ||   || ||  || |||| | |
Sbjct   61   T-AAGTCAGTGGTGAAATACTGCCGCTCAACGGTAGAACTGCCATTGATACT-GCATCTC   118

Query  119   TGGAGTACGGTAGGGGATTGTGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGG   178
             | || | | ||| || |  ||||||||||||||||||||||||| ||||||||||| ||
Sbjct  119   TTGAATATGGTTGAGGTAGGCGGAATGTGTCGTGTAGCGGTGAAATGCATAGATATGACA   178

Query  179   AAGAACACCGGCGGCGAAAGCGACAATCTAAGCCGTTACTGACACTCAAGAGCGAAAGCG   238
             | |||||| |||| |||||||||  || |||||| ||| |||| |||||||||||||||
Sbjct  179   CAGAACGCCGATTGCGAAGGCAGCTTACTAAGCCATTATTGACGCTGAGGCACGAAAGCG   238

Query  239   TGGGGAGCAAAC   250
             |||||||| |||
Sbjct  239   TGGGGAGCGAAC   250
```

Supplementary Figure 8. NCBI nucleotide sequence comparisons for *Porphyrobacter* detected between Experiment 2. (OTU361) and Experiment 3. (OTU6921) demonstrating only a 73% sequence overlap between the two OTU sequences.

Supplementary Table 6. Describes the taxonomic information of the responding low tolerant taxa detected using ANCOM compositional statistical analyses for both the Total [DNA] and Active [RNA] Communities. Taxa labelled with a (*) denote reoccurring taxa between this and the previous study.

Community	OTU Number	Kingdom	Phylum	Class	Order	Family	Genus
Active [RNA]	OTU571	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales*	Unknown	Unknown
	OTU981	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Unknown	Unknown
	OTU435	Bacteria	Bacteroidetes	Cytophagia	Unknown	Unknown	Unknown
Total [DNA]	OTU427	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_incertae sedis	Porticoccus	Unknown
	OTU824	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Unknown	Unknown
	OTU15802	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Haliea
	OTU6921	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Porphyrobacter*

Chapter 3: Experiment 2

Chapter 4: Benthic Subtidal Sediment Communities Structurally Respond to the Addition of Organic Carbon and CaCO$_3$ Enrichments Under Differing Acidified Ocean Conditions

Shelly Brandt[1], Alexis Marshall[1], Kay Vopel[2], Conrad Pilditch[1], Adam Hartland[1], Charles Lee[1], and Craig Cary[1]

[1]The University of Waikato, Thermophile Research Unit
[2]The Auckland University of Technology

Abstract

Eutrophication-induced acidification is a result of excess nutrient loading from terrestrial activity. In addition to the impacts of ocean acidification (OA), eutrophication has been known to have a compounding impact on biogeochemical nutrient cycling and microbiological community dynamics within the coastal region. The susceptibility of various oceanic environments and ecosystems remains convoluted by natural system heterogeneity, variations in hydrological processes, and unique or specialized organisms within the environment. Each of these independently or together may increase system vulnerability to changes in pH. A carefully controlled mesocosm approach was used with the application of diel (day/night) cycling patterns, microelectrode profiling of O$_2$ and H$^+$, and microbial community analysis using 16S rRNA to assess the response of the microbial community to different organic, CaCO$_3$ amendments under low and ambient pH conditions. Results showed that the organically enriched active cDNA community structurally responded to the addition of CaCO$_3$, effectively buffering the effects of pH drawdown caused by excess nutrient loading. The microbial community displayed no structural differences to the effects of CO$_2$ sediment porewater carbonation, where the amendments had a decisively greater effect on microbial dynamics than the effects of OA alone. This study provides a first look at the combined interaction between eutrophication and CaCO$_3$ addition on microbial community structure and composition in nearshore benthic sediments under projected ocean acidification scenarios.

Introduction

The acidification of global seawater pH through the continued uptake of atmospheric CO_2 (Ocean Acidification (OA)) remains a well-known and prominent environmental and ecological issue (Mason, 2010). Though the chemical process of OA is well defined, much of the current research focuses on understanding the responsiveness of the pelagic environment to OA (Hurd et al., 2020; Nelson et al., 2020; Riebesell et al., 2013; Roy et al., 2013; Shi et al., 2019), with very little attention on investigating the benthic sedimentary ecosystem (Currie et al., 2017; Fink et al., 2017; Rassmann et al., 2018a; Ravaglioli et al., 2019; Simeone et al., 2018). Modern global ocean seawater pH (pH: 8.1) is ~0.1 pH units less than preindustrial, where recent data trends indicate a further decrease in surface water pH by 0.14-0.4 units by the year 2100, and with even greater decreases predicted for nearshore or coastal environments (Basso, 2012; Cai et al., 2011; Gattuso et al., 2015; Hagens et al., 2015). However, changes in coastal or nearshore oceanic pH levels are likely a result of a multitude of regional or local drivers such as excess nutrient loading, watershed processes, and changes in ecosystem structure or metabolism (Duarte, Hendriks, et al., 2013).

The interaction between atmospheric CO_2 emissions and the dynamic regional/local drivers within the coastal ecosystem enhances the complexity of pH regulation within coastal waters, where changes in water alkalinity of CO_2 fluxes, combined with microbial metabolic activity and natural ocean dynamics result in decadal changes of up to 0.5 pH units (Duarte, Hendriks, et al., 2013). The compounding effects of both ocean acidification and eutrophication induced acidification on coastal and nearshore environments demonstrate that these systems will experience a reduction in seawater pH that extend beyond what is predicted for open ocean seawater pH from OA alone (Melzner et al., 2013; Provoost et al., 2010; Sunda & Cai, 2012). This also suggests that given their predisposition to natural and anthropogenic stressors, these environments are likely to exhibit increased resilience to changes in oceanic seawater pH.

An estimated 50% of the global population lives within 100km of a coastline, meaning these regions are exposed to a high degree of human-related activity and stressors, with particular emphasis on excess nutrient loading and runoff (Ivan Valiela, 2009). Nearshore zones are chronically subjected to additional anthropogenic input in the form of high nutrient

loading (i.e., N, P), resulting in an increase in productivity and the rate of organic carbon supplied to the coastal and nearshore waters (Nixon, 1995). The introduction of excess nutrients within these environments results in a spike in the growth rate of algal biomass, where the resulting die-off deposits organic matter to the benthic sediments (i.e., Eutrophication Induced Acidification) (Körtzinger et al., 2001). This organic material is then rapidly consumed via heterotrophic microbially-driven aerobic respiration, which draws down O_2 (i.e., hypoxia) and subsequently releases additional CO_2, further promoting stochastic acidification events (Cai et al., 2011; Cloern, 2001; Heisler et al., 2008; Wallace et al., 2014). Oxygen depletion or *hypoxia* changes system dynamics and microbial/biogeochemical interactions, where the process of eutrophication can result in significant structural or functional changes at the microbial level (Cai et al., 2011). These compositional shifts would effectively disrupt natural nearshore ecosystem processes (Cai et al., 2011).

In nearshore environments, the concentration of pCO_2 is often much higher than what can be attributed to CO_2 atmospheric gas exchange alone. These environments are also exposed to increased levels of terrestrial carbon input, which leads to various other environmental side-effects such as eutrophication induced acidification, hypoxic regimes, and localized acidifications effects (Andersson et al., 2005; Cai et al., 2011; Gattuso et al., 2015; Regnier et al., 2013). The combined natural and anthropogenic impact on seawater pH is therefore likely to be more pronounced in nearshore environments than open ocean systems (Cai et al., 2011; Hagens et al., 2015; Orr, 2011).

For marine sediments, pH is a major determining factor associated with the highly complex transport-reaction network in terms of early diagenetic reactions (Boudreau et al., 1992; Jourabchi et al., 2005). Significant alterations to bottom-water pH are, therefore, likely to affect the biogeochemical processes occurring at the sediment-water interface as well as in the deeper, more stratified layers of the sediments (Rassmann et al., 2018a). These modifications in the essential biogeochemical pathways would ultimately result in negative feedback for carbonate chemistry processes within the water column and, in extension, influence the air-sea CO_2 exchange (Rassmann et al., 2018a). Modifications in bottom water chemistry may have dramatic consequences for benthic organisms where sedimentary carbonates dissolve as a consequence of reductions in water column pH (Andersson &

MacKenzie, 2012; Andersson et al., 2007; Andersson, 2015; Gazeau et al., 2014; Mackenzie et al., 2011).

Marine biominerals (e.g., $CaCO_3$) are exported from the ocean surface along various pathways, including being incorporated into additional biogenic particles (i.e., marine snow aggregates, fecal pellets etc.), which are then redistributed throughout the water column, with a fraction that settles out on the sediments below (40-60 Tmol $CaCO_3$ per year) (Battaglia et a., 2016; Berelson et al., 2007; Sulpis et al., 2018; Sulpis et al., 2021). Given the solubility of the different calcite species (calcite, aragonite, magnesium (Mg) calcites) and their particle flux concentrations as they settle through the water column, they are likely to be first responders to the effects of OA (Morse et al., 2006b). The saturation and alkalinity of seawater with respect to each $CaCO_3$ mineral is the determining factor behind mineral precipitation or dissolution (Mucci, 1983). Sediments enriched in $CaCO_3$ are expected to uptake excess CO_2, effectively buffering the effects of lower pH conditions and helping to maintain a more homeostatic environment for the sediment microbial communities (Morse et al., 2006a). A study by Bosak & Newman, 2003 demonstrated that the relationship between $CaCO_3$ and the microbial consortia may be more mutualistic than previously thought, where sulfate-reducing bacteria aid in stimulating the precipitation of carbonates through microbial nucleation of $CaCO_3$, which helps form modern-day stromatolites.

In nearshore sediments, microbial communities operate as the biological life force of the marine ecosystem, working to maintain the vast and complex network of biogeochemical cycles that aid in the preservation and regulation of major ecosystem processes (i.e., primary productivity). Previous studies have demonstrated both the sensitivity of microbial communities to the effects of OA (Borrero-Santiago et a., 2017; Currie et al., 2017; Hassenruck et al., 2016; Witt et al., 2011), as well as their inherent resilience to changes in pH (Edmonds et al., 2009; Kitidis et al., 2011a; Oliver et al., 2014;Chapter 2. and Chapter 3.). The varying response of different taxa to OA implies a more species-specific effect (Das & Mangwani, 2015; Joint, Doney, & Karl, 2011; Liu et al., 2010). Much work has gone into examining the effects of OA and of organic carbon enrichment (i.e., eutrophication induced acidification) (Aparicio et al., 2016; Malits et al., 2021; Provoost, Van Heuven, et al., 2010), and $CaCO_3$ as a potential system buffer (Drylie et al., 2019; Egleston, Sabine, & Morel, 2010; Harvey, 2008). Albeit these processes have all been studied independently, where arguably

this is not truly reflective of a natural marine environment. Respectively, these environments are likely subjected to different combinations of all three factors, particularly when addressing coastal or nearshore ecosystems.

It has long been assumed that the high alkalinity and buffering capacity of marine sediments will mitigate any impact of OA on the resident microbial community and their functional capacity as coastal biogeochemical mediators. Addressing whether coastal sediments are biologically resilient or geochemically resilient to OA remains a challenging element of any ecological study, where the tolerance of an organism may be due to its presenting physiology, or it may be attributed to the robustness of the environment in which it lives. Decoupling these processes is essential for assessing the realized impact of OA on coastal ecosystems. Here we hypothesize that buffering the sediments with $CaCO_3$ will resolve if the sediments microbial communities are biologically resistant to OA or the geochemical environment is providing transient protection. In this study, we examine the combined impacts of organic carbon loading (i.e., eutrophication) with a reduction in seawater pH (i.e., ocean acidification) on subtidal sediment microbial community diversity, composition, and structure under typical diel (day/night) cycles). We then apply $CaCO_3$ amendments to determine the effectiveness of calcite in buffering pH at the sediment surface and allowing for a potential stabilized environment for the resident microbial communities.

Methods and Materials

Field Sampling and Site Selection

A total of 48 intact subtidal sediment cores were SCUBA diver collected on June 11th, 2019, from a depth of 10 m using a modified universal gravity core system with core barrel dimensions of 30 cm x 9 cm from Man O' War Bay, Waiheke Island, located in the Hauraki Gulf, on the North Island of New Zealand (S 36° 47´ 38´´, E 175° 10´ 14´´) as specified in (Vopel et al., 2018; Vopel et al., 2021) (**Figure 1**.). The sampling site was selected for its well-characterized fine-grained silty sediment composition with naturally low levels of infaunation. Cores were sampled within a 1,200 m^2 site radius, taking precautions to avoid distinct macrofaunal habitats (i.e., shrimp burrows, sea urchin tracks). Once the sediment profiling (vertical depth = 20 cm) was complete and the intact core and overlying water column filled head space was secured within the acrylic tube (30 x 10 cm), both ends were capped using O-ring sealed lids to avoid sedimentary displacement as the core was carefully brought to the surface. Cores were then stored on ice aboard the vessel (~2.5-hour) to keep them below 15°C until they could be transported to the AUT (Auckland University of Technology) Marine Laboratory Facility. Upon arrival, the sediment cores were randomly allocated across four pre-established mesocosm flow chambers, submerged, and left to establish.

Flow Chamber Monitoring and System Properties

Four specially equipped flow-through mesocosm chambers, as previously described by (Vopel et al., 2018; Vopel, Laverock, et al., 2021), were used for the simulation of different pH environments and conditions. In brief, a 560 L volume of natural seawater was continuously recirculated in each independent flow tank system, navigating between an overhead mixing barrel and back into the flow tank below. There are arguments for and against the use of artificial seawater (ASW) over natural seawater (NSW) in manipulation studies, where one of the primary benefits is the standardized seawater chemical formula that allows for easy reproducibility between experiments. However, there has been increasing speculation that the use of ASW in complex mesocosm studies may unknowingly adjust the seawater alkalinity concentrations above the desired range, possibly introducing undesirable errors when measuring system seawater chemistry (Vopel et al., 2018). For this

study we aimed to maintain as much natural environmental complexity as possible. For that purpose, we used natural seawater collected proximal to the sediment sampling site.

Each of the flow chambers was pre-set to a controlled pH of 8.0 (pCO_2= 472 µatm). Tank temperature, salinity, and pH were carefully monitored daily under a 12-hour day/night circadian cycle simulation. The pH was controlled and monitored regularly through the automatic injection of CO_2-enriched air (5% CO_2, 21% O_2 in nitrogen), allowing for a procedural reduction in pH by 0.04 units per day (for a 10-day period) until the pH reached 7.7 (pCO_2 = 1216 µatm), where it was stabilized and maintained (for a further nine days) for the remainder of the experiment. Where the entire study was run over a course of 21 days from the time the cores were added to the tanks until the final sampling timepoint. The influx of carbonation within the seawater was regulated by the CapCtr Software Program (Loligo Systems Aps), with the use of a SenTix HWD electrode connected to a calibrated pH 3310-meter electrode (WTW), with a solenoid valve (Vopel et al., 2018). Artificial turbidity was achieved through turbulence caused by the particle filter and seawater jets returning water from the mixing barrel.

Seawater salinity measurements within each flow chamber were taken daily and subsequently adjusted over the course of the experiment. This was achieved using a conductivity meter (Knick Portamess® Conductivity Meter) and the addition of ultrapure H_2O to maintain the salinity within the range of 35.0 ± 0.3 (ppt (‰) parts per thousand) based on the field data collected at the sampling site, which showed a measured salinity reading of 34.5°C. Seawater temperature was also regularly monitored daily, where the average system seawater temperature was 16.3 ± 0.4°C. LED flood lights (Intensity ~equivalent to sunlight at midday based on measurements taken at the sampling site by (Vopel, Marshall, et al., 2021). were used for daylight simulation (7 am -7 pm), which introduced ~130 µmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) to the surface of the submerged subtidal sediment cores. Weekly seawater measurements were taken from each experimental unit (EU), where 1 L of seawater was sampled and analyzed for DIC (Dissolved Inorganic Carbon) using coulometry and total alkalinity (TA) using potentiometric titration. This was based on the SOP's (2 & 3a) described by Dickson et al., 2007 and Pierrot's adaptation of the $CO_2Sys.BAS$ program previously prescribed by Lewis & Wallace (1998) for seawater pCO_2 and pH (seawater scale, mol kg-SW^{-1}) computation. The HSO_4^- dissociation constant was based on

methodology from Dickson, 1990, the K_1 and K_2 values for carbonic acid measurements were based on Mehrbach et al. (1973) and refitted based on Dickson & Millero (1987). The CO_2Sys.BAS computations results showed that the incremental increase in injected CO_2-enriched air effectively increased seawater $p$$CO_2$ by a factor of ~2.6 and subsequently decreased seawater pH from 8.0 down to 7.7 (**Table 1**.: (Vopel, Marshall, et al., 2021).
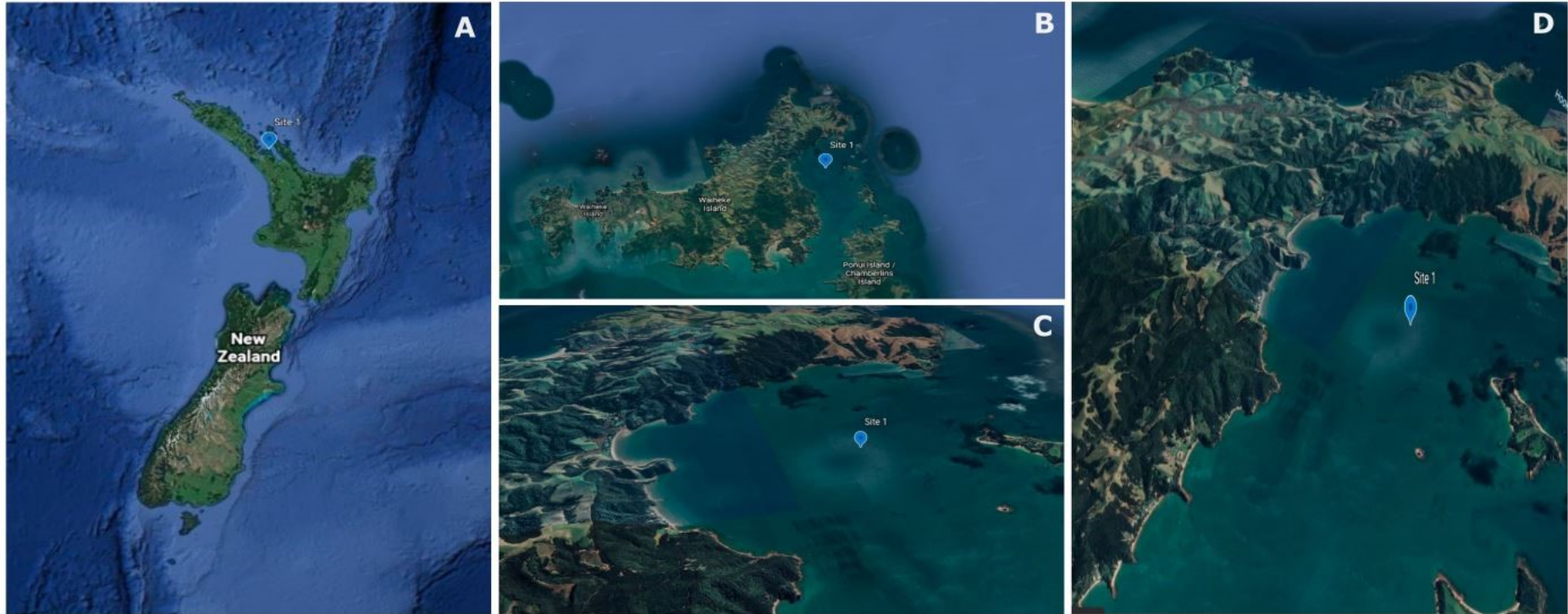
Figure 1. A.) Demonstrates the location of the sampling site in reference to the North Island of New Zealand. **B.)** Shows the sampling site proximal to Waiheke Island. **C.)** Indicates the subtidal region sampled in Man O' War Bay off the coast of Auckland. **D.)** Provides an aerial view of the sampling dive site located within the Hauraki Gulf (S 36° 47′ 38′′, E 175° 10′ 14′). All images are generated and used with permission (Google Earth Open Access).

Table 1. System properties for Ambient (pH 8.0) and CO_2 Enriched (pH 7.7) seawater averaged between the different mesocosm flow chambers (Mean ± SD). Temperature (C^o) and salinity were calculated using a combined average of the daily measurements taken over 22 days. Dissolved inorganic carbon (DIC) and total alkalinity (TA) ($\mu mol\ kg\ SW^{-1}$) were calculated using five measurements collected over the final ten days of the study. Seawater pH (total scale), $[H^+]$ ($nmol\ L^{-1}$), pCO_2 (μatm), $[CO_3^{2-}]$ ($\mu mol\ kg\ SW^{-1}$), Ω_{CA} ($CaCO_3$ Saturation) and Ω_{AR} (Aragonite Saturation) were based on the measured parameter for a temperature of $15^o C$).

	Ambient (pH 8.0 = 472 µatm)	CO_2 Enriched (pH 7.7 = 1216 µatm)
Measured Parameters:		
Temperature (°C)	14.6 ± 0.15	14.7 ± 0.16
Salinity (‰)	34.5 ± 0.07	34.5 ± 0.06
DIC ($\mu mol\ kg\ SW^{-1}$)	2112 ± 5	2261 ± 13
TA ($\mu mol\ kg\ SW^{-1}$)	2302 ± 10	2314 ± 10
Calculated Parameters at $15^o C$:		
pH_T (Total Scale)	7.99 ± 0.01	7.62 ± 0.03
$[H^+]$ ($nmol\ L^{-1}$)	10.3 ± 0.2	23.6 ± 0.9
pCO_2 (μatm)	472 ± 7	1216 ± 88
$[CO_3^{2-}]$ ($\mu mol\ kg\ SW^{-1}$)	139 ± 3	66 ± 4
Ω_{CA}	3.32 ± 0.06	1.57 ± 0.09
Ω_{AR}	2.13 ± 0.04	1.01 ± 0.06

Chapter 4: Experiment 3

Experimental Design and Amendment Application

Experimental Layout

Of the 48 intact undisturbed subtidal sediment cores collected, 12 cores we allocated per individual tank. Each pH treatment was represented by two tanks. In one tank, the sediment received a 1 mm layer of Ashed Sand (AS) as an amendment (procedural) control (Figures 2a. and 2b.). The Ashed Sand particles were heated at 550°C for 4 hours to remove trace organic carbon, sieved to exclude particles >125 μm, and then re-heated for a second time at 840°C for 1 hour to remove residual $CaCO_3$ (Vopel, Marshall, et al., 2021). Within the second tank the cores received a 1 mm layer of $CaCO_3$ (Tank 1 (pH 8.0): AS n=6; Tank 2 (pH 8.0): $CaCO_3$ n=6; Tank 3 (pH 7.7): AS n=6; Tank 4 (pH 7.7): $CaCO_3$ n=6) (Figures 2a. and 2b.). To achieve this, we briefly ground (Omni Ruptor 4000 Ultrasonic Homogenizer) bleach sterilized and rinsed oyster shells (biogenic calcite FTIR (Fourier Transform Infrared Spectroscopy) and sieved the material to remove particles >125 μm. We weighed 2 g of this material into 12 seawater-filled 100mm diameter Petri-dishes to create a consistent 1 mm thick layer at the bottom of the petri dish. The content of each petri dish was then frozen at -80C. To resulting frozen seawater/calcite disks were placed into the headwater space in cores within Tank 1 (n = 12) and Tank 3 (n = 12). As the disk thawed, an even layer of calcite was distributed onto the surface of the sediment core (**Supp. Figure 7**.).

To avoid disturbance of settling particles, the cores were isolated from their surrounding seawater until the surface layer of calcite had formed (~20 min). A 1 mm surface layer of 125 μm Ashed Sand particles were added to the cores within Tank 2 (n=12) and Tank 4 (n=12) following the same technique. Here, each petri dish received 4 g of sand particles that had been heated for 4 h at 550°C to remove organic carbon and then sieved to remove particles >125 microns. After sieving, the sand was re-heated for a second time for 1 h at 840°C to remove any remaining $CaCO_3$. Once the amendment layers were added, the cores were left to establish over a 10-day period, where during this time the pH was incrementally decreased in two of the mesocosm tanks by 0.04 units per day to stabilize at a final pH of 7.7 at pCO_2 1216 μatm. Vertical O_2 microprofiles measurements were used to determine diffusive sediment seawater O_2 exchange and verify the depth integrated sediment O_2 production through sediment porewater analysis (Vopel, Marshall, et al., 2021).

An organic matter amendment containing 2.60 g wet weight Thrive Natural Seaweed 100% Bull Kelp was additionally added to three cores in each tank on both day 11 and day 15 to address the added impacts of organic matter enrichments under acidified conditions with and without the presence of $CaCO_3$ (**Table 2**.). This amendment was applied by freezing the liquid seaweed material at -80°C into 12, 100 mm diameter Petri-dishes to create a consistent, 1 mm thick layer at the bottom of the petri dish. Once frozen, 10 ml of seawater from the control tanks was added and frozen at -80°C. The frozen discs were gradually defrosted within the head water of each core with the organic matter layer facing down, approximately 5 cm above the sediment surface with $CaCO_3$ or Ashed Sand. Freezing the disc in two steps enabled the organic matter layer to detach from the frozen water layer to generate a consistent layer of intact amendment. The OC amendment remained in place on each of the cores for four days prior to sampling. The average wet weight after sampling was 2.31 g (RE1: 2.27 g; RE2: 2.26 g; RE3: 2.36 g; RE4: 2.34 g).
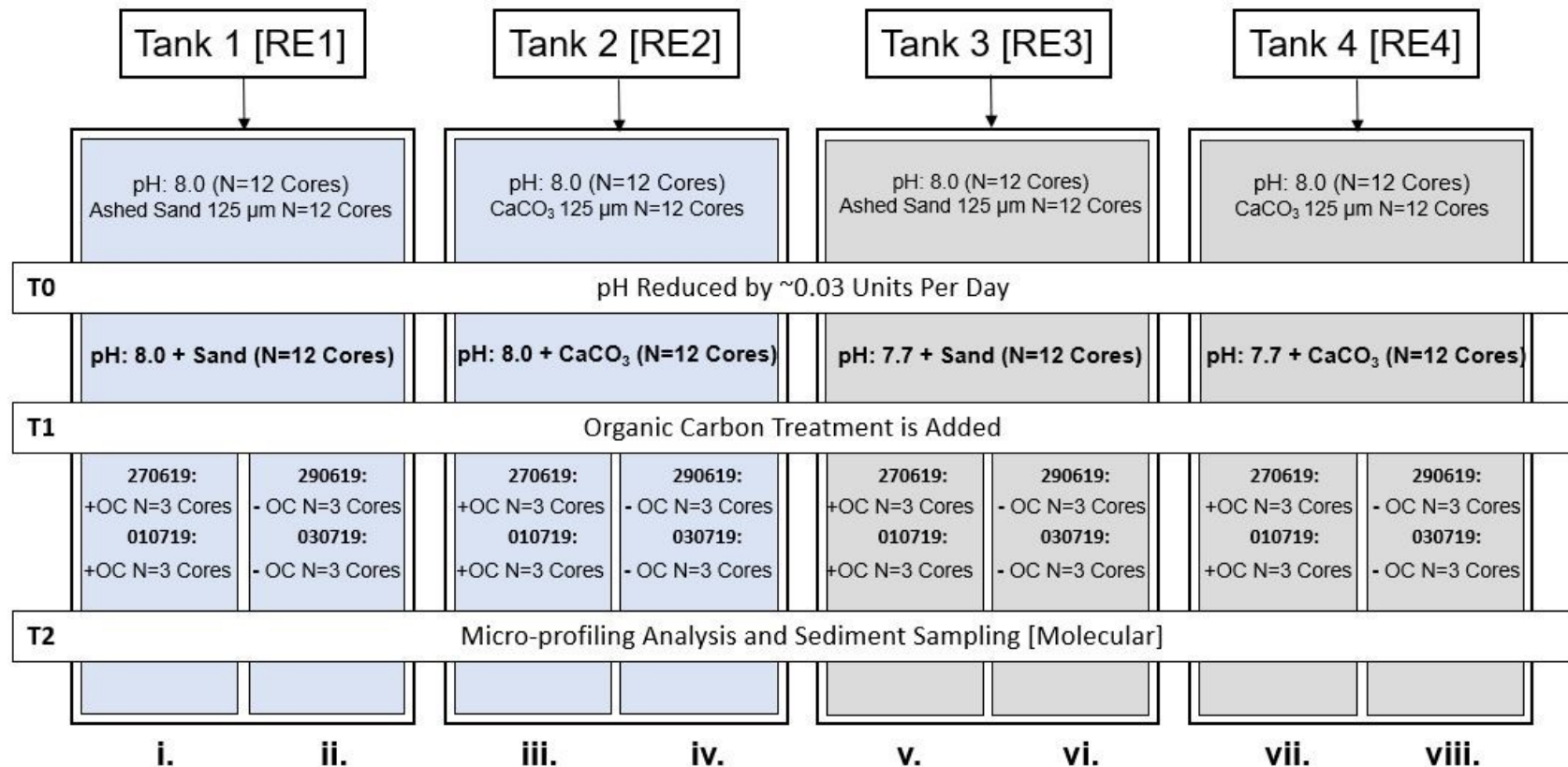
Figure 2a. Describes the experimental framework for the study, in terms of the allocation of sediment cores (12 per flow tank) (RE1-RE4), their associated pH treatments (Ambient: 8.0 pH; Enriched: 7.7 pH), and their respective amendment additions. T0, marks the start of the experiment where *all* sediment cores were added to their respective flow chambers, at a universal ambient pH (8.0 pH). At this time, selected cores also received either Ashed Sand (AS) or CaCO₃ (CC) additions. The system was left to establish for 10 days, where during this time the pH was incrementally reduced in both RE3 and RE4 flow chambers. On day 10 (T1), the organic carbon (OC) enrichment was added. The 270619 and 010719 sampling dates represent the treatments (AS/OC + CC/OC), where the 290619 and 030719 sampling dates signify the nontreatment controls (AS/NTC + CC/NTC).

i.   n=6 pH 8.0 Ashed Sand (AS)(T0); addition Organic Carbon (OC)(T1)
ii.   n=6 pH 8.0 Ashed Sand (AS)(T0); without Organic Carbon (OC)(T1)
iii.   n=6 pH 8.0 Calcium Carbonate (CC)(T0); addition Organic Carbon (OC)(T1)
iv.   n=6 pH 8.0 Calcium Carbonate (CC)(T0); without Organic Carbon (OC)(T1)
v.   n=6 pH 7.7 Ashed Sand (AS)(T0); addition Organic Carbon (OC)(T1)
vi.   n=6 pH 7.7 Ashed Sand (AS)(T0); without Organic Carbon (OC)(T1)
vii.   n=6 pH 7.7 Calcium Carbonate (CC)(T0); addition Organic Carbon (OC)(T1)
viii. n=6 pH 7.7 Calcium Carbonate (CC)(T0); without Organic Carbon (OC)(T1)

234
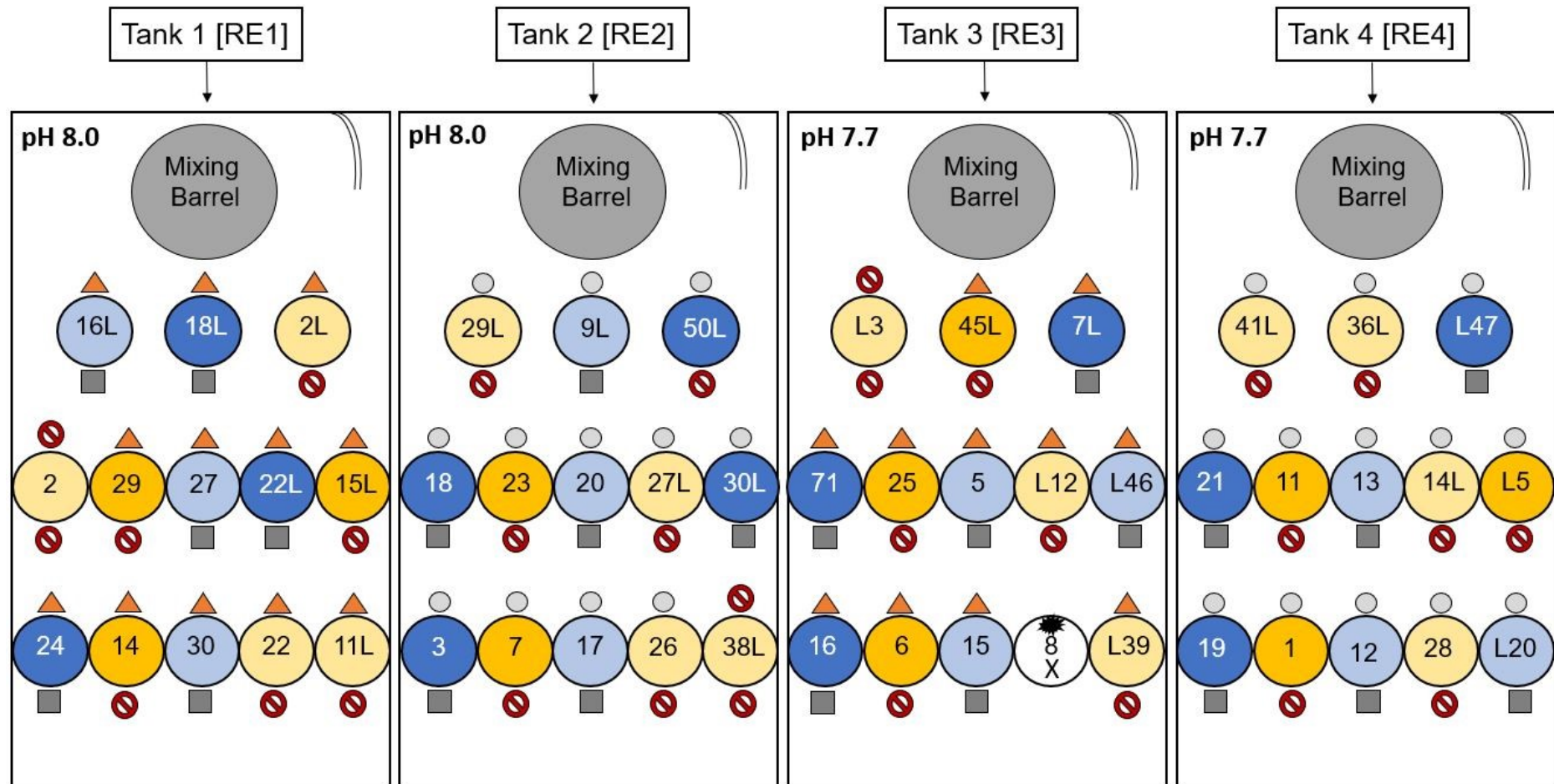
Figure 2b. Demonstrates which cores were sampled on which days for both the Ashed Sand (AS)/Organic Carbon (OC) and Calcium Carbonate CaCO$_3$ (CC)/Organic Carbon (OC) treatments (Blue), and the Ashed Sand (AS)/No Treatment Control (NTC) and Calcium Carbonate CaCO$_3$ (CC)/No Treatment Control (NTC) controls (Yellow). It also denotes the treatments received by each core, where the symbol located above the core shows "Treatment One" and the symbol located underneath the core shows "Treatment Two." The mixing barrel is located above the flow tank, with adjacent inflow tube located to the right.

Sediment Profiling for Molecular Analysis

Sediment Collection and Preservation for DNA and RNA

Cores were visually inspected prior to sampling, and all observations of the core's characteristics were recorded (i.e., amendment integration, worm lines, diatom formation, surface sediment, or treatment heterogeneity). The surface of the silt sediment (~4 mm) was carefully sampled using modified pre-packaged sterile transfer pipettes. These devices enabled us to sub-sample small sections of sediment to capture inter-core variation whilst avoiding the oxic/anoxic sediment interface distinguishable by a visual change in sediment color. The sampled sediment was split into triplicates (~2 - 3g wet weight sediment per subsample) in 15 ml falcon tubes containing 3 ml of MoBio Lifeguard™ RNA Preservation Agent (©Qiagen 2013-20). Samples were homogenized and frozen at -80°C to await nucleic acid extraction.

Physicochemical Sediment Analyses

Sediment and pore water were sampled using a 50 ml modified pre-cut sterilized plunge corer to profile a ~10 cm x 3 cm core from within the undisturbed section of the larger core for physicochemical analysis. Loss of weight was determined using combustion analysis on the upper 10 mm of the silt sediment layer by first drying the sediment at 60°C and then combusting it at 550°C to assess porewater, wet weight, dry weight, organic content (OC), calcium carbonate content ($CaCO_3$ (CC)), and pigments (Chlorophyll a/Phaeopigments). Particle size fractionation (Grain size = 63 μm - Man O' War Bay sediments) was conducted using ~10 ml sample of the homogenized silt sediment and analyzing it with the Malvern Mastersizer 2000© (Malvern Panalytical Ltd., United Kingdom). A second sub-core core was carefully removed intact and capped with a well-fitted rubber stopper, wrapped in parafilm, and transported on ice (Waikato University, Thermophile Research Unit), and stored frozen at -80°C until further physicochemical sediment property analysis. This was collected at the same time as the molecular sediment samples. Core number 8, from the RE3 tank (pH 7.7), was found to be confounded by a large sea urchin and was removed from the study.

Chapter 4: Experiment 3

Nucleic Acid Co-Extraction, Quantification & RT-PCR

Frozen sediment samples were defrosted on ice and centrifuged (2,500 RPM: 5 minutes) to separate off sediment pore water and the added Lifeguard preservation agent. Total RNA and DNA were extracted from 48 samples and the organic matter amendment using the MoBio RNA Powersoil Total RNA Isolation Kit and the DNA Elution Accessory Kit based on the manufacturer's instructions (Qiagen Inc.: Germantown, MD, USA). DNA and RNA were quantified (Quant-it ^{TM} PicoGreen High Sensitivity RNA and HS DNA Assay Kits) (Invitrogen, Thermo Fisher Scientific, Basingstoke, UK)) and quality was visually inspected via gel electrophoresis and using the Nanodrop^{TM} 1000 Spectrophotometer (Thermo Fisher Scientific). Total RNA was treated with the Turbo DNA-Free^{TM} Kit (Thermo Fisher Scientific) to remove DNA carryover within the samples based on the manufacturer's instructions. Low residual DNA carryover was confirmed using qPCR (Mackay, 2004). The production and synthesis of cDNA from single-stranded RNA were processed using the SuperScript® IV First-Strand Synthesis System (Thermo Fisher Scientific) with random hexamers based on the manufacturer's instructions.

PCR Amplification and Ion Torrent PGM Sequencing

Amplification of the V4 region of the 16S rRNA gene using PCR (Polymerase Chain Reaction) was done using the Earth Microbiome Ion Torrent Fusion Primers as described by Parada et al., 2016 and Quince et al., 2011. Reverse transcribed cDNA and Total DNA was amplified in triplicate using a 5 ng concentration of each nucleic acid type with a PCR reaction volume of 25 µl/per 1 reaction for the following master mix volumes and concentrations H_2O (Adjusted to 25 µl Total); 0.8 µl BSA (Bovine Serum Albumin) (0.4 mg/ml) (Promega Corporations, USA); 3 µl of DNTP's (2 mM) (Invitrogen Ltd. New Zealand); 3 µl of 10× PCR Buffer (Invitrogen Ltd. New Zealand); 3 µl of M_gCl_2 (50 mM); 0.12 µl of Taq Polymerase Enzyme (0.12 µl) (Invitrogen Ltd. New Zealand); 0.5 µl of Forward Primer (10 mM) and 0.5 µl of Reverse Primer (10 mM) (Integrated DNA Technologies Inc.); 2 µl of cDNA or DNA (5ng/µl) respectively. Thermocycler conditions were set at 94ºC for 3 minutes followed by 30 cycles of 94ºC for 45 seconds, 50 °C for 1 min and 72 °C for 1.5 minutes, and a final extension step of 10 minutes at 72 °C with an infinite hold at 4 °C. The produced PCR amplicons were pooled together and visually analyzed on a 1% agarose gel (1% TAE, SYBER safe) (Invitrogen). The

SequelPrep[TM] Normalization Kit (96 well plate format) was used to normalize PCR product concentrations. Normalized products were then pooled together in a 1.5 ml microcentrifuge tube and re-quantified prior to sequencing (Quant-it [TM] PicoGreen High Sensitivity RNA and DNA Assay Kits) and stored at 4°C prior to sequencing. Sequence amplification quality was assessed using the Ion PGM[TM] IA 500 Kit (Thermo Fisher Scientific, Massachusetts, USA) and sequenced using the Ion Torrent PGM[TM] System with the use of an Ion 318v2 chip (Thermo Fisher Scientific, Massachusetts, USA) at The University of Waikato Sequencing Facility, Hamilton, New Zealand.

Sequence reads without full-length forward or reverse primers were discarded (10% error allowance, Cutadapt v1.9; Martin 2011). A sliding window of 50 bp, Q-score average 33 were applied (Trimmomatic v0.32; Bolger et al. 2014). All read lengths below set quality filter parameters were discarded. Trimmed reads were processed following the recommended DADA2 pipeline (v1.14.1) (Callahan et al. 2016) to call amplicon sequence variants (ASV). For the past decade, OTU's (Operational Taxonomic Units) provided the most standard approach for identifying and clustering raw sequence data using a 97% cutoff threshold. However, more recent evolutions in bioinformatic based approaches have identified the use of ASV's (Amplicon Sequence Variants) as more the robust and effective methodology. ASV's avoid the 97% OTU cutoff limitation and are able to differentiate even minor differences in sequence reads by as much as a single nucleotide variation in order to identify unique ASV's (Callahan, McMurdie, & Holmes, 2017). Here we apply the ASV approach as the desired method of atomic unit analysis to our dataset for higher resolution and greater reproducibility. The table of quality-controlled read counts was filtered using a minimum ASV abundance cut-off threshold of 0.005% as recommended when a mock community is not incorporated (Bokulich et al., 2013). Taxonomy prediction was called against the SILVA database v138 (Quast et al. 2013) with DECIPHER 2.14.0 (Wright 2016) and a confidence threshold cut-off at 0.8. The package "Decontam" was used to identify and remove procedural contaminants (**Supp. Figures 2a.-3b**.) resulting in a final dataset represented by 1,734 [DNA] and 2,184 [RNA] ASVs. The unweighted pair group method with arithmetic mean (UPGMA) was used to construct the phylogenetic tree from the list of ASVs remaining after the threshold abundance cut-off was applied in MUSCLE (Edgar 2004a, b).

Chapter 4: Experiment 3

Post Sequencing QC and Bioinformatic Analyses

Sequence data was imported into the R software program (Version 4.0.3) (R Core Team 2020-10-10) and statistically analyzed using the corresponding packages and their subsequent dependencies for microbial compositional analysis: Phyloseq (McMurdie & Holmes, 2013); ggplot2 (Villanueva & Chen, 2019); vegan (Oksanen et al., 2008); tidyverse (Wickham et al., 2019); microbiomeSeq (Ssekagiri et al., 2017); ecodist (Goslee & Urban, 2007); factoextra (A. Kassambara, 2016); picante (Kembel et al., 2010); viridis (Garnier, 2017); adespatial (Jombart et al., 2020); tibble (Wickham et al., 2020); ampvis2 (Andersen et al., 2018); grid(Murrell, 2003); gridextra(Auguie & Antonov, 2017); rfUtilities (Evans and Murphy, 2019); caret (Kuhn, 2011); philr (Washburne et al., 2018); ape (Paradis et al., 2004); CoDaSeq (Fernandes et al., 2018); plyr (Wickham & Wickham, 2020); randomForest (Liaw & Wiener, 2002); e1071; decontam (Davis et al., 2018); knitr (Xie, 2016); tibble (Wickham et al., 2021). The Phyloseq package in R was used to conduct alpha diversity indices (Shannon, Observed, Chao1) to identify changes in species richness, diversity, and evenness between the different treatment amendments (CaCO$_3$ (CC), Organic Carbon (OC)) compared to the nontreatment controls. Quantile Quantile (QQ) plots were used to test for normal distribution within the data. T-way Analysis of Variance (ANOVA) statistics was then used to examine significant differences in species diversity and abundance between the different pH treatments and their respective additional amendments (CaCO$_3$ (CC), Organic Carbon (OC)) by treatment or control date (Treatments: 270619 & 010719; Controls: 290619 & 030719). This was followed by Tukey HSD (Honestly Significant Difference) tests for post-hoc "true significance" verification to avoid the reporting of erroneous Type I errors (R Version 4.0.3). Therefore, only significant differences were reported if the Tukey HSD analyses were determined as also being significant.

Beta diversity indices were analyzed using the PhILR (Phylogenetic Isometric Log Ratio Transform) ordinations for the analysis of compositional data, which considers the evolutionary tree (i.e., phylogenetic tree) to transform microbiological data more effectively into an unconstrained coordinate system, where the relationship of the neighboring bacterial clade/group is incorporated into the analysis and interpretation of the data (Silverman et al., 2017). Given the relative nature of microbial abundance data in ecological and microbiological

studies, the PhILR approach is desirable for avoiding other spurious statistical analyses (Gloor et al., 2017). ANOSIM (Analysis of Similarity) statistical tests were conducted on the Phylogenetic ILR Transformed data (PhILR) using Euclidean distances calculated with 999 permutations for each of the PhILR PCoA ordinations. This was done to test for similarities between the different groups in terms of pH environment and the addition of treatment amendment to cores within the different flow tank systems, applying a ranked dissimilarity matrix.

Compositional analyses were achieved using "Random Forest" classification algorithms, which employ a combination of tree predictors, where each generated tree is dependent on the values of a random vector that is independently sampled that demonstrates the same distribution for each tree produced in the theoretical forest (Breiman, 2001). The Random Forest classification method is designed around generating various "decision trees" that are built out of different subsets of the data that are randomly sampled and with replacement from the original dataset, which is then designed to train the algorithm (Breiman, 2001). The resulting decision trees are then utilized to identify a classification consensus via selecting the most frequent output (i.e., mode) (Breiman, 2001). Random forest analyses were conducted on the Active [RNA] dataset alone, using the randomForest package in R, with a set seed value of 57 and 10,000 trees. This was also done with the removal of both the Procedural Control Blank and the Organic Carbon (OC) Blank to avoid skewing the data by overestimating the contribution of certain variables to the detection of measurable differences between factors. This approach improved the accuracy of the confusion matrix algorithm and reduced the potential for high error rates.

1   Results

1.1   Physicochemical Sediment Properties

Surface (0-1cm) sediment organic content and $CaCO_3$ concentration was markedly higher in the $CaCO_3$ treatment (OC 10.2 ± 0.2; $CaCO_3$ 4.8 ± 0.2) than the Ashed Sand treatment (OC 8.5 ± 0.2; $CaCO_3$ 3.8 ± 0.3) (**Table 2.**). No impacts of acidification were detected on organic content (OC) or $CaCO_3$ (CC) concentration (e.g., RE1 (pH 8.0) vs. RE3 (pH 7.7) or RE2 (pH 8.0) vs. RE4 (pH 7.7)). No clear differences in Chlorophyll a, Phaeopigment, Chlorophyll a/Phaeopigment ratio, or sediment moisture content were measured between any of the pH treatments (**Table 2.**).

1.2   Taxonomy

Taxonomic classification of the top 10 most abundant ASV's at the level of order showed that the Active [RNA] community was dominated across all experiments (010719; 270619; 290619; 030719) by *Flavobacteriales* (4.4%), *Nitrosopumilales* (4.2%), *BD7-8* (3.0%), *Unclassifiable* (1.1%), *Cytophagales* (<1%) and *SVa 1033* (<1%) (**Table 3.**) (**Supp. Table 1.**) (**Supp. Figures 4a**. **and 4b**.). The Total [DNA] community was also shown to be dominated by *Flavobacteriales* (7.53%)*, Unclassifiable* (3.3%)*, Nitrosopumilales* (4.9%)*, Bacteroidetes VC2. 1 Bac22* (1.5%)*, BD7-8* (1.42%)*, Sva1033* (1.3%)*, Verrucomicrobiales* (1.4%) (**Table 4.**) (**Supp. Table 1.**) (**Supp. Figures 4c. and 4d**.).

Table 2. Physicochemical sediment properties (*Chlorophyll, Phaeopigments, Moisture Content (MC), Organic Content and CaCO₃ Concentrations*) from each of the mesocosm treatment tanks (RE1-RE4) for the treatments (270619/010719: AS/OC and CC/OC) and the nontreatment controls (290619/030719: AS/NTC and CC/NTC) under ambient (pH 8.0) and low (pH 7.7 pH) conditions.

pH	Tank	Treatment one	Treatment two	Date sampled	Chlorophyll (µg/g dw)	Phaeo (µg/g dw)	Chl a / Phaeo	MC	Organic content (%)	CaCO$_3$ (%)
		Ashed Sand (AS) Control			0.0	0.1	0.0	0.0	0.0	0.0
		CaCO$_3$ (CC) Control			0.3	0.8	0.4	0.0	4.6	42.3
		Organic Carbon (OC) Control			22.2	41.4	0.5	0.9	63.7	59.9
8	RE1	AS	-	290619	11.2	16.8	0.7	0.7	8.5	4.1
				030719	11.8	20.9	0.6	0.7	8.6	3.8
			OC	270619	12.2	17.5	0.7	0.7	8.2	3.5
				010719	10.0	17.0	0.6	0.7	8.3	21.7
		NTC	-	030719	10.4	17.8	0.6	0.7	9.1	3.8
	RE2	CC	-	290619	11.1	20.9	0.5	0.7	10.4	5.1
			-	030719	11.7	20.8	0.6	0.7	10.4	4.9
			OC	270619	21.1	22.1	1.0	0.7	10.3	5.5
				010719	13.8	20.9	0.7	0.7	10.4	4.6
		NTC	-	030719	14.2	22.0	0.6	0.7	9.9	4.2
7.7	RE3	AS	-	290619	12.8	17.7	0.7	0.7	8.7	4.0
				030719	12.9	16.4	0.8	0.6	8.5	3.5
			OC	270619	21.2	20.6	1.0	0.7	8.5	3.9
				010719	13.2	19.3	0.7	0.7	8.6	3.7
		NTC	-	030719	12.8	21.7	0.6	0.7	9.6	4.2
	RE4	CC	-	290619	15.0	20.2	0.7	0.7	9.8	4.6
				030719	12.2	18.9	0.6	0.6	10.1	4.5
			OC	270619	18.5	22.6	0.8	0.7	10.1	4.8
				010719	14.9	16.1	0.9	0.7	10.1	4.6

Table 3. Shows a taxonomic profile of the Active [RNA] for both the treatment (270619/010719: AS/OC and CC/OC) and nontreatment control (290619/030719: AS/NTC and CC/NTC) sediment communities under ambient (pH 8.0) and low (pH 7.7) pH conditions. Relative abundance values are derived from Supp. Figure 4b.

pH	Most Abundant Taxa (ASV's)	270619 (Abundance (%))	010719 (Abundance (%))	270619/010719 (Average ±Std.Dev.)	290619 (Abundance (%))	030719 (Abundance (%))	290619/ 030719 (Average ±Std. Dev.)
8	Flavobacteriales	2.03%	2.60%	2.32±0.002	2.40%	2.18%	2.29±0.001
	Sva1033	1.70%	1.70%	1.70±0	0.50%	0.06%	0.28±0.002
	Nitrosopumilales	1.41%	1.13%	1.27±0.001	1.40%	1.50%	1.45±0.000
	BD7-8	1.60%	1.60%	1.60±0	1.13%	1.52%	1.33±0.001
	Unknown	1.00%	1.00%	1.00±0	1.00%	1.30%	1.15±0.001
	Cytophagales	0.50%	0.60%	0.55±0.000	1.90%	1.00%	1.45±0.004
7.7	Flavobacteriales	1.50%	2.30%	1.90±0.004	1.92%	2.40%	2.16±0.002
	Sva1033	0.90%	0.90%	0.90±0	0.40%	0.50%	0.45±0.000
	Nitrosopumilales	1.40%	1.30%	1.35±0.000	1.40%	1.50%	1.45±0.000
	BD7-8	1.70%	1.90%	1.80±0.001	1.50%	1.40%	1.45±0.000
	Unknown	1.00%	1.00%	1.00±0	1.20%	1.20%	1.20±0
	Cytophagales	0.60%	0.90%	0.75±0.001	1.60%	0.90%	1.25±0.003

Table 4. Shows a taxonomic profile of the Total [DNA] for both the treatment (270619/010719: AS/OC and CC/OC) and nontreatment control (290619/030719: AS/NTC and CC/NTC) sediment communities under ambient (pH 8.0) and low (pH 7.7) pH conditions. Relative abundance values are derived from Supp. Figure 4d.

pH	Most Abundant Taxa (ASV's)	270619 (Abundance (%))	010719 (Abundance (%))	270619/010719 (Average ±Std.Dev.)	290619 (Abundance (%))	030719 (Abundance (%))	290619/030719 (Average ±Std.Dev.)
8	Flavobacteriales	0.03	0.045	3.75±0.007	0.035	0.043	3.90±0.40
	Unknown	0.032	0.028	3.00±0.002	0.035	0.033	3.40±0.10
	Nitrosopumilales	0.0171	0.016	1.66±0.000	0.0153	0.02	1.77±0.24
	Verrucomicrobiales	0.016	0.012	1.40±0.002	0.0145	0.008	1.13±0.33
	Bacteroidetes VC2. 1 Bac22	0.015	0.0115	1.33±0.001	0.016	0.014	1.50±0.10
	BD7-8	0.014	0.013	1.35±0.000	0.013	0.016	1.45±0.15
	Sva1033	0.011	0.02	1.55±0.000	0.008	0.01	0.90±0.10
7.7	Flavobacteriales	0.03	0.04	3.50±0.005	0.032	0.042	3.70±0.50
	Unknown	0.033	0.032	3.25±0.000	0.037	0.034	3.55±0.15
	Nitrosopumilales	0.016	0.016	1.60±0	0.016	0.0163	1.62±0.01
	Verrucomicrobiales	0.018	0.013	1.55±0.000	0.018	0.01	1.40±0.40
	Bacteroidetes VC2. 1 Bac22	0.015	0.013	1.40±0.001	0.015	0.016	1.55±0.05
	BD7-8	0.013	0.014	1.35±0.000	0.015	0.014	1.45±0.05
	Sva1033	0.015	0.014	1.45±0.000	0.009	0.008	0.85±-.05

1.3 The response of the microbial community to a buffering Capacity of $CaCO_3$ Under Acidification

The extent of the buffering capabilities of $CaCO_3$ on microbial community structure and composition under acidified seawater conditions (ambient: pH 8.0; CO_2 enriched: pH 7.7) were tested on the Ashed Sand No Treatment Control (AS/NTC) and $CaCO_3$ No Treatment Control (CC/NTC) samples collected on 290619 and on 030719 (**Figures 2a. and 2b**.).

1.3.1 Alpha Diversity

On both the 290619 and the 030719, in both amendments the measure of microbial community diversity (Shannon Indices) was comparatively lower within the Active [RNA] community under acidified conditions (pH 7.7) than that of ambient (pH 8.0) (**Figure 3b.).** Within the Ashed Sand amendment on the 030719 microbial diversity decreased under acidified conditions (RE1 (pH 8.0) vs. RE3 (pH 7.7)) (**Figure 3b**.). However, no statistical impact on microbial diversity was identified on either 290619 or 030719 sampling dates under acidified conditions when $CaCO_3$ was added (RE2 (pH 8.0) vs. RE4 (pH 7.7)). In summation, species diversity in the Active [RNA] community that received only the Ashed Sand amendment notably decreased under low pH conditions (pH 7.7), whereas the Active bacterial communities who received both Ashed Sand and $CaCO_3$ amendments appeared to not be affected by acidified conditions (pH 7.7), indicating that the addition of $CaCO_3$ had a positive correlation with microbial species diversity and richness. Species diversity of the Total [DNA] microbial community did not change with treatment amendment or pH (**Supp**. **Figure 8b.). Supplementary Table 2a. Supplementary Table 2b**.).

1.3.2 Beta Diversity

No significant differences in the Active [RNA] or Total [DNA] community structure were observed between the Ashed Sand and $CaCO_3$ amended samples at ambient (pH 8.0) or enriched (pH 7.7) pH on either 290619 or the 0307019 sampling dates (**Figure 4b**. **and Supp. Figure 9b.) (Supp. Table 3a**. **and 3c**.).

1.3.3 Compositional Analysis

Random Forest (RF) analysis was used to describe microbial community compositional differences between the AS/NTC and the CC/NTC nontreatment controls, where the most critical ASV's (Top 20 based on GINI Coefficient (node purity) are used to discriminate

between the AS/NTC community and the CC/NTC community. This is also used to assess differences in ambient (pH 8.0) and enriched (pH 7.7) pH AS/NTC and CC/NTC communities.

1.3.3.1   Important Discriminating ASV's on the structure of the microbial community between the Ashed Sand and CaCO$_3$ amendment: 290619

Here we identify the top 20 most important ASV's that were used to explain the differences between the AS/NTC amended sediment communities, and CC/NTC nontreatment sediment communities. The purpose of which was to better understand what is taxonomically different/unique about each of these communities and how their composition is directly impacted by the addition of different control amendments from their original pre-amendment state. Results showed that *Psuedoalteromonas* (ASV 1082) was the most important ASV to discriminate between the two nontreatment controls (**Figure 6a**.) (**Supp. Table 6a**.). The compositional diversity of the residual top 20 ASV's showed that 25% of the ASV's were *unclassifiable* (*Unknown* (ASV 1457; 2564; 1608; 1243; 1053)), 20% belonged to the Class *Gammaproteobacteria* (*Pseudoalteromonas* (ASV 1082); *Alteromonadales* (ASV 858); *Gammaproteobacteria* (ASV 1906); *Aliikangiella* (ASV 2339 from the Order *Oceanospirillales*)), 20% were associated with *Bacteroidia* (*Aquibacter* (ASV 42); *Microscilla* (ASV 971); *Crocinitomicaceae* (ASV 598); *Bacteroidia* (ASV 2252)), 10% belonged to *Alphaproteobacteria* (*Rhodobacteraceae* (ASV 417; 802)), ≤5% belonged to *BD7-11* (ASV 1684), *Sva0081 sediment group* (ASV 255), *Cyanobacteriales* (ASV 1419), *Cerasicoccus* (ASV 1591), and *Pirellulaceae* (ASV 1689) (**Figure 6a**.) (**Supp. Table 6a**.). Of the top, 20 most important ASV's that distinguished between the AS/NTC community and the CC/NTC community, 20% were associated with the previously identified top ten most abundant ASV's within the *original Non-Treatment Control (NTC)* sediment community belonging to the Order *Flavobacteriales* (ASV 42; 598)*, Cytophagales* (ASV 971), and the Class *Verrucomicrobiae* (ASV 1591) (**Supp. Figure 4b**.) (**Supp. Table 6a**.). *Aquibacter* (ASV 42) shared commonality between the top 20 ASV's identified in the 290619 community and the top 20 ASV's identified in the 030719 community (**Supp. Tables 6a. and 6c**.).

1.3.3.2 Important Discriminating ASV's on the structure of the microbial community between the sand and CaCO₃ amendment: 030719

The top 20 most important ASV's that were shown to discriminate between AS/NTC amended sediment communities and CC/NTC nontreatment sediment communities classified *SCGC AAA164-E04* (ASV 2901) as the most prominent taxa that contributed to the differences between the AS/NTC and CC/NTC nontreatment control communities (**Figure 6b**.) (**Supp. Table 6c.**). The Class *Bacteroidia* accounted for 35% of the top 20 most important ASV's (*Crocinitomicaceae* (ASV 521); *Bacteroidia* (ASV 2935; 1359); *Bacteroidetes VC2.1 Bac22* (ASV 52); *Cytophagales* (ASV 1353); *Aquibacter* (ASV 42; 170)), 35% belonged to the Class *Gammaproteobacteria* (*Gammaproteobacteria* (ASV 2336; 1499; 85); BD1-7 clade (ASV 2695); *BD7-8* (ASV 591); *Halieaceae* (ASV 136); *Porticoccus* (ASV 443)), 10% belonged to the Class *Alphaproteobacteria* (*Rhodobacteraceae* (ASV 2808); *Alphaproteobacteria* (ASV 1445)), and ≤5% associated with *Cerasicoccus* (ASV 768), *unclassifiable* (*Unknown* (ASV 8), and *Chlamydiales* (ASV 1450) (**Figure 6b**.) (**Supp. Table 6c.**). Of the top 20 most important ASV's identified as differentiating between the AS/NTC and CC/NTC communities, 40% belonged to the previously classified top 10 most abundant ASV's from the Class *Verrucomicrobiae* (ASV 2901; 768), the Order *Flavobacteriales* (ASV 521; 42; 170), *Cytophagales* (ASV 1353), *BD7-8* (ASV 591) (**Supp. Figure 4b**.) (**Supp. Table 6c**.). As previously mentioned, *Aquibacte*r (ASV 42) was seen to overlap with the top 20 ASV's identified within the *original* 290619 AS/NTC and CC/NTC sediment community composition (**Supp. Tables 6a. and 6c**.).

1.3.3.3 290619: (AS/NTC and CC/NTC) Important Discriminating ASV's by pH

RF tree analysis of the AS/NTC and CAR/NTC amendment additions by pH identified *Polyangia* (ASV 19) as the most dominant contributing taxa discriminating between the two nontreatment controls by pH treatment (**Figure 6a**.) (**Supp. Table 6b**.). Where the compositional profile of the top 20 ASV's showed that 25% of the ASV's were *unclassifiable* below the level of Phylum (*Unknown* (ASV 1221; 2323; 1834; 1134; 2655)), 15% belonged to the Class *Gammaproteobacteria* (*Gammaproteobacteria* (ASV 2340; 262; 548)), ≤5% were associated with *BD2-11 terrestrial group* (ASV 452), *Desulfosarcinaceae* (ASV 2734), *Bacteriovoracaceae* (ASV 1487), *Rhodopirellula* (ASV 2283), *Sva0081 sediment group* (81), *Roseibacillus* (ASV 1454), *Cytophagales* (ASV 1972), *Rhodobacteraceae* (ASV 1746), *Nannocystaceae* (ASV 661), *Anaerolineae* (ASV 762), and *Ilumatobacter* (ASV 840) (**Figure 6a**.)

(**Supp. Table 6b**.). From the top 20 ASV's that were shown to differentiate between the AS/NTC community and the CC/NTC community by ambient (pH 8.0) and low (pH 7.7), 10% belonged to the previously identified top 10 most abundant ASV's within the nontreatment control sediment composition associated with the Order *Verrucomicrobiales* (ASV 1454), *Cytophagales* (ASV 1972) (**Supp. Figure 4b**.) (**Supp. Table 6b**.). The *unclassifiable* taxon *Unknown* (ASV 2323) was co-occurring between the top 20 ASV's identified in the original 290619 community and the top 20 ASV's identified in the original 030719 community by pH (**Supp. Tables 6b. and 6d**.).

1.3.3.4   030719: (AS/NTC and CC/NTC) Important Discriminating ASV's by pH

RF tree analysis of the AS/NTC and CAR/NTC amendment additions by pH showed that an *unclassifiable* taxon was the most important ASV seen to discriminate between the nontreatment control sediment communities (*Unknown* (ASV 2096)) (**Figure 6b**.) (**Supp. Table 6d**.). The compositional profile of the top 20 ASV's showed that 30% of the ASV's belonged to the Class *Bacteroidia* (*Lewinella* (ASV 2078); *Bacteroidia* (ASV 1554; 2824); *Bacteroidetes VC2.1 Bac22* (ASV 2029; 10); *Lutibacter* (ASV 1754)), 20% belonged to the Class *Gammaproteobacteria* (*Alteromonadaceae* (ASV 219); *Gammaproteobacteria* (ASV 736); *Cellvibrionales* (ASV 1856); *Aliikangiella* (ASV 2339 from the Order *Oceanospirillales*)), 20% of the taxa were *unclassifiable* below Phylum level (*Unknown* (ASV 2096; 2520; 201; 2323)), and ≤5% belonged to *Rhodobacteraceae* (ASV 1048), *Puniceicoccaceae* (ASV 2602), Blrii41(ASV 1111), *Subgroup 10* (ASV 2519), *AT-s3-28* (ASV 1839), *SG8-4* (ASV 2127) (**Figure 8b**.) (**Supp. Figure 6b**.). The top 20 ASV's identified within the 030719 community by pH showed that 20% were associated with the previously identified top 10 most abundant taxa (ASV's) belonging to the Class *Verrucomicrobiae* (ASV 2602), the Order *Bacteroidetes VC2, 1 Bac22* (ASV 2029; 10), and *Flavobacteriales* (ASV 1754) (**Supp. Figure 4b**.) (**Supp. Table 6b**.). As previously mentioned, the *unclassifiable* taxon *Unknown* (ASV 2323) was consistent between the original 030719 and original 290619 top 20 responding ASV's between both communities by pH (**Supp. Table 6d. and 6b**.).

1.4   Buffering Capacity of Calcium Carbonate Under Acidification and Eutrophication Effects

The Ashed Sand/Organic Carbon (AS/OC) and the CaCO$_3$/Organic Carbon (CC/OC) (270619/010719) treatments were to assess the buffering capabilities of CaCO$_3$ on microbial

community structure and composition on organically enriched (Eutrophication-induced-acidification) subtidal sediments under additional acidified seawater conditions (i.e., OA) (ambient: pH 8.0; CO_2 enriched: pH 7.7).

1.4.1 Alpha Diversity

On both the 270619 and the 010719, microbial community diversity (Shannon Indices) increased within the Active [RNA] and Total [DNA] communities under $CaCO_3$ and organic matter enrichment and acidified conditions (RE2 (pH 8.0) vs. RE4 (pH 7.7)) (**Figure 3a. and Supp. Figure 8a**.). The combined interaction of both $CaCO_3$ and Organic Carbon enrichment together appeared to have a positive correlation with microbial species diversity and richness. Within the Ashed Sand amendment and Organic Carbon enrichment, species diversity was also (Shannon indices) shown to increase within the Active [RNA] and Total [DNA] community on 270619, but subsequently decreased on 010719 in response to acidification (**Figure 3a. and Supp. Figure 8a.**), where the combination of Ashed Sand and Organic Carbon had markedly less of a positive impact on species diversity compared to AS/OC

1.4.2 Beta Diversity

Both the Active [RNA] and Total [DNA] communities displayed changes in community structure in the presence of $CaCO_3$ under acidified pH conditions (pH 7.7) on both 270619 and 010719 sampling dates (**Figure 4a. and Supp. Figure 9a.**). Only the Active [RNA] community detected significant differences in community structure on 010719 (**Figure 4a.**) (ANOSIM: *$p \leq 0.05$; R: 285*) and **Supp. Table 3a-c.**). In summation, the Active [RNA] communities which received the $CaCO_3$, and Organic Carbon enrichments (CC/OC) were significantly different in structure compared to the sediment communities which received only Ashed Sand and Organic Carbon (AS/OC). The complex interaction of $CaCO_3$ with Organic Carbon induced a clear measurable response from the Active [RNA] sediment microbial community that was not detected in samples where $CaCO_3$ was absent (i.e., AS/OC).

1.4.3 Compositional Analysis

1.4.3.1 *Important Discriminating ASV's on the structure of the microbial community between the sand and $CaCO_3$ amendment under eutrophication: 270619*

The top 20 most important ASV's that differentiated between AS/OC amended sediment communities, and CC/OC sediment communities for the 270619 treatment

sampling date classified *Saprospiraceae* (ASV 2686) as the primary taxonomic variant that contributed to the differences between the AS/OC and CC/OC amended communities (**Figure 5a.**) (**Supp. Table 5a**.). Of the top 20, 45% belonged to the Class *Gammaproteobacteria* (*Alteromondaceae* (ASV 56); *Alteromondaceae* (ASV 219); *EPR3968-O8a-Bc78* (ASV 727); *Gammaproteobacteria* (ASV 1360); *Endothiovibrio* (ASV 995); *Halieaceae* (ASV 1200)*; UBA 10353 marine group* (ASV 839)*; Woeseia* (ASV 337); *Psuedoalteromonas* (ASV 1082)), 20% Unclassifiable below Kingdom or Phylum level (ASV: *Bacteria* (ASV 1099); *NB1-j* (ASV 2799); *Bacteria* (ASV 1195); *Bacteria* (ASV 1006)), 15% to the Class *Bacteroidia* (*Saprospiraceae* (ASV 2686)*; Winogradskyella* (ASV 2163); *Bacteroidetes BD2-2* (ASV 1128)), and less than 1% of ASV's belonging to the Phylum *Verrucomicrobiota* (*WCHB1-41* (ASV 1861); *DEV007* (ASV 1316)), and ≤10% to *Desulfatiglans* (ASV 1159), *Phycisphaera* (ASV 205) (**Figure 5a.**) (**Supp. Table 5a**.). The top 20 ASV's observed between the AS/OC and CC/OC sediment communities showed that only 10% belonged to the previously identified original top 10 most abundant taxa within the treatment communities associated with the Order *Flavobacteriales* (ASV 2163), and *Verrucomicrobiales* (ASV 1316) (**Supp. Figure 4b.**) (**Supp. Table 5a.**). *Alteromonadaceae* (ASV 219) was shown to be co-occurring within the top 20 ASV's identified between both the 270619 and 010719 treatments (**Supp. Table 5a. and 5c**.).

1.4.3.2   *Important Discriminating ASV's on the structure of the microbial community between the sand and CaCO₃ amendment under eutrophication: 010719*

The top 20 most important ASV's that were shown to discriminate between AS/OC amended sediment communities, and CC/OC sediment communities for the 010719 treatment sampling date classified *vadinHA49* (ASV 2231) as the primary taxonomic variant that contributed to the differences between the AS/OC and CAR/OC amended communities (**Figure 5b.**) (**Supp. Table 5c**.). Of the top 20, 25% were associated with the Class *Bacteoidia* (*Microscilla* (ASV 110); *Flavobacteriaceae* (ASV 173); *NS7 marine group* (ASV 2616); *Flavobacteriaceae* (ASV 797); *Cyclobacteriaceae* (ASV 2001)), 15% belonged to the Class *Gammaproteobacteria* (*Alteromondaceae* (ASV 219); *Psychromonas* (ASV 447); *Chromatiales* (ASV 1950)), 15% were *unclassifiable* (*Unknown* (ASV 2617; 1517; 2603)), 10% belonged to the Class *Desulfuromonadia* (*Sva1033* (ASV 11); *Desulfuromusca* (ASV 2775)), 10% belonged to the Class *Desulfobacteria* (*Desulfosarcinaceae* (ASV 1938); *Desulfatirhabdium* (ASV 2809)), and ≤5% *Cyanobacteriales* (ASV 1419), *Rhodobacteraceae* (ASV 600), *Nannocystaceae* (ASV 2350), *Spirochaeta* (ASV 1687) (**Figure 5b.**) (**Supp. Table 5c**.). From the top 20 ASV's observed,

25% we associated with the top 10 (previously identified) most abundant community taxa from the AS/OC and CC/OC communities belonging to the Order *Cytophagales* (ASV 110; 2001), and *Flavobacteriales* (ASV 173; 2616; 797) (**Supp. Figure 4b**.) (**Supp. Table 5c**.). As previously mentioned, *Alteromonadaceae* (ASV 219) displayed co-occurrence between both original 010719 and 270619 communities which distinguished the top 20 ASV's (**Supp. Table 5c. and 5a**.).

1.4.3.3 *Important Discriminating ASV's on the structure of the microbial community to acidified conditions between the sand and CaCO₃ amendment under eutrophication: 270619*

RF tree analysis of the AS/OC and CAR/OC amendment additions by pH identified *Lewinella* (ASV 2078) from the Family *Saprospiraceae* as the most dominant contributing taxa discriminating between the two amendments by pH treatment (**Figure 5a**.) (**Supp. Table 5b**.). Where the compositional profile of the top 20 ASV's showed that 25% of the ASV's belonged to the Class *Gammaproteobacteria* (*KI89A clade* (ASV 2480), *Gammaproteobacteria* (ASV 635; 1089; 2842); *BD7-8* (ASV 1417); 25% to the Class *Bacteroidia* (*Lewinella* (ASV 2078); *Flavobacteriaceae* (ASV 173); *Ekhidna* (ASV 1220); *Bacteroidia* (ASV 1027; 518), and ≤1% to the Class *Alphaproteobacteria* (*Rhodobacteraceae* (ASV 2330; 1492)), ≤10% to the Class *Desulfuromonadia* (*Geothermobacter* (ASV 615); *PB19* (ASV 1548)), ≤10% *Unclassified* below Phylum level (*Chloroflexi* (ASV 827); *Bacteria* (ASV 130)), and <10% for *Subgroup 10* (ASV 2437), *Pirellulaceae* (ASV 1531), *SGST604* (ASV 1003)), and *MidBa8* (ASV 1097) (**Figure 5a**.) (**Supp. Table 5b**.). The top 20 ASV's shown to differentiate between the AS/OC and CC/OC treatments by pH showed that 15% were associated with the previously identified original top 10 most abundant ASV's with the treated sediment communities belonging to the Order *Flavobacteriales* (ASV 173), *Cytophagales* (ASV 1220), and *BD7-8* (1417) (**Supp. Figure 4b**.) (**Supp. Table 5b**.). No ASV's were seen to overlap between the first 207619 and the second 010719 AS/OC and CC/OC treatments (**Supp. Table 5b. and 5d**.).

1.4.3.4 *Important Discriminating ASV's on the structure of the microbial community to acidified conditions between the sand and CaCO₃ amendment under eutrophication: 010719*

RF tree analysis of the AS/OC and CC/OC amendment additions by pH treatment identified *Porticoccus* (ASV 604) and *Reichenbachiella* (ASV 33) as the two major ASV's discriminating between the two amendments by pH (**Figure 5b**.) (**Supp. Table 5d.**). The

remaining community was compositionally dominated by at Class level by 35% *Gammaproteobacteria* (*Porticoccus* (ASV 604; 443); *Alteromonadaceae* (ASV 56); Given F17 (ASV 2229 From the Order *Oceanospirillales*); *Thiohalophilus* (ASV 84); *Gammaproteobacteria* (ASV 1458); *Ectothiorhodospirales* (ASV 353)), 15% *unclassifiable* (*Unknown* (ASV 1299; 2118; 2898)), 15% *Bacteroidia* (*Reichenbachiella* (ASV 33); *Flavobacteriaceae* (ASV 2016); *Cytophagales* (ASV 1345)), 15% belonged to the Class *Desulfobacteria* (*Desulfosarcinaceae* (ASV 2530; 524); *Sva0081 sediment group* (ASV 248)), 10% belonged to the Class *Alphaproteobacteria* (*Rhodobacteraceae* (ASV 417; 866)), and ≤5% *Pedosphaeraceae* (ASV 1203), *Bathyarchaeia* (ASV 393) (**Figure 5b**.) (**Supp. Table 5d.**). From the top 20 ASV's identified, 20% were associated with the original top 10 most abundant ASV's belonging to the Order *Cytophagales* (ASV 33; 1345), *Flavobacteriales* (ASV 2016), and the Class *Verrucomicrobiae* (ASV 1203) (**Supp. Figure 4b**.) (**Supp. Table 5d**.). No ASV's showed commonality between the first 270619 and the second 010719 treatment communities in relation to the top 20 most important contributing taxa (**Supp. Table 5b. and 5a.**).
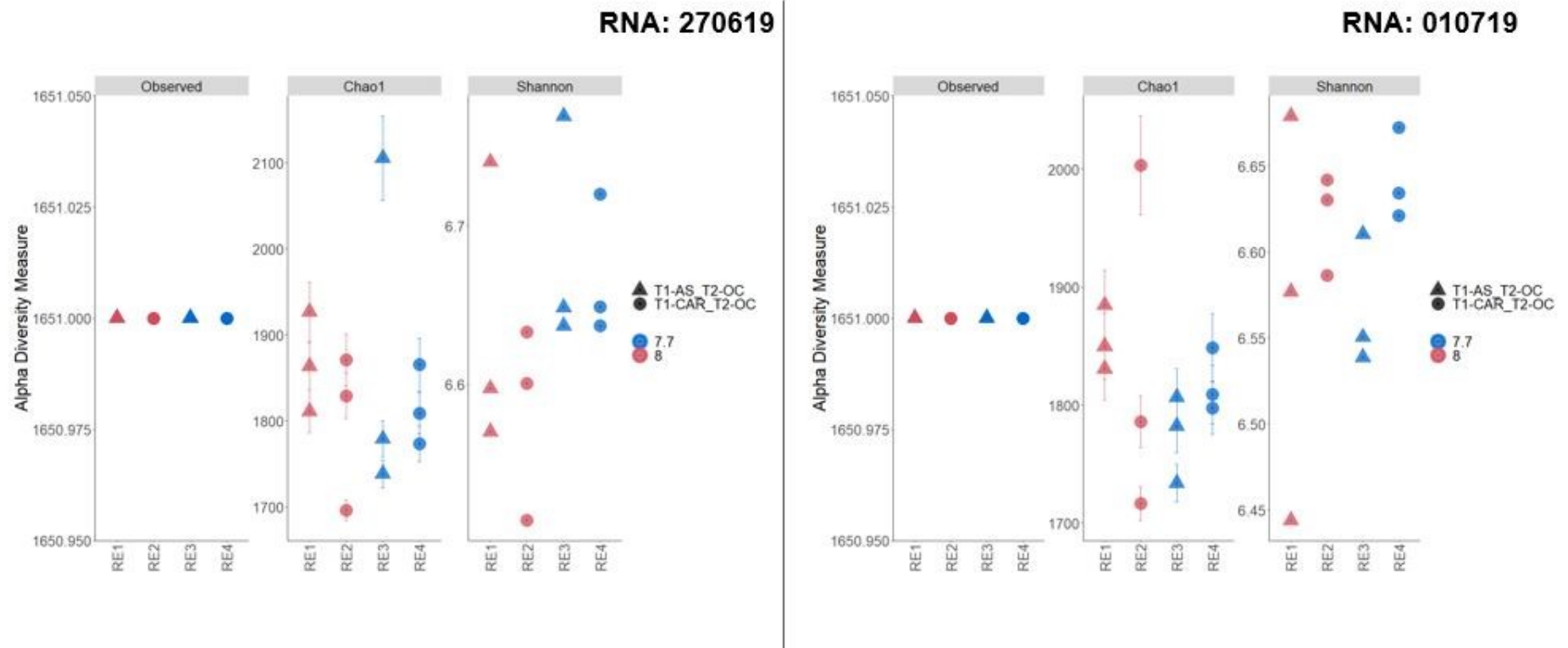
Figure 3a. Alpha diversity matrices (Observed, Chao1, Shannon) for the Active [RNA] dataset for the AS/OC and CC/OC treatments (270619 and 010719), across each of the mesocosm treatments (RE1 (8.0 pH); RE2 (8.0 pH); RE3 (7.7 pH); RE4 (7.7 pH)), colored by pH (Red: 8.0; Blue:7.7) treatment, where triangular and circular symbols indicate the amendment addition. T1 and T2 denote "Treatments 1" and "Treatments 2."
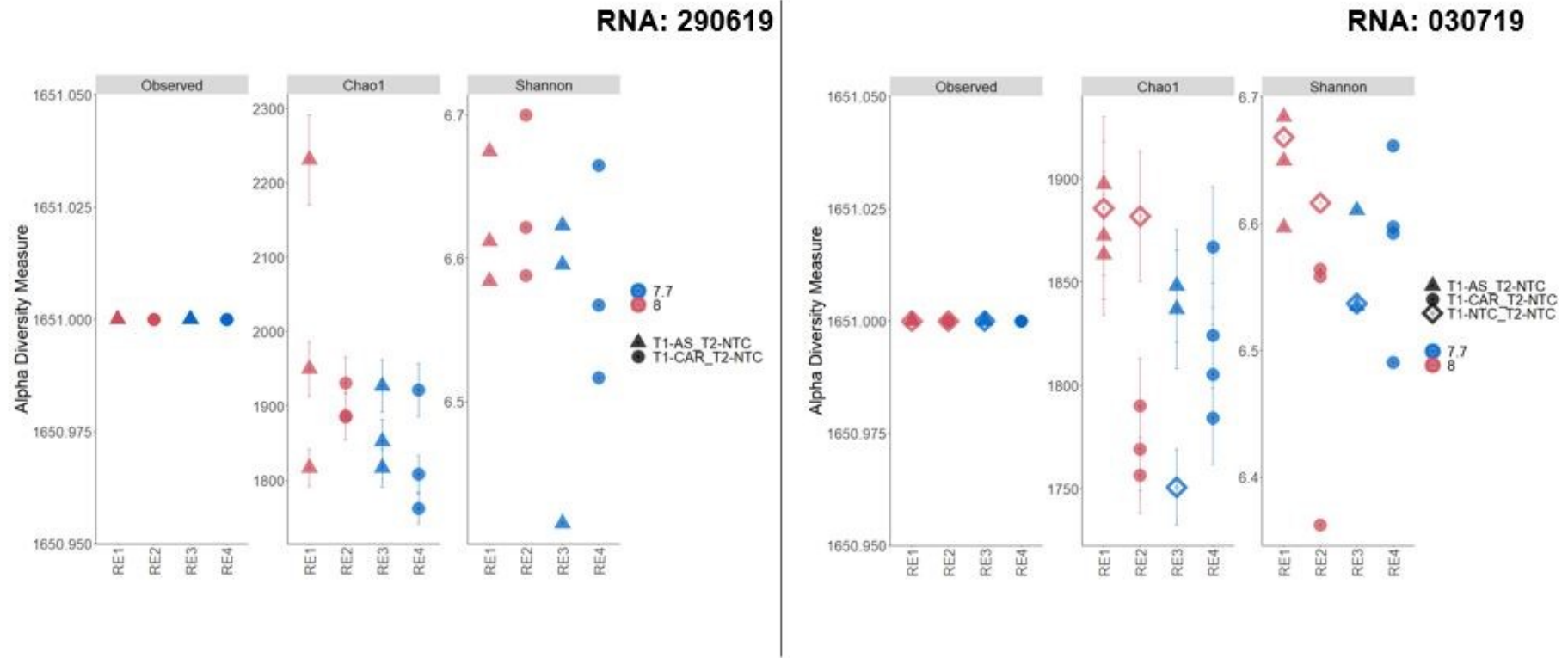
Figure 3b. Alpha diversity matrices (Observed, Chao1, Shannon) for the Active [RNA] dataset for the AS/NTC and CC/NTC treatment controls (290619 and 030719), across each of the mesocosm treatments (RE1 (8.0 pH); RE2 (8.0 pH); RE3(7.7 pH); RE4 (7.7 pH)), colored by pH (Red: 8.0; Blue: 7.7) treatment, where triangular and circular symbols indicate the amendment addition. T1 and T2 denote "Treatments 1" and "Treatments 2."
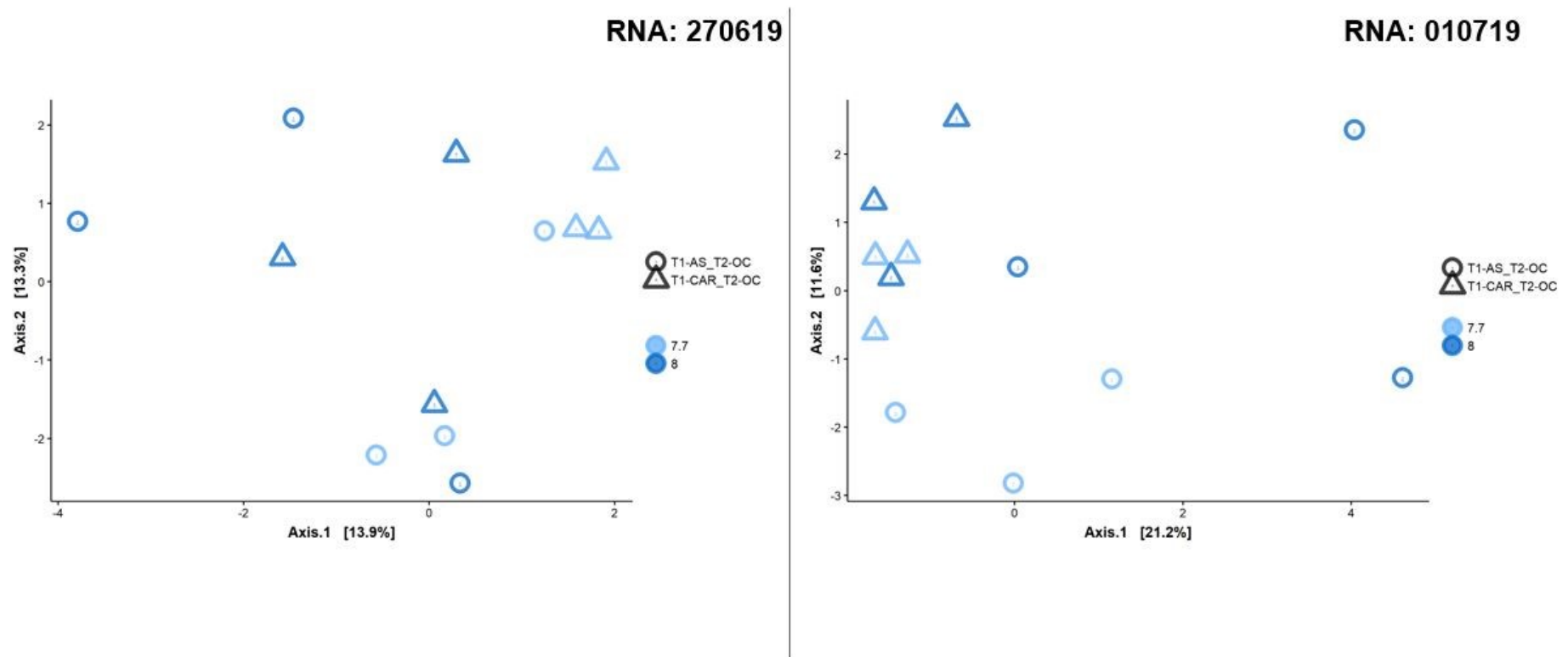
Figure 4a. PCoA ordinations of the PhILR log-transformed Active [RNA] data matrix for the AS/OC and CC/OC treatments (270619 and 010719). Symbols indicate the amendment addition (Ashed Sand (AS); CaCO$_3$ (CC); Organic Carbon (OC); where T1 and T2 represent Treatment One and Treatment Two per each sample, and the color of each symbol describes pH (8.0; 7.7).
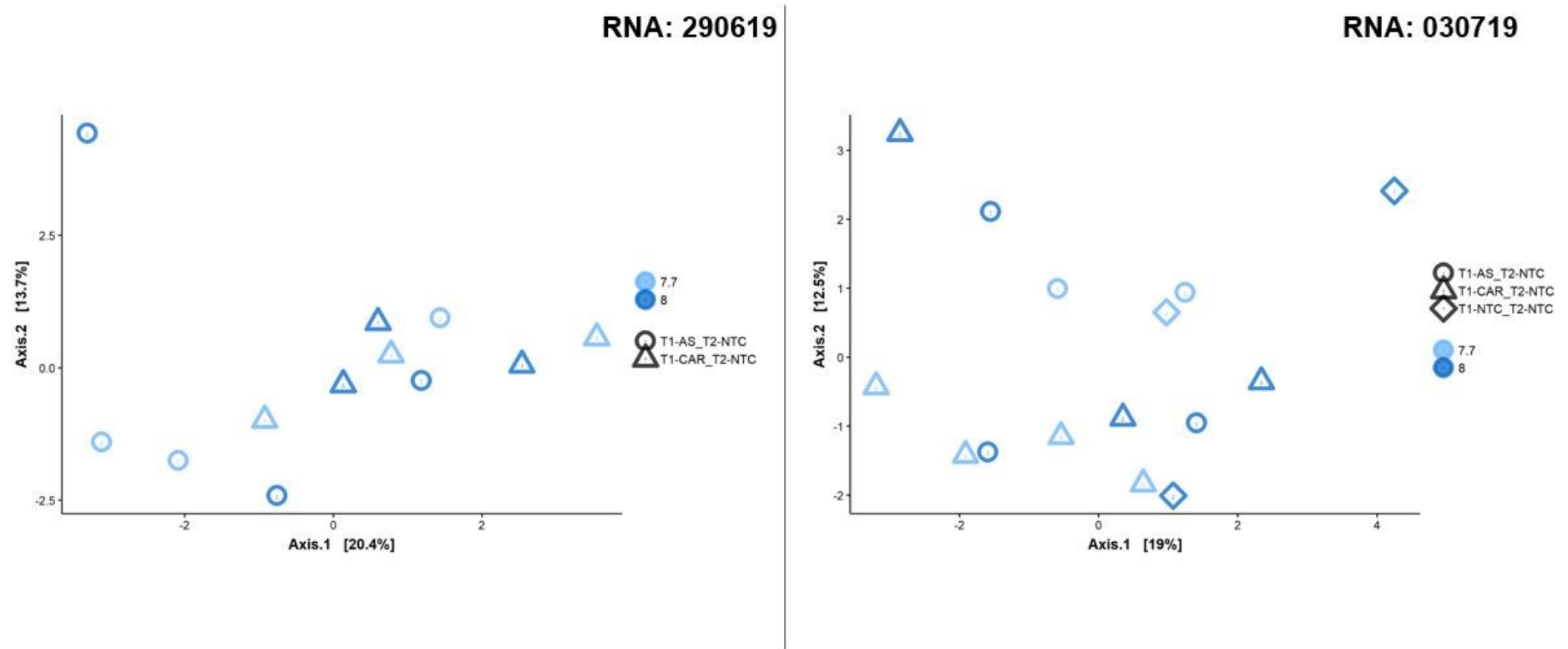
Figure 4b. PCoA ordinations of the PhILR log-transformed Active [RNA] data matrix for the AS/NTC and CC/NTC treatment controls (290619 and 030719). Symbols indicate the amendment addition (Ashed Sand (AS); CaCO₃ (CC); Organic Carbon (OC); No Treatment Control (NTC)), where T1 and T2 represent Treatment One and Treatment Two per each sample, and the color of each symbol describes pH (8.0; 7.7).

Treatment: 270619
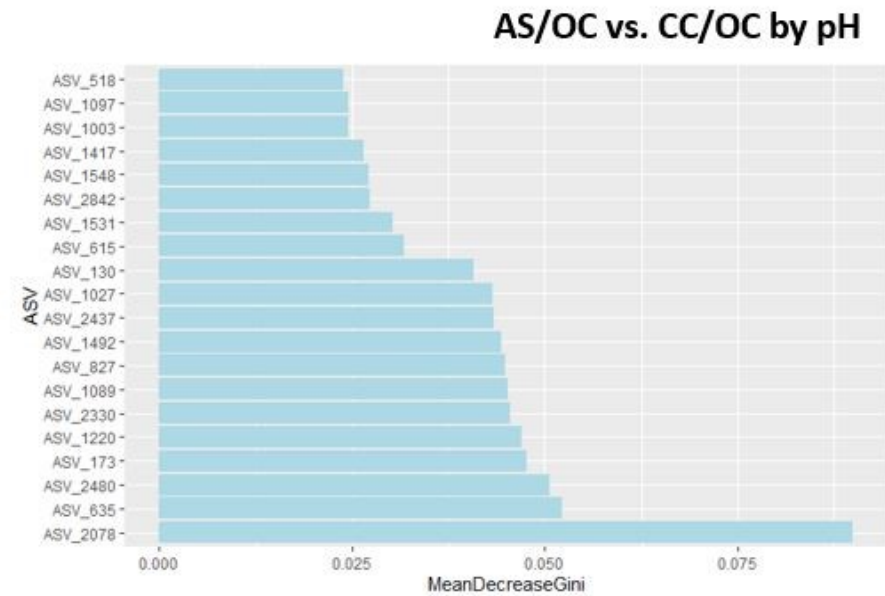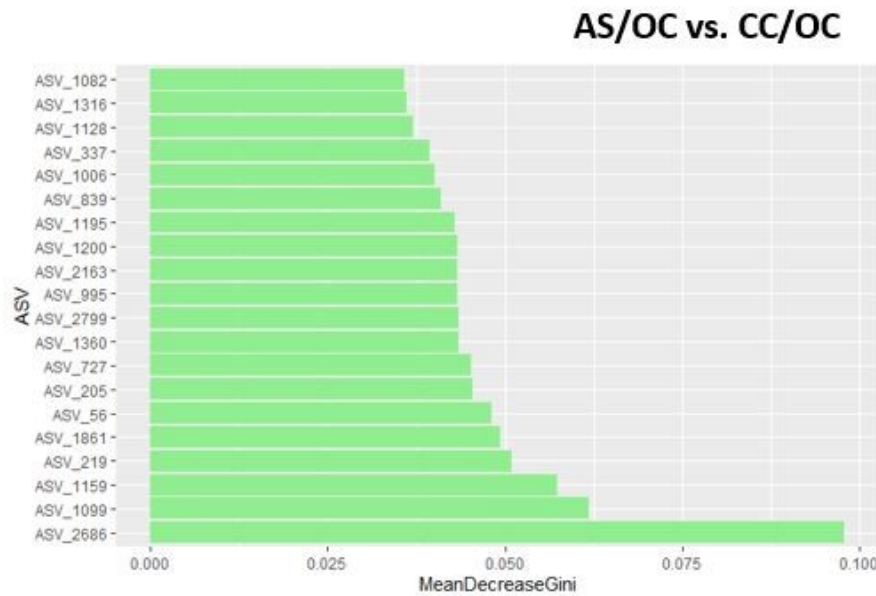
Figure 5a. An R generated plot (R package: ggplot) of the Random Forest classification tree output for the AS/OC and CC/OC treatment run on 270619, identifying the top 20 most important ASV's that differentiate between the AS/OC and the CC/OC amended sediment communities (left), and between the two amendments by pH (right). The y axis indicates the ASV's as predictors, and the x-axis describes the mean decrease GINI coefficient (i.e., node purity) used to predict the relative importance of a particular ASV in discriminating between the two factors.
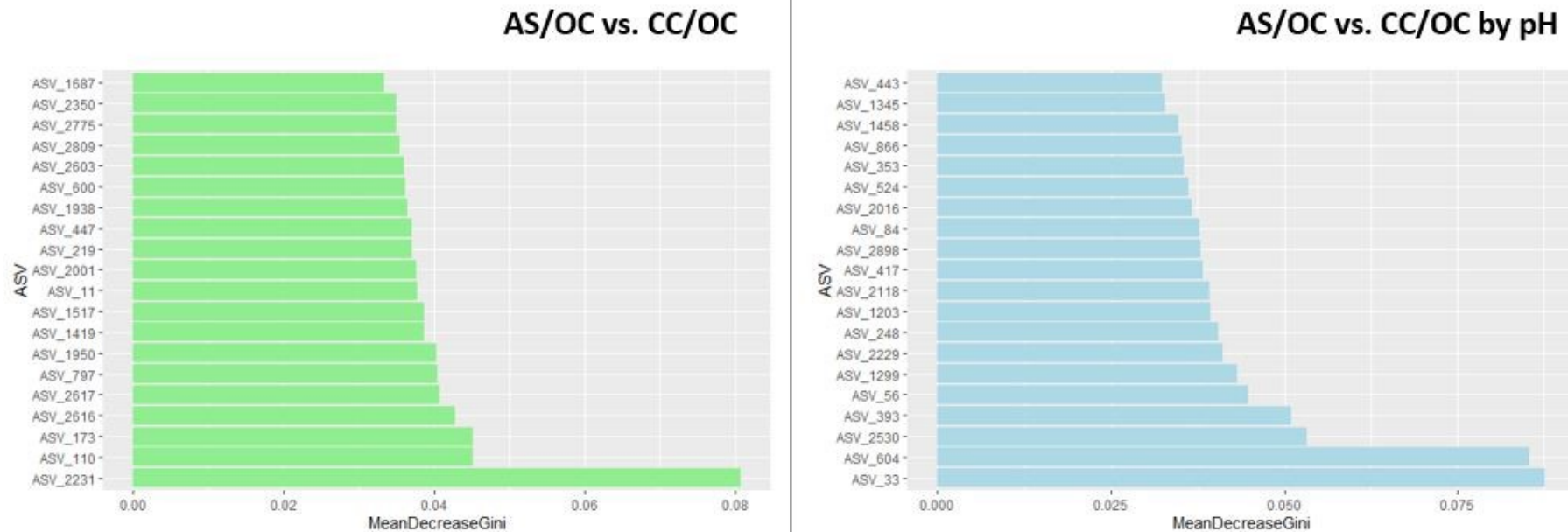
Figure 5b. An R generated plot (R package: ggplot) of the Random Forest classification tree output for the AS/OC and CC/OC treatment run on 010719, identifying the top 20 most important ASV's that differentiate between the AS/OC and the CC/OC amended sediment communities (left), and between the two amendments by pH treatment (right). The y axis indicates the ASV's as predictors, and the x-axis describes the mean decrease GINI coefficient (i.e., node purity) used to predict the relative importance of a particular ASV in discriminating between the two factors.

Control: 290619
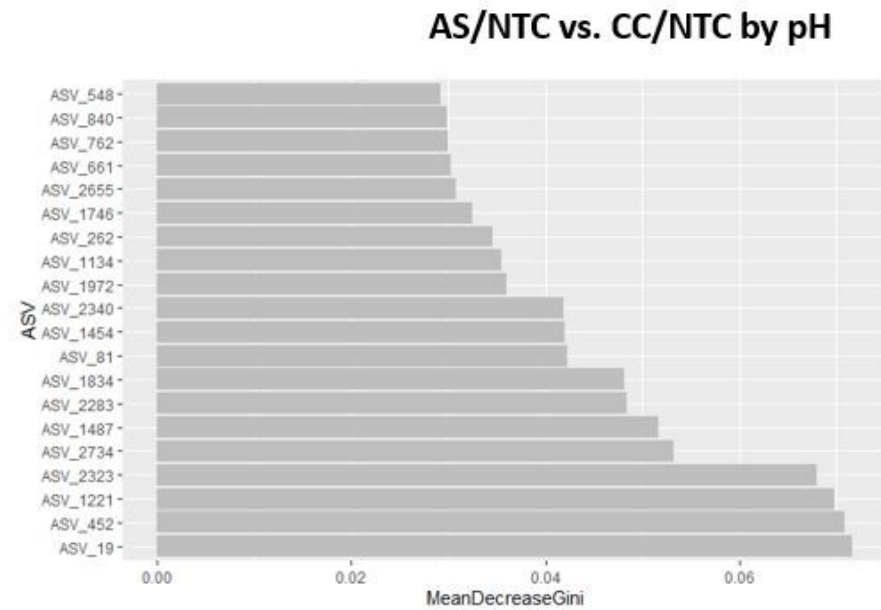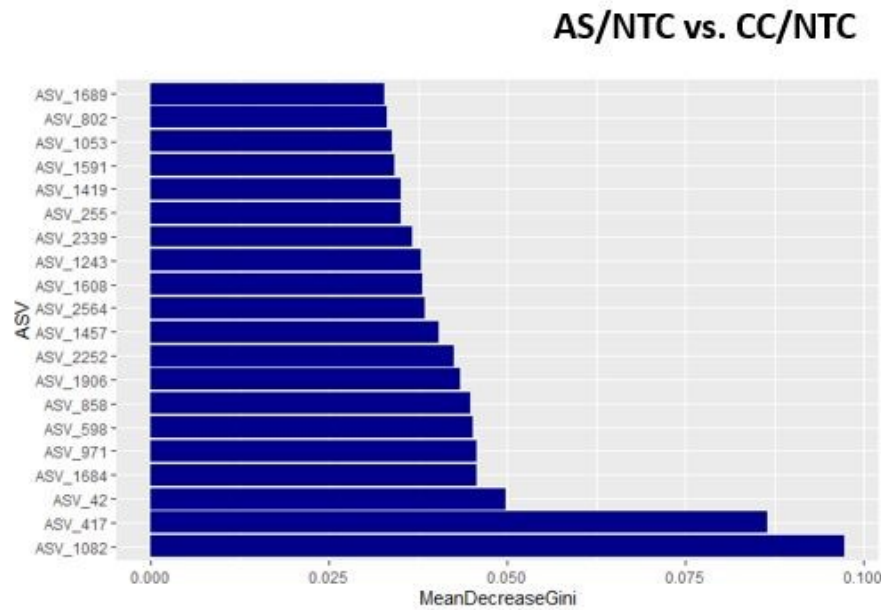
Figure 6a. An R generated plot (R package: ggplot) of the Random Forest classification tree output for the AS/NTC and CC/NTC treatment control run on 290619, identifying the top 20 most important ASV's that differentiate between the AS/NTC and the CC/NTC amended sediment communities (left), and between the two amendments by pH (right). The y axis indicates the ASV's as predictors, and the x-axis describes the mean decrease GINI coefficient (i.e., node purity) used to predict the relative importance of a particular ASV in discriminating between the two factors.

Control: 030719
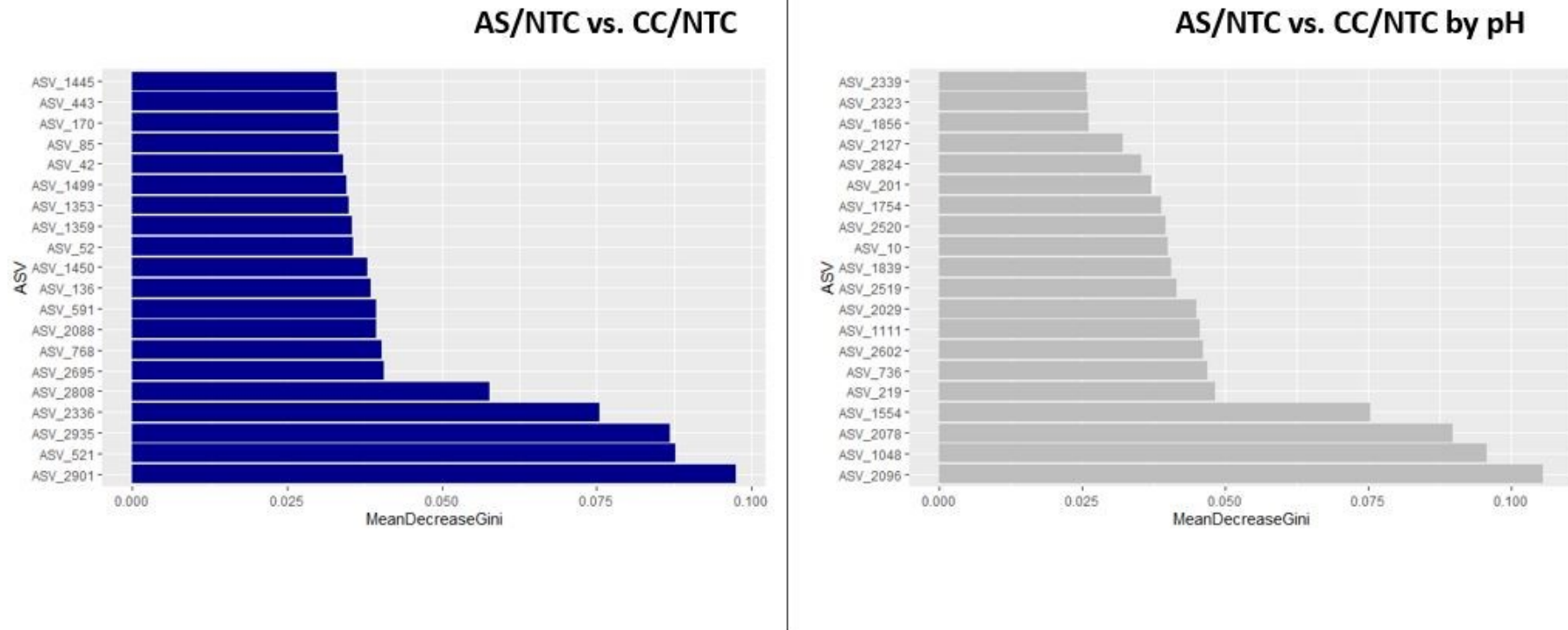
Figure 6b. An R generated plot (R package: ggplot) of the Random Forest classification tree output for the AS/NTC and CC/NTC treatment control run on 030719, identifying the top 20 most important ASV's that differentiate between the AS/NTC and the CC/NTC amended sediment communities (left), and between the two amendments by pH treatment (right). The y axis indicates the ASV's as predictors, and the x-axis describes the mean decrease GINI coefficient (i.e., node purity) used to predict the relative importance of a particular ASV in discriminating between the two factors.

Discussion

Coastal and nearshore environments describe a multi-stressor ecosystem that is chronically exposed to various natural and anthropogenic impacts. Previous studies have clearly demonstrated that these environments are regularly subjected to drastic oscillations in seawater pH within a typical diel/diurnal cycling event (Joint et al., 2011; Liu et al., 2010; Widdicombe et al., 2009). In addition to the large-scale global atmospheric CO_2 input into the surface of the ocean (i.e., ocean acidification), coastal regions are also exposed to smaller-scale localized impacts of terrestrial nutrient loading (i.e., eutrophication-induced-acidification), which further drives down seawater pH (Körtzinger et al., 2001; Nixon, 1995). The coupling of these two processes results in a compounding effect on ecosystem pH, further increasing the vulnerability of these environments to additional ecological disturbances (Gobler & Baumann, 2016; Guan et al., 2020; Kessouri et al., 2021; Laurent et al., 2017; Wallace et al., 2014). Within the coastal zone, eutrophication-induced-acidification often has greater consequences for the marine ecosystem in terms of seawater pH than that of ocean acidification alone (Borgesa & Gypensb, 2010; Melzner et al., 2013; Provoost et al., 2010; Sunda & Cai, 2012; Wallace et al., 2014). However, some selective environments may be uniquely poised to evade, or perhaps even mitigate changes in global ocean pH, where organisms within these environments unknowingly contribute to the natural resistance of the ecosystem to climatic changes (Rassmann et al., 2018b; Tynan & Opdyke, 2011).

This study was designed to investigate the response of benthic sediment microbial communities to the effects of organic carbon enrichment (eutrophication-induced acidification) and $CaCO_3$ amendment additions as a potential system buffer against changes in pH under different ocean acidification scenarios. In what is perhaps one of the first studies to address all three disturbance and mitigation factors on benthic sediment biogeochemistry and microbial community structure and composition. It was hypothesized that nearshore benthic microbial communities that receive both organic carbon and $CaCO_3$ enrichments would be structurally and compositionally different from the controls, where both additions will counteract one another in terms of acidification (i.e., pH reduction). Additionally, sediment communities that are exposed to ambient pH will be structurally dissimilar from sediment communities that receive increased CO_2 enrichment.

Chapter 4: Experiment 3

Dissolution of CaCO₃ Decreased Porewater [H+] Concentrations While OA Reduced OPD

        To better understand the implications of OA in combination with eutrophication it is necessary to put these experiments in context with the carbonate geochemistry. In a companion study conducted at the same time by (Vopel, Marshall, et al., 2021) it was shown that the addition of biogenic calcite (i.e., $CaCO_3$) to the surface of the sediments resulted in a spike in the flux of [H^+] transitioning from the bottom of the oxic sediment layer (H+ flux$_{sub}$) to the surface of the sediment under both light and dark conditions. This was observed in both ambient (pH 8.0) and CO_2 enriched (pH 7.7) treatments (Vopel, Marshall, et al., 2021). Here, the dissolution of $CaCO_3$ effectively decreased porewater [H^+] concentrations below what was observed within the overlying seawater within the CO_2 enriched (pH 7.7) treatment. This same effect was not detected under ambient (pH 8.0) conditions, where the impact of photosynthetic processes was greater, and therefore the addition of $CaCO_3$ exerted less of an influence on photosynthetically induced [H^+] oscillations. This chemical process (Ca^{2+} + $2HCO_3^-$ <-> $CaCO_3$+ CO_2 +H_2O) is more formally described by Long et al. (2015), where carbonate dissolution and precipitation (i.e., net calcification (NEC) are strong contributors to sedimentary carbon cycling within the coastal environment. Here the carbonate geochemistry was sufficiently buffered with the addition of $CaCO_3$ under enriched CO_2 conditions, this is also reflected in our findings where the microbial communities amended with calcite displayed increased structural stability compared to the non-mitigated AS/OC enriched communities.

        Photosynthetic processes result in the uptake of CO_2 which subsequently shifts the following equation: CO_2 + H_2O <-> H^+ + HCO_3^- <-> $2H^+$ + CO_3^{2-} to the left, effectively decreasing H^+ concentrations and increasing pH, whilst simultaneously causing a downward flux (i.e., consumption) in [H^+] in the photosynthetic zone of the sediments. Similar shifts in carbonate equilibria were observed under carbonate dissolution/calcification and additional redox processes (Long et al., 2015). By enriching the overlying water column with CO_2 for the pH 7.7 treatment, both the $CaCO_3$ amendments and Ashed Sand procedural controls observed notable reductions in oxygen penetration depths (OPD) under both light and dark conditions. A response that was not detected within the ambient (pH 8.0) treatments under the same diel cycles. This is perhaps attributed to the microbial reoxidation of the present reduced

solutes (i.e., Fe, Mn, S) in terms of aerobic mineralization and nitrification, which generate free [H^+] and consume bioavailable O_2. One such example would be the increase and subsequent oxidation of Fe^{2+}, which increases both the demand for O_2 as well as porewater [H^+] concentrations within the sediment surface. This process further complicates the following chemical interaction: $4Fe^{2+} + HCO_3^- + 10H_2O \rightarrow 7H^+ + 4Fe(OH)_3 + CH_2O$, ultimately effecting the pH-dependent sensitivity factor and corresponding net exchange of charge (Vopel, Marshall, et al., 2021). Other studies have inferred a similar biological response in terms of microbial mineralization processes across various marine sediment types based on variations in OPD and total O_2 flux (D M Alongi et al., 2005; Bosselmann, 2007; Rysgaard, Fossing, & Jensen, 2001). However, it cannot be said with confidence that the microbial activity is dictating the biogeochemical patterned observed here without measuring redox profiles in full.

CaCO3 Amendment Addition Possibly Mitigates the Added effects of OC Enrichment

Across the experiments, the addition of $CaCO_3$ appeared to slightly constrain the magnitude of the structural response of the microbial community to both acidification and organic carbon enrichment. This is shown best in the Active [RNA] community, possibly suggesting the potential for $CaCO_3$ dissolution to impact how the microbial community responds to acidification. However, even though this response from the Active community implied that in the presence of $CaCO_3$, the community structure demonstrated greater stability, this interaction could not be clearly reduced to a 1:1 cause and effect relationship where the addition of $CaCO_3$ combined with Organic Carbon could be effectively decoupled. Though this result is an interesting observation where the presence of $CaCO_3$ appears to illicit a greater response from the Active community than when absent, we strongly attest that this requires more testing. These findings do however align with our recent publication where we addressed the impacts of the dissolution of $CaCO_3$ in the surface sediment under acidified conditions (Vopel, Marshall, et al., 2021). This study and its companion study (Vopel, Marshall, et al., 2021) reinforce the possibility that $CaCO_3$ is an important player within the carbonate system in terms of buffering the effects of OA, which has been well documented by other recent publications (Egleston et al., 2010; Morse et al., 2006b; Tynan & Opdyke, 2011). This observation is further supported by Rodil et al., (2013) who showed that addition

of $CaCO_3$ resulted in a direct effect on sediment biogeochemical properties, insinuating microbiological impacts which govern biogeochemical processes. Furthermore, It was also anticipated that the pH in the overlying water column of our study would be substantially different from the pH underneath the thin layer of the organic material used for the OC amendment, based on the model of Ben-Yaakov, 1973.

Important Taxa Shown to Discriminate between OC Control and OC/CaCO₃ Sediment Communities

Using Random Forest analysis, major ASV's that differentiated between AS/OC amended communities from that of the CC/OC amended communities identified various taxa, that as anticipated, were associated with DOM (Dissolved Organic Matter) breakdown where the common denominator between these two communities is the organic carbon amendment. In both experiments, *Saprospiraceae* was classified as the most important discriminating ASV between the two communities. Previous taxonomic studies have demonstrated its importance as well as *Lewinella* in the breakdown of complex organic compounds previously isolated from the marine environment (McIlroy & Nielsen, 2014). *Alteromondaceae* belonging to the Class *Gammaproteobacteria* were also determined to be one of the most important differentiating taxa across both the 270619 and 010719 AS/OC and CC/OC treatments. McCarren et al., 2010 demonstrated under high-molecular-weight DOM amendment additions, *Alteromondaceae* showed transcriptional responses in upregulation of genes associated with assimilatory and dissimilatory single-carbon compound utilization, indicating its importance in chemical transformations associated with the breakdown of DOM. A study by Mahmoudi et al., 2014 also observed the same compositional response, where, in a nutrient-enriched environment (Caspian Sea), many of the responsive taxa were shown to belong to the order of Gammaproteobacteria (*Alteromonadales, Pseudoalteromonadaceae, Oceanospirillales*), further supporting our experimental observations. This finding is also consistent with this and our previous work (Chapter 2. & Chapter 3.), which acknowledges both *Altermonadales* and *Oceanospirillales* as "responsive" and possible bio-indicative taxa to changes in seawater pH.

Various sulfate-reducing bacteria (SRB's) were also found to be present within the top 20 discriminatory ASV's, likely spurred on by the introduction of organic material.

Desulfosarcinaceae and *Sva0081* from the Class *Desulfobacteria* were also present, and where previous work has elucidated their roles as critical players in carbon and Sulphur cycling within organically enriched marine sediments during the remineralization process (Paliaga, Felja, Budiša, & Ivančić, 2019). As anticipated, the presence and abundance of SRBs were notably greater in the second experiment compared to the first, demonstrating the natural temporal progression of nutrient turnover within the system. A prolonged experimental timeline would have likely seen an even greater increase in SRB concentrations, given the natural depreciation of available nutrients (Hoehler, Alperin, Albert, & Martens, 2001).

The Effects of Ocean Acidification Displays Only Minor Secondary Impacts

Four mesocosm flow chambers were circulated with carbonated seawater to achieve both ambient (pH 8.0) and the more CO_2 enriched (pH 7.7) pH treatments. The effects of pH were identifiable in the beta diversity matrices, which showed clear though non-significant differences in microbial structure governed by pH. However, it is important to note that though these differences were not recognized in terms of statistical significance ($p \leq 0.05$), the structure of the microbial communities displayed using PCoA ordinations visibly showed the impact of pH. Additionally, in terms of the statistics, the difference between reporting quantifiable significance versus non-significance in both the first 270619 and second 010719 (AS/OC and CC/OC) experiments by pH was distilled down to only a ≤ 0.02 difference. This implies that the effect of pH still had a measurable impact, though was not pronounced enough to be statistically significant. Therefore, we attest that its influence on microbial community composition and structure cannot be ruled out. Rather than the impact of the amendment additions in terms of organic carbon enrichment and $CaCO_3$ additions, were likely the more dominant driver for shifts on the microbial level than that of ocean acidification. As previously mentioned, in a coastal or nearshore environment, the local or regional impacts on seawater pH are likely to be the more pervasive of the two processes (Borgesa & Gypensb, 2010; Melzner et al., 2013; Provoost et al., 2010; Sunda & Cai, 2012; Wallace et al., 2014). Our observation in this study supports this general theory, where the response that we captured during this experiment from the microbial communities is perhaps a more accurate representation of a natural system that is exposed to more than one oceanic stressor.

Conclusion

Here we identify that the addition of $CaCO_3$ appears to effectively buffer the drawdown in pH due to organic matter addition. This suggests that sediments that are naturally rich in calcium carbonate may be better physically and biogeochemically equipped to deal with changes in water column pH. The co-occurrence of ocean acidification and eutrophication-induced acidification pose a unique challenge for coastal and nearshore environments, where universal mitigation strategies to combat such problems are still in their infancy. Despite our evidence to suggest that seeding benthic subtidal sediments with calcite ($CaCO_3$) minimizes the effects of low pH, we do not recommend this as a large-scale mitigation strategy. This approach requires an extensive understanding of the intricacy, heterogeneity, and variability of nearshore environments that are further complexed by different regional and local drivers. These contributing factors make it difficult to predict the effectiveness of this type of strategy magnified up on a larger scale. Particularly when interpreting the delicate interconnectivity between the microbial fraction and the biogeochemical cycling processes, which maintain the health and regulation of the marine ecosystem. Shifting this balance too far one way may have unforeseen consequences from the bottom up. Additionally, physically adding ground-up shell hash as a $CaCO_3$ buffer is not considered a cost-effective or ecological approach. More research is required to fundamentally understand the significance and the diversity of microbial communities in benthic sediments on a functional level as system drivers, but also how they respond to both terrestrially and atmospherically induced changes in seawater pH.

References

Alongi, D. M., Pfitzner, J., Trott, L. A., Tirendi, F., Dixon, P., & Klumpp, D. W. (2005). Rapid sediment accumulation and microbial mineralization in forests of the mangrove Kandelia candel in the Jiulongjiang Estuary, China. *Estuarine, Coastal and Shelf Science*, *63*(4), 605–618.

Andersen, K. S., Kirkegaard, R. H., Karst, S. M., & Albertsen, M. (2018). ampvis2: An R package to analyse and visualise 16S rRNA amplicon data. *BioRxiv*, 10–11. https://doi.org/10.1101/299537

Andersson, A. J., & MacKenzie, F. T. (2012). Revisiting four scientific debates in ocean acidification research. *Biogeosciences*, *9*(3), 893–905. https://doi.org/10.5194/bg-9-893-2012

Andersson, Andreas J., Bates, N. R., & Mackenzie, F. T. (2007). Dissolution of carbonate sediments under rising pCO2 and ocean acidification: Observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, *13*(3), 237–264. https://doi.org/10.1007/s10498-007-9018-8

Andersson, Andreas J. (2015). A fundamental paradigm for coral reef carbonate sediment dissolution. *Frontiers in Marine Science*, *2*, 52.

Andersson, Andreas J, Mackenzie, F. T., & Lerman, A. (2005). Coastal ocean and carbonate systems in the high CO2 world of the Anthropocene. *American Journal of Science*, *305*(9), 875–918.

Aparicio, F. L., Nieto-Cid, M., Borrull, E., Calvo, E., Pelejero, C., Sala, M. M., … Marrasé, C. (2016). Eutrophication and acidification: Do they induce changes in the dissolved organic matter dynamics in the coastal Mediterranean Sea? *Science of the Total Environment*, *563*, 179–189.

Auguie, B., & Antonov, A. (2017). *gridExtra*. 1–9. Retrieved from https://cran.r-project.org/web/packages/gridExtra/gridExtra.pdf

Basso, D. (2012). Carbonate production by calcareous red algae and global change. *Geodiversitas*, *34*(1), 13–33.

Battaglia, G., Steinacher, M., & Joos, F. (2016). A probabilistic assessment of calcium carbonate export and dissolution in the modern ocean. *Biogeosciences*, *13*(9), 2823–2848.

Ben-Yaakov, S. (1973). pH BUFFERING OF PORE WATER OF RECENT ANOXIC MARINE SEDIMENTS. *Limnology and Oceanography*, *18*(1), 86–94. https://doi.org/10.4319/lo.1973.18.1.0086

Berelson, W. M., Balch, W. M., Najjar, R., Feely, R. A., Sabine, C., & Lee, K. (2007). Relating estimates of CaCO3 production, export, and dissolution in the water column to measurements of CaCO3 rain into sediment traps and dissolution on the sea floor: A revised global carbonate budget. *Global Biogeochemical Cycles*, *21*(1).

Borgesa, A. V, & Gypensb, N. (2010). Carbonate chemistry in the coastal zone responds more strongly to eutrophication than ocean acidification. *Limnology and Oceanography*, *55*(1), 346–353.

Borrero-Santiago, A. R., Bautista-Chamizo, E., DelValls, T., & Riba, I. (2017). A possible CO2 leakage event: Can the marine microbial community be recovered? *Marine Pollution Bulletin*, *117*(1–2), 380–385. https://doi.org/10.1016/j.marpolbul.2017.02.027

Bosak, T., & Newman, D. K. (2003). Microbial nucleation of calcium carbonate in the Precambrian. *Geology*, *31*(7), 577–580. https://doi.org/10.1130/0091-7613(2003)031<0577:MNOCCI>2.0.CO;2

Bosselmann, K. (2007). *Sulfate reduction and iron-manganese cycling in intertidal surface sediments of the southern North Sea*. Universität Oldenburg.

Boudreau, B. P., Canfeld, D. E., & Mucci, A. (1992). Early diagenesis in a marine sapropel, Mangrove Lake, Bermuda. *Limnology and Oceanography*, *37*(8), 1738–1753.

Breiman, L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32.

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., … Wang, Y. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, *4*(11), 766–770.

Cai, W. J., Hu, X., Huang, W. J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., … Gong, G. C.

(2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, *4*(11), 766–770. https://doi.org/10.1038/ngeo1297

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Cloern, J. E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, *210*, 223–253.

Currie, A. R., Tait, K., Parry, H., de Francisco-Mora, B., Hicks, N., Mark Osborn, A., … Stahl, H. (2017). Marine microbial gene abundance and community composition in response to ocean acidification and elevated temperature in two contrasting coastal marine sediments. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.01599

Das, S., & Mangwani, N. (2015). Ocean acidification and marine microorganisms: responses and consequences. *Oceanologia*, *57*(4), 349–361. https://doi.org/10.1016/J.OCEANO.2015.07.003

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 1–14.

Dickson, A., Sabine, C., & Christian, J. (2007). Guide to Best Practices for Ocean C02 Measurments. In *North Pacific Marine Science Organization* (Vol. 3). Retrieved from www.pices.int

Dickson, A G, & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(10), 1733–1743.

Dickson, Andrew G. (1990). Standard potential of the reaction: AgCl (s)+ 12H2 (g)= Ag (s)+ HCl (aq), and and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, *22*(2), 113–127.

Drylie, T. P., Needham, H. R., Lohrer, A. M., Hartland, A., & Pilditch, C. A. (2019). Calcium carbonate alters the functional response of coastal sediments to eutrophication-

induced acidification. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-48549-8

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., … McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts*, *36*(2), 221–236.

Edmonds, J. W., Weston, N. B., Joye, S. B., Mou, X., & Moran, M. A. (2009). Microbial community response to seawater amendment in low-salinity tidal sediments. *Microbial Ecology*, *58*(3), 558–568. https://doi.org/10.1007/s00248-009-9556-2

Egleston, E. S., Sabine, C. L., & Morel, F. M. M. (2010). Revelle revisited: Buffer factors that quantify the response of ocean chemistry to changes in DIC and alkalinity. *Global Biogeochemical Cycles*, *24*(1), 1–9. https://doi.org/10.1029/2008GB003407

Fernandes, A. D., Vu, M. T. H. Q., Edward, L. M., Macklaim, J. M., & Gloor, G. B. (2018). A reproducible effect size is more useful than an irreproducible hypothesis test to analyze high throughput sequencing datasets. *ArXiv*, 1–15.

Fink, A., den Haan, J., Chennu, A., Uthicke, S., & de Beer, D. (2017). Ocean Acidification Changes Abiotic Processes but Not Biotic Processes in Coral Reef Sediments. *Frontiers in Marine Science*. https://doi.org/10.3389/fmars.2017.00073

Garnier, S. (2017). viridis: Default Color Maps from "matplotlib." *R Package Version 0.5.1*, 1–6. Retrieved from https://cran.r-project.org/package=viridis

Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., … Eakin, C. M. (2015). Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. *Science*, *349*(6243).

Gazeau, F., Van Rijswijk, P., Pozzato, L., & Middelburg, J. J. (2014). Impacts of ocean acidification on sediment processes in shallow waters of the Arctic Ocean. *PLoS One*, *9*(4), e94068.

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: and this is not optional. *Frontiers in Microbiology*, *8*, 2224.

Gobler, C. J., & Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled

dynamics and effects on marine life. *Biology Letters*, *12*(5), 20150976.

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. In *Journal of Statistical Software* (Vol. 22). https://doi.org/10.18637/jss.v022.i07

Guan, Y., Hohn, S., Wild, C., & Merico, A. (2020). Vulnerability of global coral reef habitat suitability to ocean warming, acidification and eutrophication. *Global Change Biology*, *26*(10), 5646–5660.

Hagens, M., Slomp, C. P., Meysman, F. J. R., Seitaj, D., Harlay, J., Borges, A. V, & Middelburg, J. J. (2015). Biogeochemical processes and buffering capacity concurrently affect acidification in a seasonally hypoxic coastal marine basin. *Biogeosciences*, *12*(5), 1561–1583.

Harvey, L. D. D. (2008). Mitigating the atmospheric CO2 increase and ocean acidification by adding limestone powder to upwelling regions. *Journal of Geophysical Research: Oceans*, *113*(C4).

Hassenruck, C., Fink, A., Lichtschlag, A., Tegetmeyer, H. E., de Beer, D., & Ramette, A. (2016). Quantification of the effects of ocean acidification on sediment microbial communities in the environment: the importance of ecosystem approaches. *Fems Microbiology Ecology*, *92*(5). https://doi.org/ARTN fiw027 10.1093/femsec/fiw027

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., … Humphries, E. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae*, *8*(1), 3–13.

Hoehler, T. M., Alperin, M. J., Albert, D. B., & Martens, C. S. (2001). Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. *FEMS Microbiology Ecology*, *38*(1), 33–41.

Hurd, C. L., Beardall, J., Comeau, S., Cornwall, C. E., Havenhand, J. N., Munday, P. L., … McGraw, C. M. (2020). Ocean acidification as a multiple driver: how interactions between changing seawater carbonate parameters affect marine life. *Marine and Freshwater Research*, *71*(3), 263–274.

Joint, I., Doney, S. C., & Karl, D. M. (2011). Will ocean acidification affect marine microbes. *ISME Journal*, *5*(1), 1–7. https://doi.org/10.1038/ismej.2010.79

Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2020). *Package ' adespatial .'* https://doi.org/10.1890/11-1183.1>.Maintainer

Jourabchi, P., Van Cappellen, P., & Regnier, P. (2005). Quantitative interpretation of pH distributions in aquatic sediments: A reaction-transport modeling approach. *American Journal of Science*, *305*(9), 919–956.

Kassambara, A. (2016). Practical guide to principal component methods in R: PCA, M (CA), FAMD, MFA, HCPC, factoextra. Vol. 2. *Sthda*. Retrieved from http://www.sthda.com/english/rpkgs/factoextra BugReports

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., … Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kessouri, F., McWilliams, J. C., Bianchi, D., Sutula, M., Renault, L., Deutsch, C., … Howard, E. M. (2021). Coastal eutrophication drives acidification, oxygen loss, and ecosystem change in a major oceanic upwelling system. *Proceedings of the National Academy of Sciences*, *118*(21).

Kitidis, V., Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., … Widdicombe, S. (2011). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters*. https://doi.org/10.1029/2011GL049095

Körtzinger, A., Hedges, J. I., & Quay, P. D. (2001). Redfield ratios revisited: Removing the biasing effect of anthropogenic CO2. *Limnology and Oceanography*, *46*(4), 964–970.

Kuhn, M. (2011). The caret Package. *A Unified Interface for Predictive Models*, 1–27. Retrieved from http://cran.r-project.org/web/packages/caret/vignettes/caretTrain.pdf%0Apapers2://publication/uuid/D8CA271E-F548-44F3-B081-B129A8765F04

Laurent, A., Fennel, K., Cai, W. J., Huang, W. J., Barbero, L., & Wanninkhof, R. (2017). Eutrophication-induced acidification of coastal waters in the northern Gulf of Mexico:

Insights into origin and processes from a coupled physical-biogeochemical model. *Geophysical Research Letters*, *44*(2), 946–956. https://doi.org/10.1002/2016GL071881

Lewis, E. R., & Wallace, D. W. R. (1998). *Program developed for CO2 system calculations*. Environmental System Science Data Infrastructure for a Virtual Ecosystem.

Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R News*, *2*(3), 18–22.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J.-P. (2010a). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*, *61*(3), 291–305.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., & Gattuso, J. P. (2010b). Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquatic Microbial Ecology*. https://doi.org/10.3354/ame01446

Long, M. H., Charette, M. A., Martin, W. R., & McCorkle, D. C. (2015). Oxygen metabolism and pH in coastal ecosystems: Eddy Covariance Hydrogen ion and Oxygen Exchange System (ECHOES). *Limnology and Oceanography: Methods*, *13*(8), 438–450.

Mackay, I. M. (2004). Real-time PCR in the microbiology laboratory. *Clinical Microbiology and Infection*, *10*(3), 190–212.

Mackenzie, F. T., Andersson, A. J., Arvidson, R. S., Guidry, M. W., & Lerman, A. (2011). Land–sea carbon and nutrient fluxes and coastal ocean CO2 exchange and acidification: Past, present, and future. *Applied Geochemistry*, *26*, S298–S302.

Malits, A., Boras, J. A., Balagué, V., Calvo, E., Gasol, J. M., Marrasé, C., … Vaqué, D. (2021). Viral-mediated microbe mortality modulated by ocean acidification and eutrophication: Consequences for the carbon fluxes through the microbial food web. *Frontiers in Microbiology*, *12*.

Mason, G. (2010). *Poisoning and Acidification of the Earth's Oceans*. Nova Science Publishers.

McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R., … DeLong, E. F. (2010). Microbial community transcriptomes reveal microbes and metabolic

pathways associated with dissolved organic matter turnover in the sea. *Proceedings of the National Academy of Sciences*, *107*(38), 16420–16427.

McIlroy, S. J., & Nielsen, P. H. (2014). The family saprospiraceae. In *The prokaryotes: Other major lineages of bacteria and the Archaea* (pp. 863–889). Springer Science+ Business Media.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure 1. *Limnology and Oceanography*, *18*(6), 897–907.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., … Körtzinger, A. (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology*, *160*(8), 1875–1888.

Morse, J. W., Andersson, A. J., & Mackenzie, F. T. (2006a). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO2 and "ocean acidification": Role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, *70*(23), 5814–5830.

Morse, J. W., Andersson, A. J., & Mackenzie, F. T. (2006b). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO2 and "ocean acidification": Role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, *70*(23), 5814–5830.

Mucci, A. (1983). The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. *American Journal of Science*, *283*(7), 780–799.

Murrell, P. (2003). *The grid Graphics Package*. 1–6.

Nelson, K. S., Baltar, F., Lamare, M. D., & Morales, S. E. (2020). Ocean acidification affects microbial community and invertebrate settlement on biofilms. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-60023-4

Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future

concerns. *Ophelia*, *41*(1), 199–219.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P. M., … & Wagner, H. (2008). The vegan package. *Community Ecology Package*, (May 2014), 190. Retrieved from https://bcrc.bio.umass.edu/biometry/images/8/85/Vegan.pdf

Oliver, A. E., Newbold, L. K., Whiteley, A. S., & van der Gast, C. J. (2014). Marine bacterial communities are resistant to elevated carbon dioxide levels. *Environmental Microbiology Reports*, *6*(6), 574–582. https://doi.org/10.1111/1758-2229.12159

Orr, J. C. (2011). Recent and future changes in ocean carbonate chemistry. *Ocean Acidification*, *1*, 41–66.

Paliaga, P., Felja, I., Budiša, A., & Ivančić, I. (2019). The Impact of a Fish Cannery Wastewater Discharge on the Bacterial Community Structure and Sanitary Conditions of Marine Coastal Sediments. *Water*, *11*(12), 2566.

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Provoost, P., Heuven, S. van, Soetaert, K., Laane, R., & Middelburg, J. J. (2010). Seasonal and long-term changes in pH in the Dutch coastal zone. *Biogeosciences*, *7*(11), 3869–3878.

Provoost, P., van Heuven, S., Soetaert, K., Laane, R., & Middelburg, J. J. (2010). Long-term record of pH in the Dutch coastal zone: a major role for eutrophication-induced changes. *Biogeosciences Discussions*, *7*(3).

Provoost, P., Van Heuven, S., Soetaert, K., Laane, R. W. P. M., & Middelburg, J. J. (2010). Seasonal and long-term changes in pH in the Dutch coastal zone. *Biogeosciences*, *7*(11), 3869–3878. https://doi.org/10.5194/bg-7-3869-2010

Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing noise from

pyrosequenced amplicons. *BMC Bioinformatics*, *12*(1), 38.
https://doi.org/10.1186/1471-2105-12-38

Rassmann, J., Lansard, B., Gazeau, F., Guidi-Guilvard, L., Pozzato, L., Alliouane, S., …
Rabouille, C. (2018a). Impact of ocean acidification on the biogeochemistry and
meiofaunal assemblage of carbonate-rich sediments: Results from core incubations
(Bay of Villefranche, NW Mediterranean Sea). *Marine Chemistry*, *203*, 102–119.

Rassmann, J., Lansard, B., Gazeau, F., Guidi-Guilvard, L., Pozzato, L., Alliouane, S., …
Rabouille, C. (2018b). Impact of ocean acidification on the biogeochemistry and
meiofaunal assemblage of carbonate-rich sediments: Results from core incubations
(Bay of Villefranche, NW Mediterranean Sea). *Marine Chemistry*, *203*(May 2017), 102–
119. https://doi.org/10.1016/j.marchem.2018.05.006

Ravaglioli, C., Bulleri, F., Rühl, S., McCoy, S. J., Findlay, H. S., Widdicombe, S., & Queirós, A.
M. (2019). Ocean acidification and hypoxia alter organic carbon fluxes in marine soft
sediments. *Global Change Biology*, *25*(12), 4165–4178.
https://doi.org/10.1111/gcb.14806

Regnier, P., Friedlingstein, P., Ciais, P., Mackenzie, F. T., Gruber, N., Janssens, I. A., …
Andersson, A. J. (2013). Anthropogenic perturbation of the carbon fluxes from land to
ocean. *Nature Geoscience*, *6*(8), 597–607.

Riebesell, U., Gattuso, J.-P., Thingstad, T. F., & Middelburg, J. J. (2013). Arctic ocean
acidification: pelagic ecosystem and biogeochemical responses during a mesocosm
study. *Biogeosciences*, *10*, 5619–5626.

Rodil, I. F., Lohrer, A. M., & Thrush, S. F. (2013). Sensitivity of heterogeneous marine benthic
habitats to subtle stressors. *PloS One*, *8*(11), e81646.

Roy, A. S., Gibbons, S. M., Schunck, H., Owens, S., Caporaso, J. G., Sperling, M., … Gilbert, J.
A. (2013). Ocean acidification shows negligible impacts on high-latitude bacterial
community structure in coastal pelagic mesocosms. *Biogeosciences*, *10*(1), 555–566.
https://doi.org/10.5194/bg-10-555-2013

Rysgaard, S., Fossing, H., & Jensen, M. M. (2001). Organic matter degradation through

oxygen respiration, denitrification, and manganese, iron, and sulfate reduction in marine sediments (the Kattegat and the Skagerrak). *Ophelia*, *55*(2), 77–91. https://doi.org/10.1080/00785236.2001.10409475

Shi, D., Hong, H., Su, X., Liao, L., Chang, S., & Lin, W. (2019). The physiological response of marine diatoms to ocean acidification: differential roles of seawater pCO 2 and pH. *Journal of Phycology*, *55*(3), 521–533.

Simeone, S., Molinaroli, E., Conforti, A., & De Falco, G. (2018). Impact of ocean acidification on the carbonate sediment budget of a temperate mixed beach. *Climatic Change*, *150*(3), 227–242.

Sulpis, O., Boudreau, B. P., Mucci, A., Jenkins, C., Trossman, D. S., Arbic, B. K., & Key, R. M. (2018). Current CaCO3 dissolution at the seafloor caused by anthropogenic CO2. *Proceedings of the National Academy of Sciences*, *115*(46), 11700–11705.

Sulpis, O., Jeansson, E., Dinauer, A., Lauvset, S. K., & Middelburg, J. J. (2021). Calcium carbonate dissolution patterns in the ocean. *Nature Geoscience*, 1–6.

Sunda, W. G., & Cai, W. J. (2012). Eutrophication induced CO2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric P CO2. *Environmental Science and Technology*, *46*(19), 10651–10659. https://doi.org/10.1021/es300626f

Tynan, S., & Opdyke, B. N. (2011). Effects of lower surface ocean pH upon the stability of shallow water carbonate sediments. *Science of the Total Environment*, *409*(6), 1082–1086.

Valiela, I. (2009). *Global coastal change*. John Wiley & Sons.

Villanueva, R. A. M., & Chen, Z. J. (2019). ggplot2: Elegant Graphics for Data Analysis (2nd ed.). *Measurement: Interdisciplinary Research and Perspectives*, *17*(3), 160–167. https://doi.org/10.1080/15366367.2019.1565254

Vopel, K., Del-Río, C., & Pilditch, C. A. (2018). Effects of CO2 enrichment on benthic primary production and inorganic nitrogen fluxes in two coastal sediments. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-017-19051-w

Chapter 4: Experiment 3

Vopel, K., Laverock, B., Cary, C., & Pilditch, C. A. (2021). Effects of warming and CO2 enrichment on O2 consumption, porewater oxygenation and pH of subtidal silt sediment. *Aquatic Sciences*, *83*(1). https://doi.org/10.1007/s00027-020-00765-5

Vopel, K., Marshall, A., Brandt, S., Hartland, A., Lee, C. K., Cary, S. C., & Pilditch, C. A. (2021). Biogeochemical feedbacks to ocean acidification in a cohesive photosynthetic sediment. *Scientific Reports*, *11*(1), 22867. https://doi.org/10.1038/s41598-021-02314-y

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014a). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, *148*, 1–13. https://doi.org/10.1016/j.ecss.2014.05.027

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014b). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, *148*, 1–13.

Washburne, A. D., Morton, J. T., Sanders, J., McDonald, D., Zhu, Q., Oliverio, A. M., & Knight, R. (2018). Methods for phylogenetic analysis of microbiome. *Nature Microbiology*, *3*(6), 652–661. https://doi.org/10.1038/s41564-018-0156-0

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wickham, H., Francois, R., & Bryan, J. (2020). *Package ' tibble .'*

Wickham, H., Francois, R., & Bryan, J. (2021). *Package ' tibble .'*

Wickham, H., & Wickham, M. H. (2020). Package 'plyr.' *Obtenido Httpscran Rproject Orgwebpackagesdplyrdplyr Pdf*.

Widdicombe, S., Dashfield, S. L., McNeill, C. L., Needham, H. R., Beesley, A., McEvoy, A., … Berge, J. A. (2009). Effects of CO2 induced seawater acidification on infaunal diversity and sediment nutrient fluxes. *Marine Ecology Progress Series*, *379*, 59–75.

Witt, V., Wild, C., Anthony, K. R. N., Diaz-Pulido, G., & Uthicke, S. (2011). Effects of ocean acidification on microbial community composition of, and oxygen fluxes through,

biofilms from the Great Barrier Reef. *Environmental Microbiology*, *13*(11), 2976–2989. https://doi.org/10.1111/j.1462-2920.2011.02571.x

Xie, M. Y. (2016). *Package 'knitr.'*

Supplementary Data and Information

Treatment of the Data and Identification of Contaminants

A 0.005% cull cut-off was applied to the raw ASV counts table to remove poorly represented ASV's within the dataset (Bokulich et al., 2013). This approach results in the removal of any ASV from the ASV matrix with cumulatively less than 157 reads across all samples. Prior to filtering, a total of 27,345 ASV's were identified with a total number of reads of 3,632,100 with a minimum ASV read count of 2. Post filtering, a total of 2,939 ASV's were maintained in the data set, with a total number of reads of 2,701,916 with a minimum ASV read count of 171. Where 89.3% of the ASV's were removed from the dataset, and 74.4% of the total ASV read count was removed from the dataset following the 0.005% cull. Cyanobacteria and chloroplasts are known to co-amplify with the applied 16S rRNA primers, following standard bioinformatic protocols, cyanobacteria/chloroplasts were subset from the dataset. A generated rarefaction curve (R: Phyloseq: ggrare function) (post 0.005% cull) of the ASV matrix by nucleic acid type (i.e., DNA, cDNA/RNA) demonstrated that all samples were sequenced to full taxonomic saturation, indicating that each sample was sequenced to full depth and the diversity within each sample was considered "well represented" (**Supp. Figure 1a**.). The dataset was then split by nucleic acid type Total [DNA] dataset, and Active [cDNA/RNA] (Referred to after this point as "RNA") dataset, and bioinformatically processed independently (**Supp. Figure 1b. and c.**). It was determined that during the nucleic acid co-extraction process the procedural controls (PC: Blank) used to verify the sterility of the extraction reagents, came back positive with an unknown source of contamination. This concentration was pronounced in both the DNA (6.26 ng/μl) and the RNA (46.6 ng/μl) datasets, though comparatively less than the average concentration of an actual sediment sample (DNA: RE1: 600.07 ± 89.57; RE2: 666.70 ± 214.92; RE3: 626.80 ± 57.15; RE4: 650.97 ± 17.67; RNA: RE1: 191.08 ± 84.59; RE2: 154.70 ± 32.00; RE3: 246.48 ± 27.44; RE4: 278.55 ± 86.27). The contaminated blank samples were processed to completion and sequenced to verify their nature. Preliminary analysis showed that the composition of the contaminated blank samples was only marginally different from the actual samples, however, it was structurally unique to the actual samples where the abundances of certain ASV's were vastly different from the actual samples. Decreasing the probability of "introduced" contamination

during the extraction process, but where point source contamination could not be verified. However, based on the sequence profile of the contaminant it was evident that the contamination did not originate from the extraction or PCR reagents, but surprisingly showed characteristics that were highly similar to the other sequenced sediment samples. Meaning it was more likely that the procedural controls used to verify the sterility of the reagents were unknowingly contaminated with a sediment sample rather than exposed to an external variant. To address this known contaminant issue, both the DNA and RNA dataset were treated with the Decontam (Davis et al., 2018) package in R using the more aggressive prevalence classification threshold (threshold=0.5), to assess the extent of the contamination across the other samples, and verify what is a contaminant and what is a true sample (**Supp. Figures 2a, c; 3a, c**). This is done to avoid the introduction of microbial genetic material (i.e., DNA/RNA) that is not originally present in the sampled community, which confounds downstream analyses. Failure to account for contamination in the Active [RNA] or Total [DNA] datasets would likely result in major inaccuracies in the interpretation of the data, where introduced contamination falsely inflates with-in sample diversity.

The Decontam approach seeks to statistically identify and visualize and contaminating nucleic acid features, where once identified, can be effectively removed from the dataset (**Supp. Figures 2b, 3b**). Decontam works through the implementation of two standard de novo classification methods that are based on frequent or widely reproduced external contamination signatures which appear distinct from the samples where (1) sequences from the contaminated taxa that have frequencies that are inversely correlated to the signals from the desired sample concentrations (Fernández-Gó Mez et al., 2013; Lusk, 2014; Salter et al., 2014) and (2) sequences of the contaminated taxa are likely to have increased prevalence in the control than are present in the actual sediment samples (Adams et al.,2015; Dunn et al.,, 2013). In the Active [RNA] dataset, the Decontam protocol effectively discriminated between the *true* sediment samples, the procedural control, and the organic matter control based on contaminating taxonomic frequency as shown in **Supp. Figure 2a**., where both the procedural and organic matter controls demonstrated markedly less sequence reads compared to the true samples. The Decontam "prevalence" (i.e., presence/absence) analysis of the Active [RNA] community detected higher prevalence of *contaminated taxa* in the procedural controls versus the true sediment samples using a 0.5 threshold, which identifies all

sequences that are more prevalent in the negative control samples than the positive samples (**Supp. Figure 2b.).** Based on this stringent approach, true sediment samples are shown to separate cleanly from the contaminated controls (**Supp. Figure 2b.).** Additionally, despite the detection of contaminated taxa from a control clustering with the true samples, it was able to be effectively removed given that is displays a prevalence measurement that is much greater than all *true* samples (**Supp. Figure 2b**.). Given the results of the Decontam protocol on the Active [RNA] community we are confident in its effectiveness in removing the presence of contaminated taxonomic sequences from the dataset. However, the Total [DNA] community was not as easily corrected using the Decontam pipeline. Results of the frequency analysis (1) failed to clearly differentiate between true sediment samples and contaminated controls based on the frequency of sequence reads (**Supp. Figure 3a**.), where true samples and contaminated controls appear to express a similar number of sequences. The prevalence (2) analysis did effectively discriminate between the true sediment samples and the controls using the same stringent 0.5 prevalence threshold (**Supp. Figure 3b**.). However, given that the results showed that the Decontam protocol was only marginally effective in removing the full contamination from the Total [DNA] dataset we carefully report both Total [DNA] and Active [RNA] findings in the results, but we conservatively present (Figures etc.) only the Active [RNA] community data. For contextual reference, all Total [DNA] figures and tables have been maintained only in the supplementary data. Following Decontam analysis, a total of 1,205 ASV's [DNA] and 755 ASV's [RNA] were identified as contamination and removed from the dataset. Where the remaining "theoretically" contaminant free ASV's, 1,734 [DNA] and 2,184 [RNA] were then processed (bioinformatically) under *standard* conditions.

Post decontamination, the resulting taxonomically classified ASV's across both the DNA and RNA datasets were subset out in R using the Phyloseq package under the function subset_taxa to separate the dataset by *Bacteria* (DNA: 1,372; RNA: 1,720 ASV's), *Archaea* (DNA: 25; RNA: 34 ASV's), *Eukaryota* (DNA: 23; RNA: 36 ASV's)*, and Blank* (*unclassified*) (DNA: 314; RNA: 394 ASV's) at the domain level, and *Chloroplasts* (DNA: 66; RNA: 103 ASV's) at the order level. Any ASV that was not annotated at the domain level (i.e., unclassifiable) was removed from the dataset. Any ASV that annotated as Chloroplast at the order level was removed from the dataset Any ASV annotated as *Eukaryota* at the domain level was removed

from the dataset. Where the remaining ASV's 1,331 [DNA] and 1,651 [RNA] were used for downstream analyses. In contrast to the previous experiments, this study applied a dark/light (day/night) natural circadian simulation approach. The incorporation of "light" into the experiment ensured that natural photosynthetic processes and their corresponding biological componentry (i.e., cyanobacteria, chloroplasts) were unavoidably maintained. Given the dominating presence of cyanobacteria and algal plant cells on the subtidal sediment community, these individuals were selectively removed from the dataset prior to downstream analysis and taxonomic profiling.
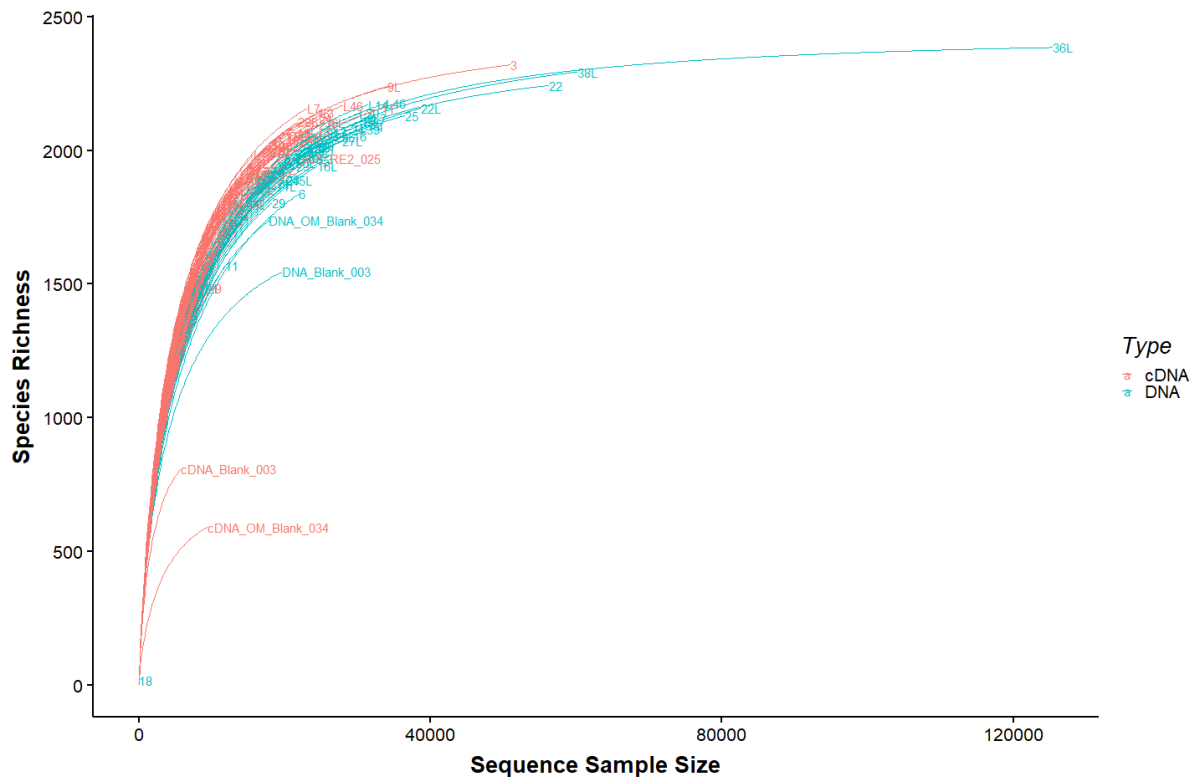
Supplementary Figure 1a. Rarefaction curve of the culled and filtered ASV matrix demonstrating each sample was sequenced to full taxonomic saturation. The Active [RNA/cDNA] samples are distinguishable (red) from the Total [DNA] (blue) samples. All blank (procedural control) samples show low sequence numbers and nominal species richness values.
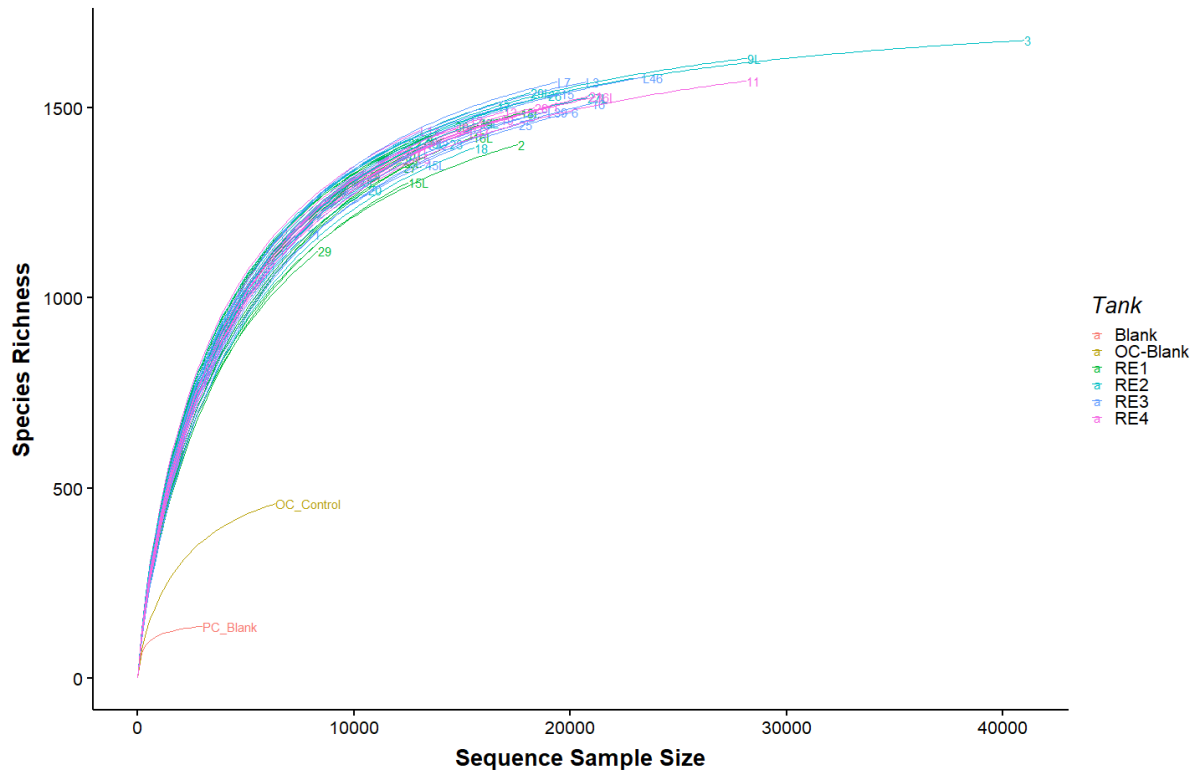
Supplementary Figure 1b. Rarefaction curve of the culled and filtered ASV matrix for the subset Active [RNA] dataset demonstrating each sample was sequenced to full taxonomic saturation.
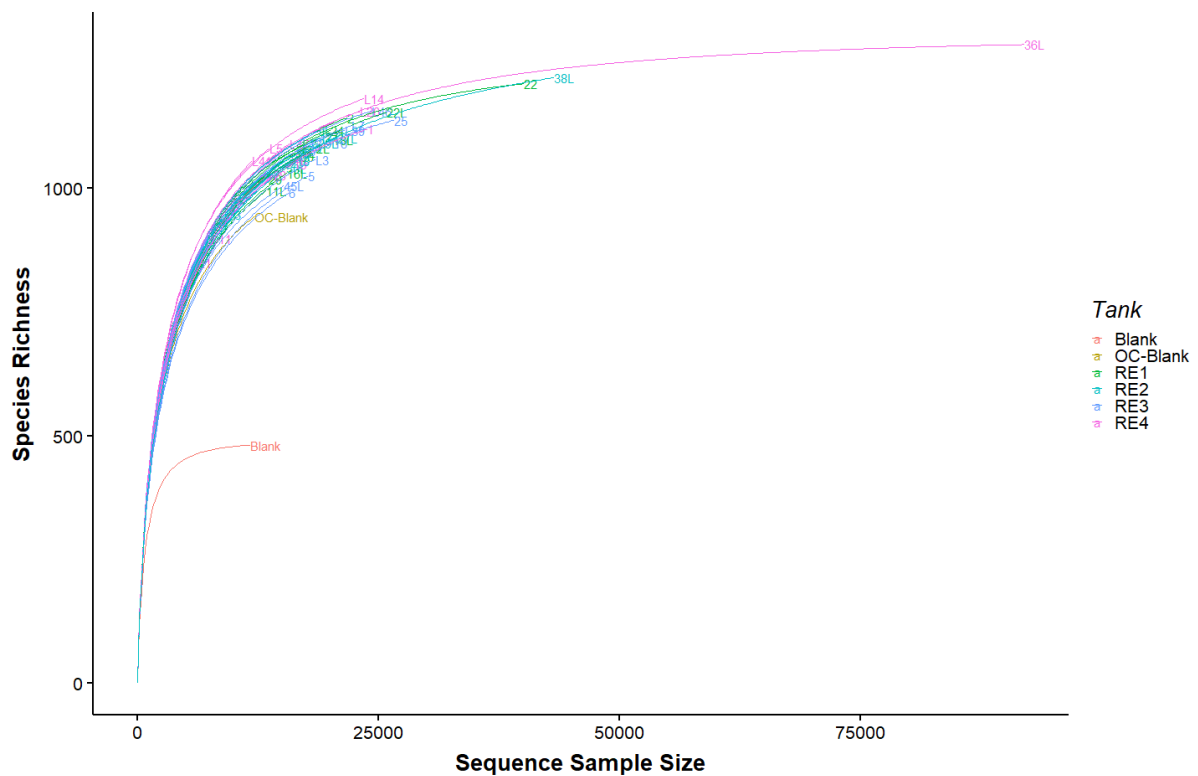
Supplementary Figure 1c. Rarefaction curve of the culled and filtered ASV matrix for the subset Total [DNA] dataset demonstrating each sample was sequenced to full taxonomic saturation.
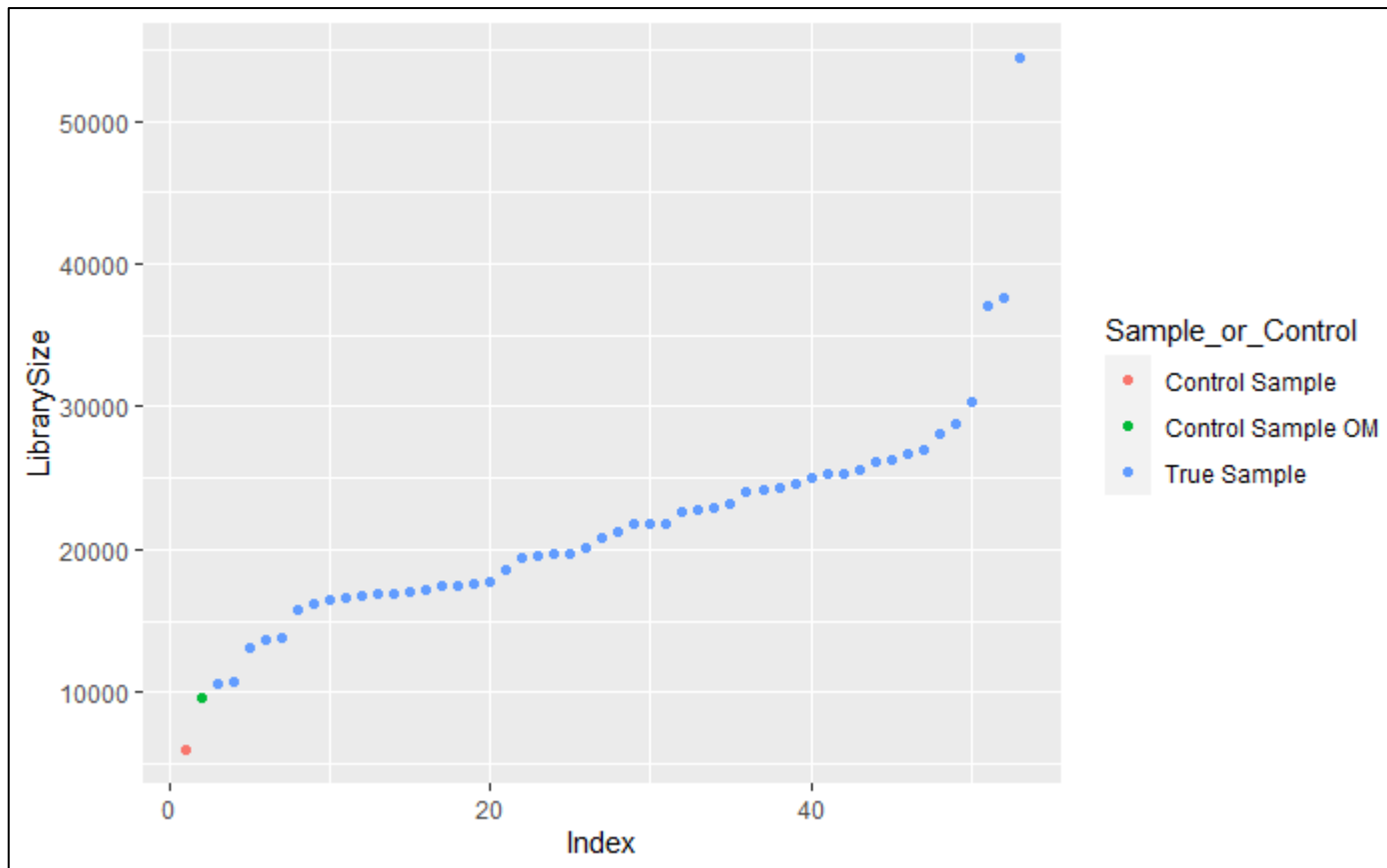
Supplementary Figure 2a. Shows the number of reads (i.e., library size) in each of the RNA samples as a function of determining what is considered a "True Sample" and what is a negative control using the decontam package in R. The PC/Blank sample shown here in red demonstrates comparatively fewer reads than True samples. The OC (Organic Carbon) control sample displayed in green also shows significantly fewer reads than the lowest True sample.
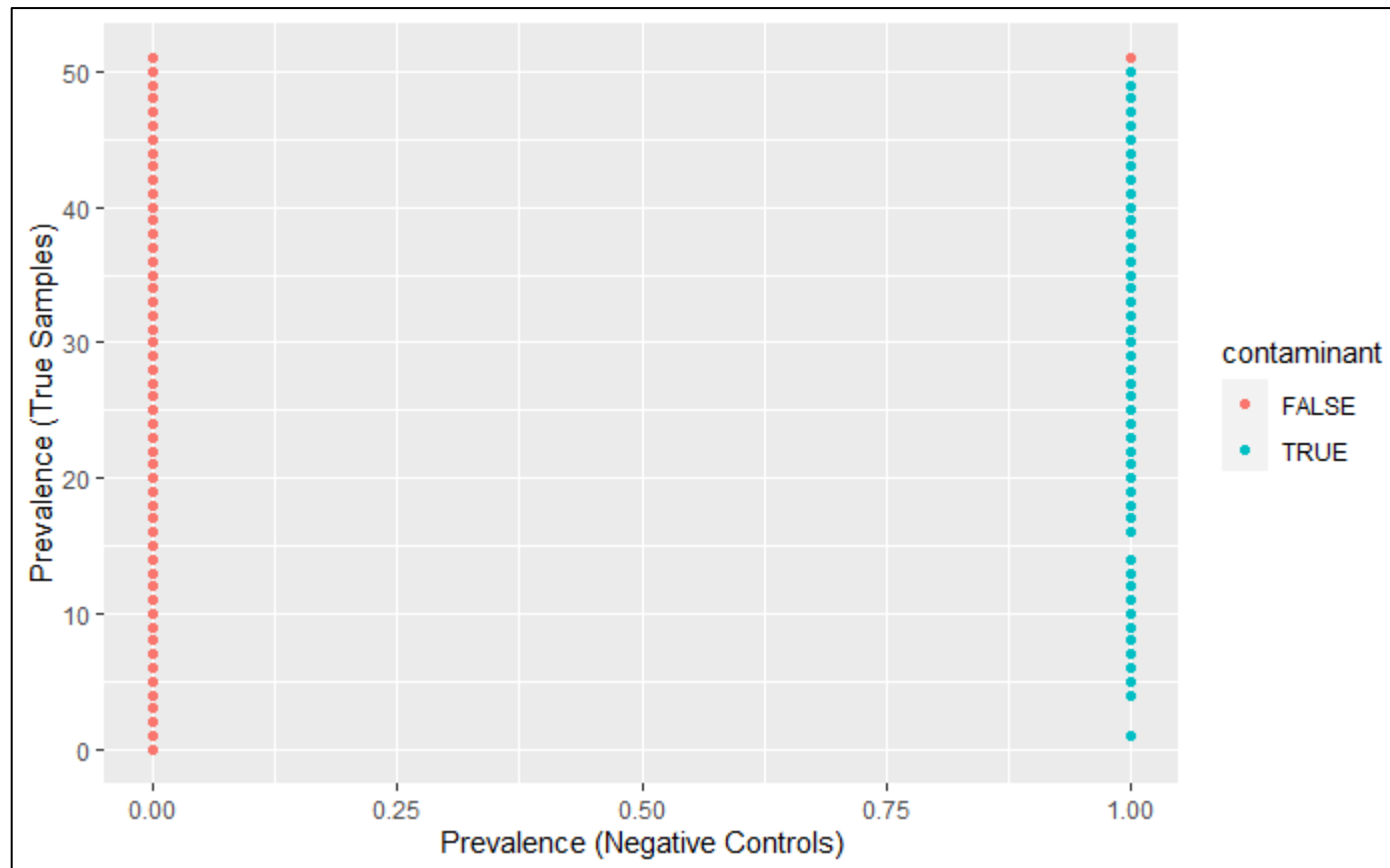
Supplementary Figure 2b. Provides a visual output of the decontam prevalence of contamination identification approach for the RNA dataset which looks at the prevalence (i.e., presence/absence) in terms of each sequence feature across all "True Samples" versus negative controls, to better identify possible contaminants with a threshold of 0.5 which identifies all sequences that are more prevalent in the negative control samples than the positive samples as contaminants. Samples are shown to split cleanly in to two separate branches where the red (FALSE) points demonstrate the prevalence of taxa that show up mostly in the True samples, versus the blue (True; logical variable for negative control) points which indicate the prevalence of taxa in the negative controls. The even separation shows that the stringent 0.5 threshold approach effectively identified taxa that were mostly in the negative (contaminated PC: Blank) samples. Allowing for clean removal.

Supplementary Figure 3a. Shows the number of reads (i.e., library size) in each of the DNA samples as a function of determining what is considered a "True Sample" and what is a negative control using the decontam package in R. The PC/Blank sample shown here in red demonstrates comparatively fewer reads than the majority of True samples. The OC (Organic Carbon) control sample displayed in green also shows significantly fewer reads than many of the True sample. Several True samples were identified here as *low-read* outliers. These low-read samples were maintained in the dataset to strengthen the algorithmic identification of contaminants.
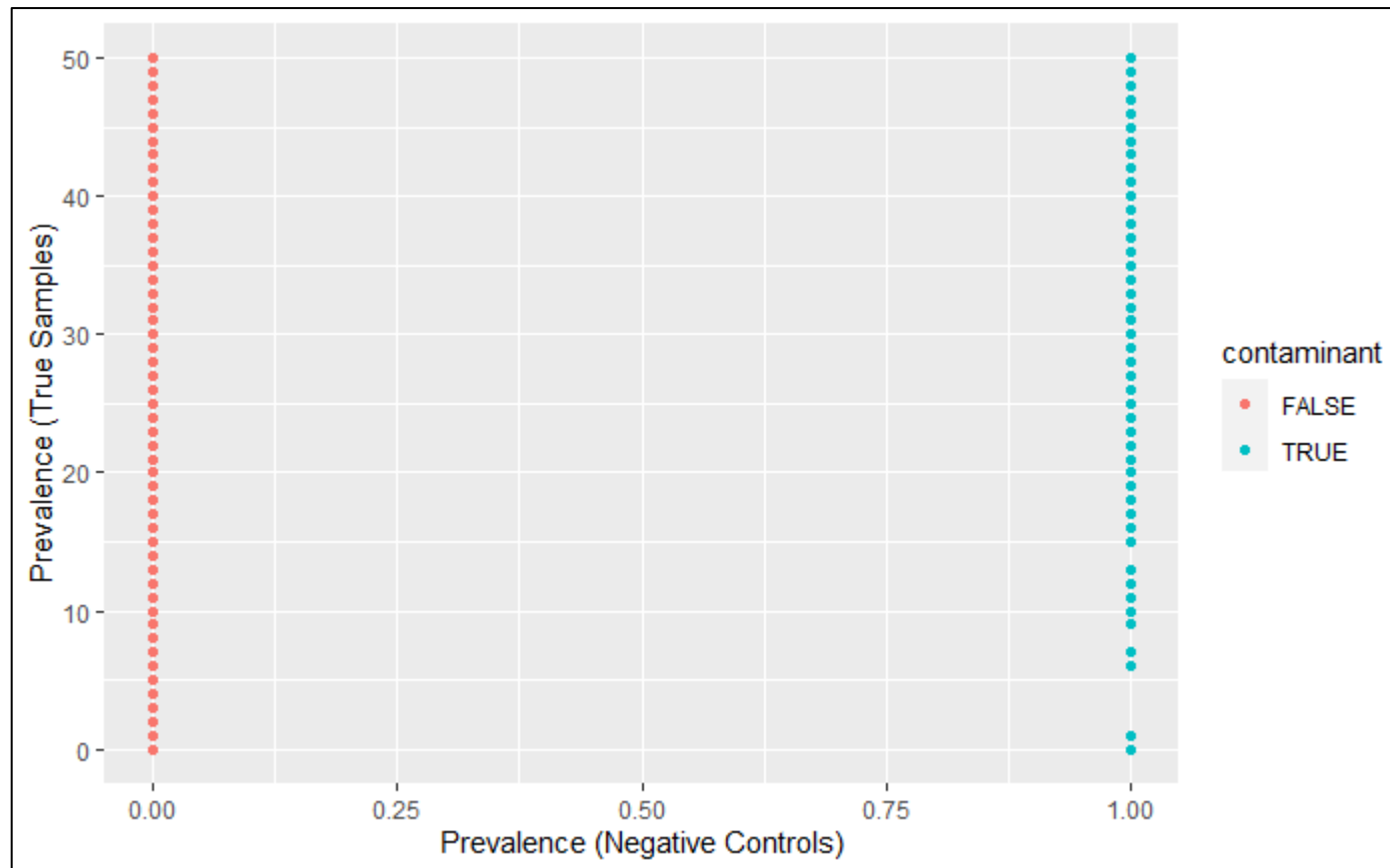
Supplementary Figure 3b. Provides a visual output of the decontam prevalence of contamination identification approach for the DNA dataset which looks at the prevalence (i.e., presence/absence) in terms of each sequence feature across all "True Samples" versus negative controls, to better identify possible contaminants with a threshold of 0.5 which identifies all sequences that are more prevalent in the negative control samples than the positive samples as contaminants. Samples are shown to split cleanly in to two separate branches where the red (FALSE) points demonstrate the prevalence of taxa that show up mostly in the True samples, versus the blue (True; logical variable for negative control) points which indicate the prevalence of taxa in the negative controls. The even separation shows that the stringent 0.5 threshold approach effectively identified taxa that were mostly in the negative (contaminated PC: Blank) samples. Allowing for clean removal.

Supplementary Figure 4a. Taxonomic heatmap of the RNA dataset of the top ten most abundant (i.e., *relative abundances*) taxa at the Phylum level, separated by the different sampling dates for the AS/OC and CC/OC treatments (270619 and 010719) and the AS/NTC and CC/NTC treatment controls (290619 and 030719), and by the individual mesocosm treatment tanks (RE1-RE4). The Procedural Control (Blank), and the Organic Carbon (OC-Blank) demonstrate distinct differences in structure compared to *true* samples.

Supplementary Figure 4b. Taxonomic heatmap of the RNA dataset of the top ten most abundant (i.e., *relative abundances*) taxa at the level of Order, separated by the different sampling dates for each of the for the AS/OC and CC/OC treatments (270619 and 010719) and the AS/NTC and CC/NTC treatment controls (290619 and 030719), and by the individual mesocosm treatment tanks (RE1-RE4). The Procedural Control (Blank), and the Organic Carbon (OC-Blank) demonstrate distinct differences in structure compared to *true* samples.

Chapter 4: Experiment 3

Supplementary Table 1. RNA (Active) and DNA (Total) abundance averages calculated from the taxonomic heatmaps (RNA: Supplementary Figure 4a; DNA: Supplementary Figure 4b) for the top 10 most (relative) abundant taxa (Order Level) across the AS/OC and CC/OC treatments (270619 and 010719) and the AS/NTC and CC/NTC treatment controls (290619 and 030719).

RNA Taxonomy	ASV	270619	010719	290619	030719
Flavobacteriales	3	2.30%	3.70%	2.03%	4.00%
BD7-8	6	2.30%	2.32%	1.80%	1. 52%
Nitrosopumilales	5	1.80%	1.60%	1.72%	2.00%
Nitrosopumilales	7	1.40%	1.20%	1.40%	1.40%
Flavobacteriales	12	1.20%	1.20%	2.30%	0.73%
Nitrosopumilales	14	1.08%	0.90%	1.06%	1.10%
Unknown (Blank)	46	1.00%	1.00%	1.08%	1.20%
BD7-8	20	1.00%	1.20%	0.90%	1.00%
Cytophagales	33	0.60%	0.80%	1.73%	1.00%
Sva1033	11	1.30%	1.30%	0.50%	0.60%

DNA Taxonomy	ASV's	270619	010719	290619	030719
Flavobacteriales	3	4.10%	7.40%	5.42%	7.23%
Unknown (Blank)	4	3.20%	3.00%	3.60%	3.30%
Nitrosopumilales	5	2.30%	2.20%	2.20%	2.30%
Flavobacteriales	9	1.80%	1.63%	1.30%	1.30%
Nitrosopumilales	7	1.40%	1.40%	1.42%	1.60%
Bacteroidetes VC2. 1 Bac22	10	1.50%	1.30%	1.60%	1.40%
BD7-8	6	1.40%	1.40%	1.40%	1.50%
Sva1033	11	2.06%	1.70%	0.82%	0.88%
Verrucomicrobiales	16	1.70%	1.30%	1.60%	0.88%
Nitrosopumilales	13	1.22%	1.20%	1.12%	1.23%

Supplementary Figure 4c. Taxonomic heatmap of the DNA dataset of the top ten most abundant (i.e., *relative abundances*) taxa at the Phylum level, separated by the different sampling dates for each of the for the AS/OC and CC/OC treatments (270619 and 010719) and the AS/NTC and CC/NTC treatment controls (290619 and 030719), and by the individual mesocosm treatment tanks (RE1-RE4). The Procedural Control (Blank), and the Organic Carbon (OC-Blank) demonstrate distinct differences in structure compared to *true* samples.

	10719				270619				290619				30719				Blank	
	RE1	RE2	RE3	RE4	RE1	RE2	RE3	RE4	RE1	RE2	RE3	RE4	RE1	RE2	RE3	RE4	Blank	OC-Blank
Flavobacteriales; ASV_3	6.3	8.2	5.8	7.2	4.4	3.5	4.6	3.9	5.4	6.3	4.4	5.6	5.9	8.9	6.8	7.3	6.2	6.3
Blank; ASV_4	2.6	2.9	3.4	2.9	3.3	3	3.3	3.2	3.6	3.3	4	3.4	3.6	2.9	3.7	3	6	4.6
Nitrosopumilales; ASV_5	2.1	2.2	2.2	2.1	2.3	2.5	2.5	1.9	2	2.2	2.4	2.1	2.4	2.6	2.2	2.3	1.2	1.4
Flavobacteriales; ASV_9	2	1.4	1.8	1.3	1.9	1.9	1.8	1.5	1.2	1.2	1.5	1.1	1.2	1.3	1.5	1.2	3	2
Nitrosopumilales; ASV_7	1.6	1.2	1.5	1.3	1.3	1.5	1.6	1.2	1.3	1.5	1.5	1.4	1.5	1.7	1.4	1.6	0.8	1.2
Bacteroidetes VC2.1 Bac22; ASV_10	1.2	1.1	1.3	1.3	1.6	1.4	1.5	1.5	1.6	1.5	1.7	1.3	1.6	1.2	1.7	1.4	1.3	1.2
BD7-8; ASV_6	1.2	1.4	1.5	1.3	1.3	1.5	1.4	1.2	1.3	1.3	1.5	1.5	1.6	1.5	1.5	1.2	1.7	1.2
Sva1033; ASV_11	2.7	1.1	1.8	1	3	2.3	1.5	1.4	0.8	0.8	1	0.7	0.9	1	0.9	0.7	3.1	2.9
Verrucomicrobiales; ASV_16	1.1	1.3	1.2	1.4	1.1	2	1.7	1.9	1.4	1.5	1.5	2	0.7	0.9	0.9	1	0.8	0.6
Nitrosopumilales; ASV_13	1.2	1.1	1.2	1.1	1.3	1.4	1.2	1	1.1	1.1	1.2	1.1	1.2	1.4	1.2	1.1	0.6	0.8

Supplementary Figure 4d. Taxonomic heatmap of the DNA dataset of the top ten most abundant (i.e., *relative abundances*) taxa at the level of Order, separated by the different sampling dates for each of the for the AS/OC and CC/OC treatments (270619 and 010719) and the AS/NTC and CC/NTC treatment controls (290619 and 030719), and by the individual mesocosm treatment tanks (RE1-RE4). The Procedural Control (Blank), and the Organic Carbon (OC-Blank) demonstrate distinct differences in structure compared to *true* samples.

294

Chapter 4: Experiment 3

Supplementary Table 2a. Alpha diversity metric analyses (Observed and Shannon) using Two-way ANOVA (Analysis of Variance) statistics for examining statical differences in species diversity between the AS/OC and CC/OC (270619 and 010719) treatments and the AS/NTC and CC/NTC (290619 and 030719) treatment controls, for both Active [RNA] and Total [DNA] datasets. Post-hoc Tukey test values are reported below the ANOVA statistics as a measure of "True Significance." Values that did not meet the assumptions of the post-hoc Tukey test were not reported in the analysis or the results.

RNA	Observed Significance ($p \leq 0.05$)	Observed F-value	Shannon Significance ($p \leq 0.05$)	Shannon F-value
AS/OC and CC/OC Treatments				
270619 (Amendment)	0.99	0.0	0.696	0.162
010719 (Amendment)	0.337	1.019	0.172	2.166
270619/010719 (Amendment)	0.5	0.47	0.189	1.835
270619/010719 (pH)	0.0826	3.307	0.138	2.369
AS/NTC and CC/NTC Nontreatment Controls				
290619 (Amendment)	0.0896	3.533	0.0466*/ Tukey: 0.046628*	5.15
030719 (Amendment)	0.921	0.083	0.46	0.828
290619/030719 (Amendment)	0.391	0.978	0.873	0.137
290619/030719 (pH)	0.482	0.509	0.457	0.571

DNA	Observed Significance ($p \leq 0.05$)	Observed F-value	Shannon Significance ($p \leq 0.05$)	Shannon F-value
AS/OC and CC/OC Treatments				
270619 (Amendment)	0.804	0.065	0.37	0.889
010719 (Amendment)	0.318	1.105	0.696	0.162
270619/010719 (Amendment)	0.441	0.616	0.395	0.755
270619/010719 (pH)	0.611	0.267	0.598	0.287
AS/NTC and CC/NTC Nontreatment Controls				
290619 (Amendment)	0.189	1.986	0.503	0.482
030719 (Amendment)	0.125	2.49	0.511	0.711
290619/030719 (Amendment)	0.165	1.942	0.607	0.51
290619/030719 (pH)	0.806	0.061	0.47	0.538

Chapter 4: Experiment 3

Supplementary Table 2b. Calculated averages (i.e.., mean, standard deviation) for the alpha diversity metric data (Observed, Chao1, Shannon) for the AS/OC and CC/OC (270619 and 010719) treatments and the AS/NTC and CC/NTC (290619 and 030719) treatment controls, separated by the different datasets for both nucleic acid types (RNA, DNA).

RNA			
Date Sampled	**Observed**	**Chao1**	**Shannon**
270619	837.0±21.2	1288.0±75.0	6.1±0.08
010719	827.0±21.0	1252.0.4±52.4	6.08±0.08
270619/010719	832.0±21.6	1270.0±67.0	6.10±0.08
290619	810.3±19.4	1246.1±38.0	6.05±0.06
030719	821.1±17.0	1268.1±76.0	6.05±0.07
290619/030719	816.3±19.0	1258.4±63.0	6.05±0.07

DNA			
Date Sampled	**Observed**	**Chao1**	**Shannon**
270619	842.4±12.6	1058.0±30.0	5.74±0.06
010719	850.3±19.3	1082.6±31.2	5.70±0.05
270619/010719	846.5±17.0	1071.0±33.0	5.72±0.06
290619	837.4±22.3	1055.4±50.3	5.70±0.06
030719	852.0±24.0	1080.5±50.0	5.70±0.10
290619/030719	845.2±24.1	1069.4±51.5	6.70±0.08

Chapter 4: Experiment 3

Supplementary Table 3a. Beta diversity analyses for the PhILR log transformed RNA dataset using the ANOSIM statistical approach (*significance: p≤0.05*) to identify differences between experimental treatment variables for the AS/OC and CC/OC (270619 and 010719) treatments and the AS/NTC and CC/NTC (290619 and 030719) treatment controls.

RNA: All Samples		
Variable	**R Value**	**Significance (p≤0.05)**
Mesocosm Tank (RE1-RE4)	0.17	0.001*
Date Sampled	0.4977	0.001*
Treatment (Amendment)	0.3734	0.001*
pH	0.174	0.002*
RNA: AS/OC and CC/OC Treatments (Combined: 270619 & 010719)		
Variable	**R Value**	**Significance (p≤0.05)**
Treatment (Amendment)	0.1434	0.022*
pH	0.1358	0.02*
RNA: AS/OC and CC/OC Treatment 270619		
Variable	**R Value**	**Significance (p≤0.05)**
Treatment (Amendment)	0.07222	0.234
pH	0.1944	0.066
RNA: AS/OC and CC/OC Treatment 010719		
Variable	**R Value**	**Significance (p≤0.05)**
Treatment (Amendment)	0.2852	0.006*
pH	0.1519	0.062
RNA: AS/NTC and CC/NTC Nontreatments Controls (Combined: 290619 & 030719)		
Variable	**R Value**	**Significance (p≤0.05)**
Treatment (Amendment)	0.05178	0.22
pH	-0.01977	0.623
RNA: AS/NTC and CC/NTC Nontreatment Control 290619		
Variable	**R Value**	**Significance (p≤0.05)**
Treatment (Amendment)	0.0963	0.179
pH	0.04074	0.301
RNA: AS/NTC and CC/NTC Nontreatment Control 030719		

Chapter 4: Experiment 3

Variable	R Value	Significance (p≤0.05)
Treatment (Amendment)	0.02568	0.381
pH	-0.04883	0.697

Chapter 4: Experiment 3

Supplementary Table 3b. Beta diversity analyses for the PhILR log transformed RNA dataset for the combined assessment of each amendment from both AS/OC and CC/OC treatments (270619/010719) treatments and the AS/NTC and CC/NTC (290619/030719) treatment controls using the ANOSIM statistical approach (*significance: p≤0.05*) to identify differences between experimental treatment variables across time. By combining the same treatments/nontreatment controls together from the different experiment sampling dates was used to identify the effects of time on community structure and composition.

Treatments and Controls	Significance *p ≤0.05* (ANOSIM)	R Value
AS/OC		
Date Sampled (Time)	0.078	0.1704
pH	0.053	0.2148
AS/NTC		
Date Sampled (Time)	0.022*	0.3493
pH	0.719	-0.08267
CC/NTC		
Date Sampled (Time)	0.069	0.1667
pH	0.394	0.0172
CC/OC		
Date Sampled (Time)	0.001*	0. 6574
pH	0.128	0.1315
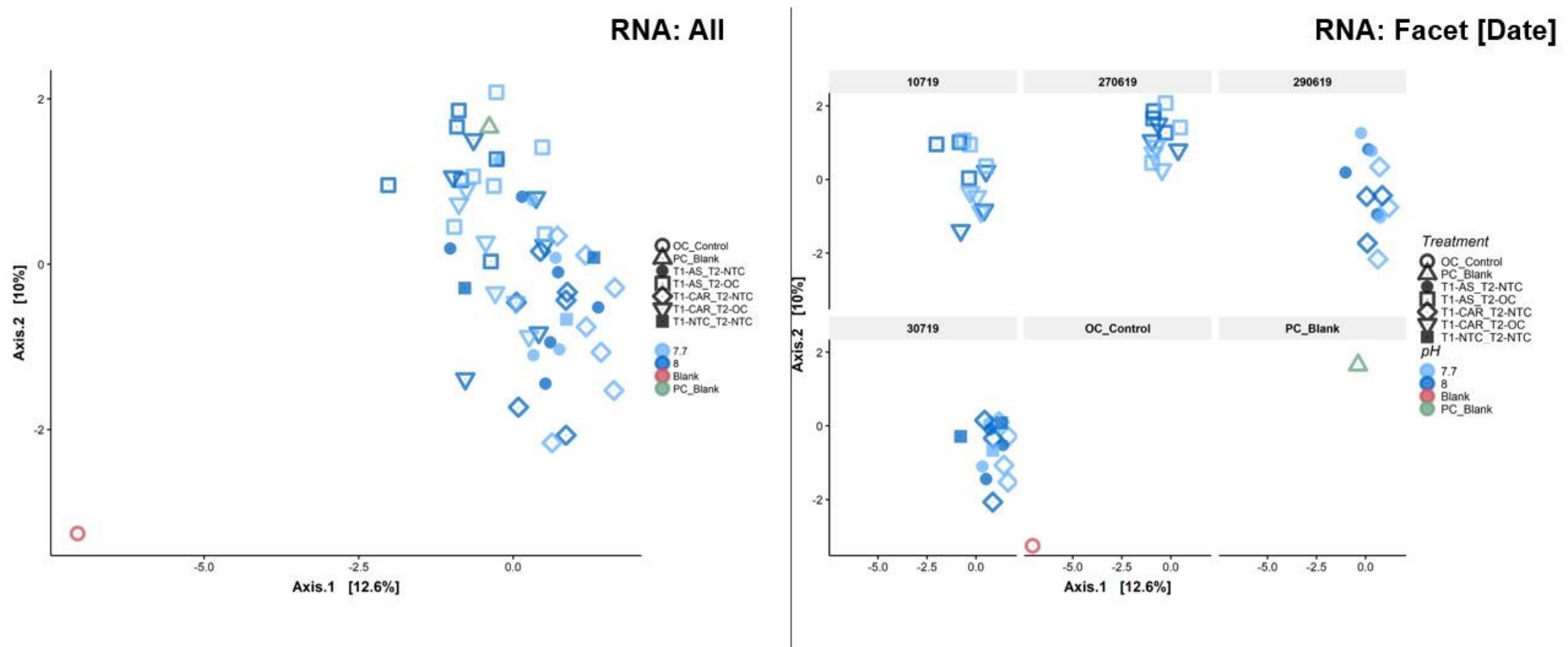
Supplementary Figure 5a. (Left) PCoA ordinations of the PhILR log transformed Active [RNA] data matrix incorporating all RNA samples across both treatments (270619 and 010719) and treatment controls (290619 and 030719), including the Blank Procedural Control (PC) and the Organic Carbon (OC) Blank samples; **(Right)** demonstrates the same PCoA ordination now facetted by Sampling Date for each of the experiments. Symbols indicate the amendment addition (Ashed Sand (AS); CaCO$_3$(CC); Organic Carbon (OC); No Treatment Control (NTC)), where T1 and T2 represent Treatment One and Treatment Two per each sample, and the color of each symbol describes pH (8.0; 7.7; Blank (PC/OC; pH N/A)).

Chapter 4: Experiment 3

Notes Regarding Separation of the Blanks from the Remaining Dataset

PCoA ordinations of the PhILR transformed data examining relative abundances for the collective Active [RNA] and Total [DNA] community across both experiments, showed the Blank (Procedural Control (PC)) ordinating independently of the actual sediment samples (Supplementary Figures 5a, 6a.), and where the Organic Carbon (OC) Blank ordinates with close structural similarity to the AS/OC, and CAR/OC samples. Statistical analyses were run on the Active dataset with and without the Blanks (PC, and OC) and the results showed not significant differences in the dataset when the Blanks are incorporated versus when they excluded. Indicating that the presence of the Procedural Control (PC) and Organic Carbon (OC) Blank data did not affect the interpretation of the experiment results. Here we report the beta diversity metric analyses without the Blanks maintained within the dataset.

Supplementary Table 3c. Beta diversity analyses for the PhILR log transformed DNA dataset using the ANOSIM statistical approach (*significance: p≤0.05*) to identify differences between experimental treatment variables the AS/OC and CC/OC (270619 and 010719) treatments and the AS/NTC and CC/NTC (290619 and 030719) treatment controls.

DNA: All Samples		
Variable	R Value	Significance (p≤0.05)
Mesocosm Tank (RE1-RE4)	0.1941	0.001*
Date Sampled	0.4599	0.001*
Treatment	0.314	0.001*
pH	0.1341	0.001*
DNA: AS/OC and CC/OC Treatments (Combined: 270619 & 010719)		
Variable	R Value	Significance (p≤0.05)
Treatment	0.2736	0.002*
pH	0.0437	0.213
DNA: AS/OC and CC/OC Sampling Date 270619		
Variable	R Value	Significance (p≤0.05)
Treatment	0.1627	0.099
pH	0.07467	0.244
DNA: AS/OC and CC/OC Sampling Date 010719		
Variable	R Value	Significance (p≤0.05)
Treatment	0.3704	0.003*
pH	-0.003704	0.493
DNA: AS/NTC and CC/NTC (Combined: 290619 & 030719)		
Variable	R Value	Significance (p≤0.05)
Treatment	0.07408	0.157
pH	0.02438	0.252
DNA: AS/NTC and CC/NTC Sampling Date 290619		
Variable	R Value	Significance (p≤0.05)
Treatment	0.02963	0.243
pH	0.1685	0.052
DNA: AS/NTC and CC/NTC Sampling Date 030719		

Chapter 4: Experiment 3

Variable	R Value	Significance (p≤0.05)
Treatment	0.09279	0.193
pH	0.06633	0.172

Chapter 4: Experiment 3

Supplementary Table 3d. Beta diversity analyses for the PhILR log transformed DNA dataset for the combined assessment of each amendment from both the AS/OC and CC/OC treatments (270619/010719) treatments and the AS/NTC and CC/NTC (290619/030719) treatment controls using the ANOSIM statistical approach (*significance: p≤0.05*) to identify differences between experimental treatment variables across time.

Treatments and Controls	Significance *p ≤0.05* (ANOSIM)	R Value
AS/OC		
Date Sampled (Time)	0.041*	0.2407
pH	0.093	0.1815
AS/NTC		
Date Sampled (Time)	0.034*	0.2507
pH	0.121	0.1413
CC/NTC		
Date Sampled (Time)	0.004*	0.4405
pH	0.864	-0.1019
CC/OC		
Date Sampled (Time)	0.003*	0. 6987
pH	0.376	0.01333
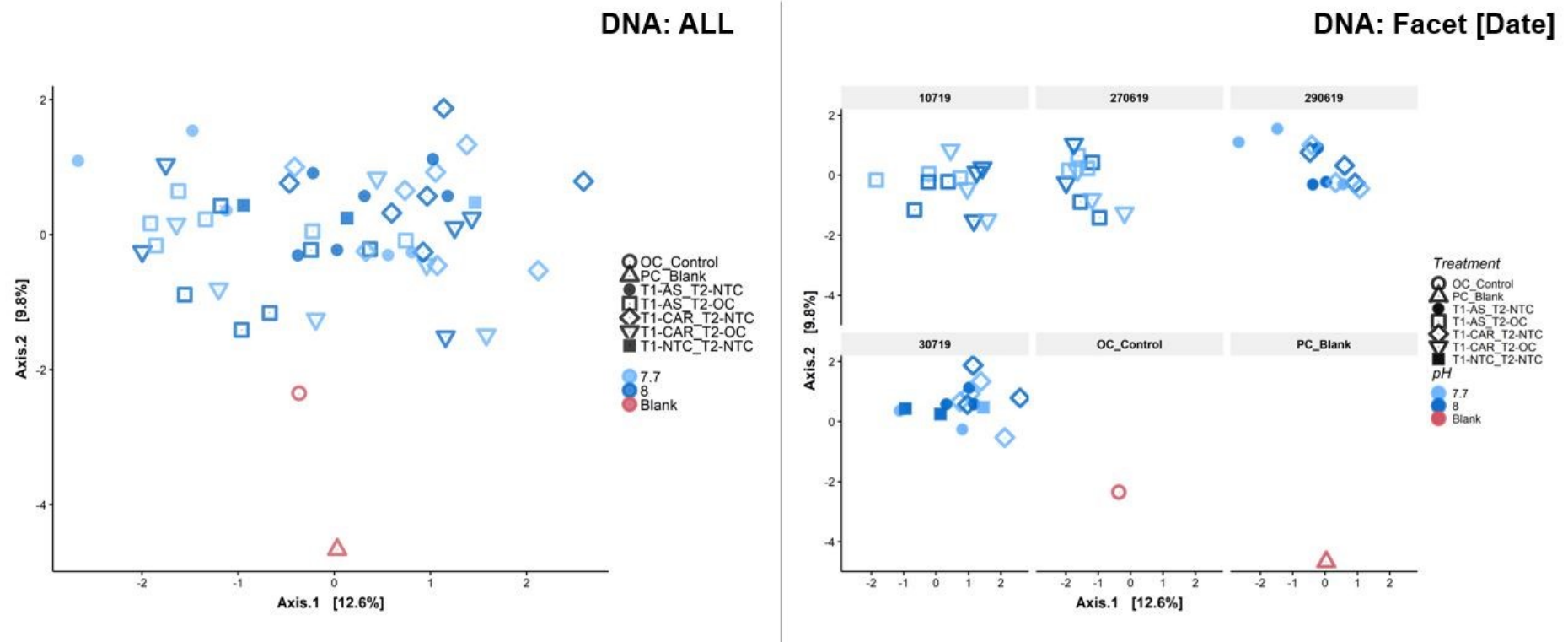
Supplementary Figure 6a. (Left) PCoA ordinations of the PhILR log transformed Total [DNA] data matrix incorporating all DNA samples across both treatments (270619 and 010719) and treatment controls (290619 and 030719), including the Blank Procedural Control (PC) and the Organic Carbon (OC) Blank samples; **(Right)** demonstrates the same PCoA ordination now facetted by Sampling Date for each of the experiments. Symbols indicate the amendment addition (Ashed Sand (AS); CaCO₃(CC); Organic Carbon (OC); No Treatment Control (NTC)), where T1 and T2 represent Treatment One and Treatment Two per each sample, and the color of each symbol describes pH (8.0; 7.7; Blank (PC/OC; pH N/A)).

Chapter 4: Experiment 3

Supplementary Table 4a. Differences in physicochemical sediment properties between the different flow chamber mesocosm treatment tanks and corresponding controls (AS, CC, OC), calculated using *t-test* inferential statistics with adjusted variances for the AS/OC and CC/OC (270619/010719) treatments.

Treatment	Chlorophyll a (µg/g dw)	Phaeo pigments (µg/g dw)	Chlorophyl a / Phaeo (µg/g dw)	Wet Weight (g)	Dry Weight (g)	Organic content (%)	CaCO₃ (%)	63	Dx (50)
RE1/RE2	0.245	0.050438	0.330824	0.014847*	0.063232	0.000456*	0.573921	0.271007	0.428841
RE1/RE3	0.282	0.092986	0.319398	0.451114	0.422555	0.081431	0.513604	0.336913	0.423418
RE1/RE4	0.082	0.618215	0.275297	0.787898	0.529868	0.025626*	0.548393	0.313252	0.461464
RE2/RE3	0.577	0.022829*	0.279608	0.378044	0.305006	0.013413*	0.517642	0.091439	1.355615
RE3/RE4	0.846	0.852283	0.959407	0.070812	0.778454	0.01424*	0.02799*	0.24838	0.855615
RE2/RE4	0.746	0.562939	0.803704	0.229032	0.377771	0.156067	0.517642	0.788431	0.625666

Supplementary Table 4b. Differences in physicochemical sediment properties between the different flow chamber mesocosm treatment tanks and corresponding controls (AS, CC, OC), calculated using *t-test* inferential statistics with adjusted variances for the AS/NTC and CC/NTC (290619/030719) treatment controls.

Treatment	Chlorophyll a (µg/g dw)	Phaeo pigments (µg/g dw)	Chlorophyl a / Phaeo (µg/g dw)	Wet Weight (g)	Dry Weight (g)	Organic content (%)	CaCO₃ (%)	63	Dx (50)
RE1/RE2	0.464568	0.194925	0.679333	0.507821	0.603752	0.041285	0.067461	0.306649	0.531805
RE1/RE3	0.046821*	0.968193	0.290644	0.357762	0.78156	0.330018	0.988873	0.896338	0.736866
RE1/RE4	0.128306	0.569218	0.202439	0.481648	0.265307	0.013802*	0.016178*	0.279422	0.570951
RE2/RE3	0.644928	0.162905	0.304863	0.960582	0.586305	0.116336	0.182311	0.056907	0.913553
RE3/RE4	0.519558	0.682766	0.94248	0.411159	0.394743	0.105991	0.103427	0.193928	0.363015
RE2/RE4	0.485711	0.103714	0.135765	0.493011	0.40018	0.291545	0.582154	0.820089	0.913553

Chapter 4: Experiment 3

Supplementary Table 4c. Calculated averages and standard deviations for physicochemical sediment properties collected between the different mesocosm flow chamber treatment tank (RE1-RE4) subtidal sediment samples for the AS/OC and CC/OC (270619/010719) treatments.

Treatment: Flow Chamber	pH	Chlorophyl a (µg/g dw)	Phaeo Pigments (µg/g dw)	Chlorophyl a & Phaeo	Wet Weight (g)	Dry Weight (g)	Organic Content (%)	CaCO₃ Content (%)	Grain Size (63)	Grain Size (Dx (50))
RE1	8.1	11.11±1.08	17.3±0.30	0.64±0.05	11.10±0.40	3.80±0.14	8.25±0.05	12.60±9.06	71.72±3.65	29.0±3.80
RE2	7.7	17.5±3.70	21.5±0.60	0.81±0.15	9.60±0.32	3.01±0.06	10.35±0.05	5.05±0.50	80.0±0.03	23.5±0.6
RE3	7.9	17.20±4.0	19.92±0.66	0.86±0.17	10.40±0.25	3.42±0.14	8.60±0.02	3.83±0.10	77.41±0.32	23.85±0.25
RE4	7.5	16.70±1.80	19.31±3.30	0.87±0.05	10.91±0.20	3.45±0.23	10.07±0.02	4.71±0.14	80.0±0.60	23.7±1.0

Supplementary Table 4d. Calculated averages and standard deviations for physicochemical sediment properties collected between the different mesocosm flow chamber treatment tank (RE1-RE4) subtidal sediment samples for the AS/NTC and CC/NTC (290619/030719) treatment controls.

Treatment: Flow Chamber	pH	Chlorophyl a (µg/g dw)	Phaeo Pigments (µg/g dw)	Chlorophyl a & Phaeo	Wet Weight (g)	Dry Weight (g)	Organic Content (%)	CaCO₃ Content (%)	Grain Size (63)	Grain Size (Dx (50))
RE1	8.1	11.15±0.05	18.50±1.75	0.61±0.04	9.15±1.60	2.9±0.43	8.71±0.25	3.91±0.12	77.0±1.8	24.4±2.0
RE2	7.7	12.31±1.34	21.23±0.60	0.60±0.05	8.31±3.02	2.70±1.01	10.22±0.2	4.75±0.38	81.6±0.6	21.6±1.15
RE3	7.9	12.83±0.06	18.61±2.27	0.70±0.08	8.40±2.28	2.83±0.81	8.94±0.50	3.91±0.30	76.6±1.0	25.7±0.85
RE4	7.5	13.60±1.40	19.60±0.70	0.70±0.05	10.63±1.60	3.64±0.46	9.96±0.11	4.53±0.07	81.0±2.0	22.0±3.05

Supplementary Figure 7. A side profile (Left) and an aerial view (right) of one of the CaCO$_3$ amendment additions after being applied to one of the subtidal sediment cores. Once defrosted the thin layer of shell hash was uniformly rained down on to the surface of the sediment, where it was effectively integrated into the sediment. Pinnate diatoms can be seen on the surface of the CaCO$_3$ layer, and on the walls of the acrylic tube, indicating the ability to successfully navigate through the material.

Supplementary Table 5a. The Random Forest analysis output for the Active [RNA] dataset for the AS/OC and CC/OC treatment 270619 with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/OC and CC/OC amendment additions, using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_2686	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Saprospiraceae	Blank	Blank	0.09789024
2	ASV_1099	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.06186429
3	ASV_1159	Bacteria	Desulfobacterota	Desulfobacteria	Desulfatiglandales	Desulfatiglandaceae	Desulfatiglans	Blank	0.05744492
4	ASV_219	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Blank	Blank	0.05090905
5	ASV_1861	Bacteria	Verrucomicrobiota	Kiritimatiellae	WCHB1-41	Blank	Blank	Blank	0.04928738
6	ASV_56	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Blank	Blank	0.04807810
7	ASV_205	Bacteria	Planctomycetota	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	Phycisphaera	Blank	0.04549230
8	ASV_727	Bacteria	Proteobacteria	Gammaproteobacteria	EPR3968-O8a-Bc78	Blank	Blank	Blank	0.04528040
9	ASV_1360	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.04346286
10	ASV_2799	Bacteria	NB1-j	Blank	Blank	Blank	Blank	Blank	0.04344786
11	ASV_995	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family_4	Endothiovibrio	Blank	0.04333952
12	ASV_2163	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Winogradskyella	Blank	0.04328698
13	ASV_1200	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	Blank	Blank	0.04327206
14	ASV_1195	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.04302524
15	ASV_839	Bacteria	Proteobacteria	Gammaproteobacteria	UBA10353 marine group	Blank	Blank	Blank	0.04094563
16	ASV_1006	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.04013651
17	ASV_337	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	Blank	0.03941135
18	ASV_1128	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidetes BD2-2	Blank	Blank	0.03702230
19	ASV_1316	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	DEV007	Blank	Blank	0.03617762
20	ASV_1082	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	Blank	0.03574024

Supplementary Table 5b. The Random Forest analysis output for the Active [RNA] dataset for the AS/OC and CC/OC treatment 270619, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/OC and CC/OC amendment additions by pH (ambient: pH 8.0; CO_2 enriched: 7.7), using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_2078	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Saprospiraceae	Lewinella	Blank	0.08992421
2	ASV_635	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.05224421
3	ASV_2480	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A clade	Blank	Blank	Blank	0.05053246
4	ASV_173	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Blank	Blank	0.04756857
5	ASV_1220	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Cyclobacteriaceae	Ekhidna	Blank	0.04697032
6	ASV_2330	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.04550476
7	ASV_1089	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.04517571
8	ASV_827	Bacteria	Chloroflexi	Blank	Blank	Blank	Blank	Blank	0.04485310
9	ASV_1492	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.04439659
10	ASV_2437	Bacteria	Acidobacteriota	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup 10	Blank	0.04342325
11	ASV_1027	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.04329492
12	ASV_130	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.04077881
13	ASV_615	Bacteria	Desulfobacterota	Desulfuromonadia	Blank	Geothermobacteraceae	Geothermobacter	Blank	0.03173214
14	ASV_1531	Bacteria	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Blank	Blank	0.03032770
15	ASV_2842	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.02729468
16	ASV_1548	Bacteria	Desulfobacterota	Desulfuromonadia	PB19	Blank	Blank	Blank	0.02713310
17	ASV_1417	Bacteria	Proteobacteria	Gammaproteobacteria	BD7-8	Blank	Blank	Blank	0.02648214
18	ASV_1003	Bacteria	Planctomycetota	SGST604	Blank	Blank	Blank	Blank	0.02447056
19	ASV_1097	Bacteria	Myxococcota	Polyangia	MidBa8	Blank	Blank	Blank	0.02446214
20	ASV_518	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.02381238

Supplementary Table 5c. The Random Forest analysis output for the Active [RNA] dataset for the AS/OC and CC/OC treatment control 010719, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/OC and CC/OC amendment additions, using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_2231	Bacteria	Planctomycetota	vadinHA49	Blank	Blank	Blank	Blank	0.08066040
2	ASV_110	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	Microscilla	Blank	0.04514071
3	ASV_173	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Blank	Blank	0.04513278
4	ASV_2616	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	NS7 marine group	Blank	Blank	0.04271921
5	ASV_2617	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.04063183
6	ASV_797	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Blank	Blank	0.04038516
7	ASV_1950	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Blank	Blank	Blank	0.04020897
8	ASV_1419	Bacteria	Cyanobacteria	Cyanobacteriia	Cyanobacteriales	Blank	Blank	Blank	0.03862643
9	ASV_1517	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03857365
10	ASV_11	Bacteria	Desulfobacterota	Desulfuromonadia	Sva1033	Blank	Blank	Blank	0.03774063
11	ASV_2001	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Cyclobacteriaceae	Blank	Blank	0.03757960
12	ASV_219	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Blank	Blank	0.03705429
13	ASV_447	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Psychromonadaceae	Psychromonas	Blank	0.03695397
14	ASV_1938	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Blank	Blank	0.03644563
15	ASV_600	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.03606190
16	ASV_2603	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03590460
17	ASV_2809	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Desulfatirhabdium	Blank	0.03536230
18	ASV_2775	Bacteria	Desulfobacterota	Desulfuromonadia	Blank	Geopsychrobacteraceae	Desulfuromusa	Blank	0.03495262
19	ASV_2350	Bacteria	Myxococcota	Polyangia	Nannocystales	Nannocystaceae	Blank	Blank	0.03488905
20	ASV_1687	Bacteria	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta 2	Blank	0.03333500

Supplementary Table 5d. The Random Forest analysis output for the Active [RNA] dataset for the AS/OC and CC/OC treatment 010719, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/OC and CC/OC amendment additions by pH (ambient: pH 8.0; CO$_2$ enriched: 7.7), using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_33	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Cyclobacteriaceae	Reichenbachiella	Blank	0.08754492
2	ASV_604	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Porticoccaceae	Porticoccus	Blank	0.08541119
3	ASV_2530	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Blank	Blank	0.05329095
4	ASV_393	Archaea	Crenarchaeota	Bathyarchaeia	Blank	Blank	Blank	Blank	0.05103079
5	ASV_56	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Blank	Blank	0.04471627
6	ASV_1299	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.04317119
7	ASV_2229	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Gven-F17	Blank	Blank	0.04116103
8	ASV_248	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Sva0081 sediment group	Blank	0.04055730
9	ASV_1203	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Blank	Blank	0.03938429
10	ASV_2118	Bacteria	Sva0485	Blank	Blank	Blank	Blank	Blank	0.03924270
11	ASV_417	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.03831452
12	ASV_2898	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03788397
13	ASV_84	Bacteria	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Thioalkalispiraceae	Thiohalophilus	Blank	0.03773262
14	ASV_2016	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Blank	Blank	0.03665905
15	ASV_524	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Blank	Blank	0.03614952
16	ASV_353	Bacteria	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Blank	Blank	Blank	0.03559540
17	ASV_866	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.03523286
18	ASV_1458	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.03475016
19	ASV_1345	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Blank	Blank	Blank	0.03288563
20	ASV_443	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Porticoccaceae	Porticoccus	Blank	0.03229310

Supplementary Table 6a. The Random Forest analysis output for the Active [RNA] dataset for the AS/NTC and CC/NTC treatment control 290619, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/NTC and CC/NTC amendment additions, using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_1082	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	Blank	0.09727881
2	ASV_417	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.08657619
3	ASV_42	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aquibacter	Blank	0.04984222
4	ASV_1684	Bacteria	Planctomycetota	BD7-11	Blank	Blank	Blank	Blank	0.04565158
5	ASV_971	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	Microscilla	Blank	0.04561825
6	ASV_598	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Blank	Blank	0.04509524
7	ASV_858	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Blank	Blank	Blank	0.04483206
8	ASV_1906	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.04339167
9	ASV_2252	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.04254365
10	ASV_1457	Bacteria	NB1-j	Blank	Blank	Blank	Blank	Blank	0.04038024
11	ASV_2564	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03836698
12	ASV_1608	Bacteria	Desulfobacterota	Blank	Blank	Blank	Blank	Blank	0.03807444
13	ASV_1243	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03791849
14	ASV_2339	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Kangiellaceae	Aliikangiella	Blank	0.03667484
15	ASV_255	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Sva0081 sediment group	Blank	0.03503571
16	ASV_1419	Bacteria	Cyanobacteria	Cyanobacteriia	Cyanobacteriales	Blank	Blank	Blank	0.03502635
17	ASV_1591	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Puniceicoccaceae	Cerasicoccus	Blank	0.03415286
18	ASV_1053	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03385071
19	ASV_802	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.03306778
20	ASV_1689	Bacteria	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Blank	Blank	0.03273373

Supplementary Table 6b. The Random Forest analysis output for the Active [RNA] dataset for the AS/NTC and CC/NTC treatment control 290619, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/NTC and CC/NTC amendment additions by pH treatment (ambient: pH 8.0; CO$_2$ enriched: 7.7), using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_19	Bacteria	Myxococcota	Polyangia	Blank	Blank	Blank	Blank	0.07166159
2	ASV_452	Bacteria	Gemmatimonadota	BD2-11 terrestrial group	Blank	Blank	Blank	Blank	0.07096921
3	ASV_1221	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.06991548
4	ASV_2323	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.06798778
5	ASV_2734	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Blank	Blank	0.05332698
6	ASV_1487	Bacteria	Bdellovibrionota	Bdellovibrionia	Bacteriovoracales	Bacteriovoracaceae	Blank	Blank	0.05166476
7	ASV_2283	Bacteria	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Rhodopirellula	Blank	0.04848373
8	ASV_1834	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.04815786
9	ASV_81	Bacteria	Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Sva0081 sediment group	Blank	0.04227619
10	ASV_1454	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Roseibacillus	Blank	0.04199675
11	ASV_2340	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.04195024
12	ASV_1972	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Blank	Blank	Blank	0.03605738
13	ASV_1134	Bacteria	NB1-j	Blank	Blank	Blank	Blank	Blank	0.03552976
14	ASV_262	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.03463476
15	ASV_1746	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.03243556
16	ASV_2655	Bacteria	Proteobacteria	Blank	Blank	Blank	Blank	Blank	0.03084579
17	ASV_661	Bacteria	Myxococcota	Polyangia	Nannocystales	Nannocystaceae	Blank	Blank	0.03021492
18	ASV_762	Bacteria	Chloroflexi	Anaerolineae	Blank	Blank	Blank	Blank	0.02998810
19	ASV_840	Bacteria	Actinobacteriota	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	Ilumatobacter	Blank	0.02991238
20	ASV_548	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.02926341

Supplementary Table 6c. The Random Forest analysis output for the Active [RNA] dataset for the AS/NTC and CC/NTC treatment control 030719, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/NTC and CC/NTC amendment additions, using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of *species*.

	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_2901	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	SCGC AAA164-E04	Blank	0.09747357
2	ASV_521	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Blank	Blank	0.08765102
3	ASV_2935	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.08689286
4	ASV_2336	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.07541452
5	ASV_2808	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.05770611
6	ASV_2695	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	BD1-7 clade	Blank	0.04066619
7	ASV_768	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Puniceicoccaceae	Cerasicoccus	Blank	0.04019960
8	ASV_2088	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03936571
9	ASV_591	Bacteria	Proteobacteria	Gammaproteobacteria	BD7-8	Blank	Blank	Blank	0.03934603
10	ASV_136	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	Blank	Blank	0.03852881
11	ASV_1450	Bacteria	Verrucomicrobiota	Chlamydiae	Chlamydiales	Blank	Blank	Blank	0.03794429
12	ASV_52	Bacteria	Bacteroidota	Bacteroidia	Bacteroidetes VC2.1 Bac22	Blank	Blank	Blank	0.03559992
13	ASV_1359	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.03544452
14	ASV_1353	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Blank	Blank	Blank	0.03491389
15	ASV_1499	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.03465683
16	ASV_42	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aquibacter	Blank	0.03400786
17	ASV_85	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.03336000
18	ASV_170	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aquibacter	Blank	0.03335563
19	ASV_443	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Porticoccaceae	Porticoccus	Blank	0.03324397
20	ASV_1445	Bacteria	Proteobacteria	Alphaproteobacteria	Blank	Blank	Blank	Blank	0.03301762

Supplementary Table 6d. The Random Forest analysis output for the Active [RNA] dataset for the AS/NTC and CC/NTC treatment control 030719, with the Blank (Procedural Blank (PC) and the Organic Carbon (OC) Blank removed) identifying the top 20 ASV's that discriminate between the AS/NTC and CC/NTC amendment additions by pH treatment (ambient: pH 8.0; CO_2 enriched: 7.7), using the mean decrease in the GINI Coefficient as a measure of the added amendment's contribution to the homogeneity of the nodes and leaves of the random forest. No ASV's were able to be described to the depth of _species_.

|	ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species	MeanDecreaseGini
1	ASV_2096	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.10562635
2	ASV_1048	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Blank	Blank	0.09587921
3	ASV_2078	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Saprospiraceae	Lewinella	Blank	0.08977754
4	ASV_1554	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.07548794
5	ASV_219	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Blank	Blank	0.04817222
6	ASV_736	Bacteria	Proteobacteria	Gammaproteobacteria	Blank	Blank	Blank	Blank	0.04689786
7	ASV_2602	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Puniceicoccaceae	Blank	Blank	0.04611484
8	ASV_1111	Bacteria	Myxococcota	Polyangia	Polyangiales	Blrii41	Blank	Blank	0.04565929
9	ASV_2029	Bacteria	Bacteroidota	Bacteroidia	Bacteroidetes VC2.1 Bac22	Blank	Blank	Blank	0.04500587
10	ASV_2519	Bacteria	Acidobacteriota	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup 10	Blank	0.04149413
11	ASV_1839	Bacteria	Acidobacteriota	AT-s3-28	Blank	Blank	Blank	Blank	0.04047127
12	ASV_10	Bacteria	Bacteroidota	Bacteroidia	Bacteroidetes VC2.1 Bac22	Blank	Blank	Blank	0.03994984
13	ASV_2520	Bacteria	NB1-j	Blank	Blank	Blank	Blank	Blank	0.03958024
14	ASV_1754	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Lutibacter	Blank	0.03877746
15	ASV_201	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.03709524
16	ASV_2824	Bacteria	Bacteroidota	Bacteroidia	Blank	Blank	Blank	Blank	0.03527563
17	ASV_2127	Bacteria	Planctomycetota	Phycisphaerae	MSBL9	SG8-4	Blank	Blank	0.03202103
18	ASV_1856	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Blank	Blank	Blank	0.02611619
19	ASV_2323	Bacteria	Blank	Blank	Blank	Blank	Blank	Blank	0.02587024
20	ASV_2339	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Kangiellaceae	Aliikangiella	Blank	0.02576532
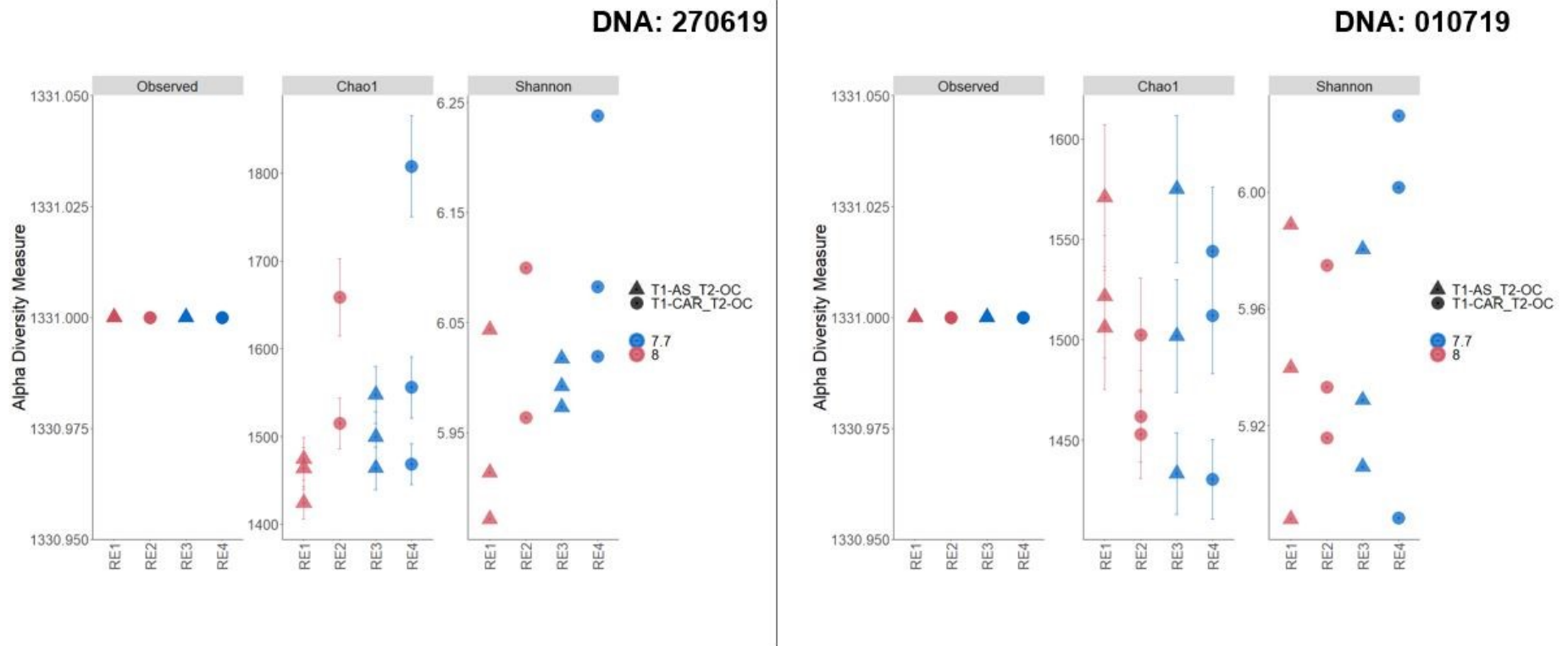
Supplementary Figure 8a. Alpha diversity matrices (Observed, Chao1, Shannon) for the Total [DNA] dataset for the AS/OC and CC/OC treatments (270619 and 010719), across each of the mesocosm treatments (RE1; RE2; RE3; RE4), colored by pH (8.0; 7.7) treatment, where triangular and circular symbols indicate the amendment addition. T1 and T2 denote "Treatments 1" and "Treatments 2."
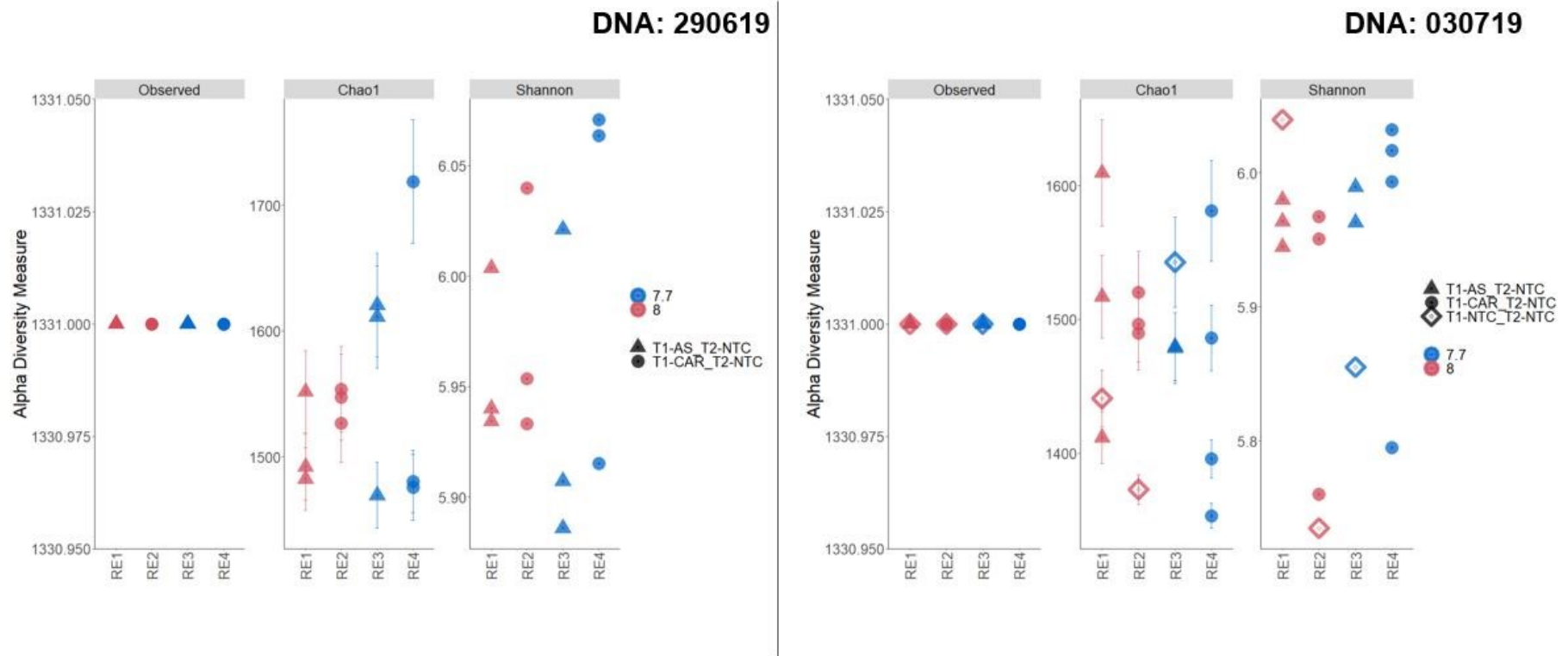
Supplementary Figure 8b. Alpha diversity matrices (Observed, Chao1, Shannon) for the Total [DNA] dataset for the AS/NTC and CC/NTC treatment controls (290619 and 030719), across each of the mesocosm treatments (RE1; RE2; RE3; RE4), colored by pH (8.0; 7.7) treatment, where triangular and circular symbols indicate the amendment addition. T1 and T2 denote "Treatments 1" and "Treatments 2."
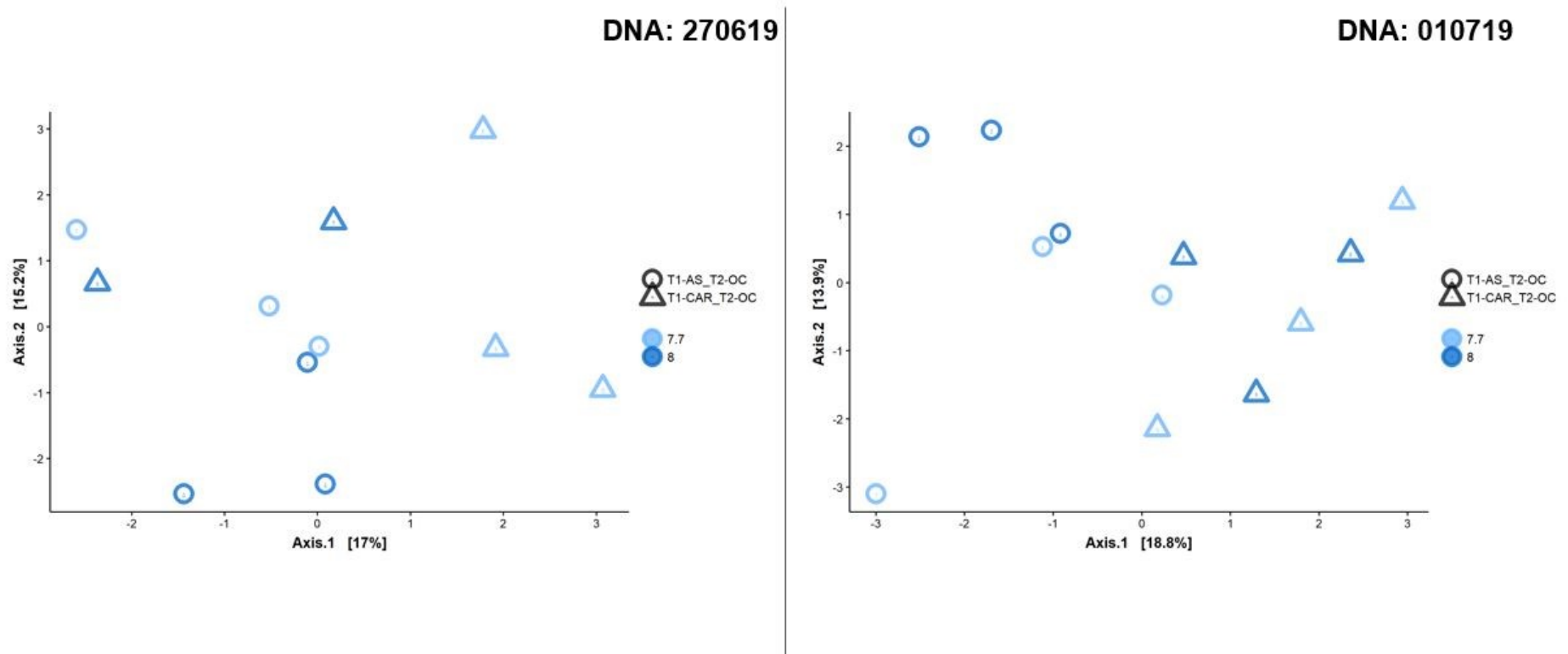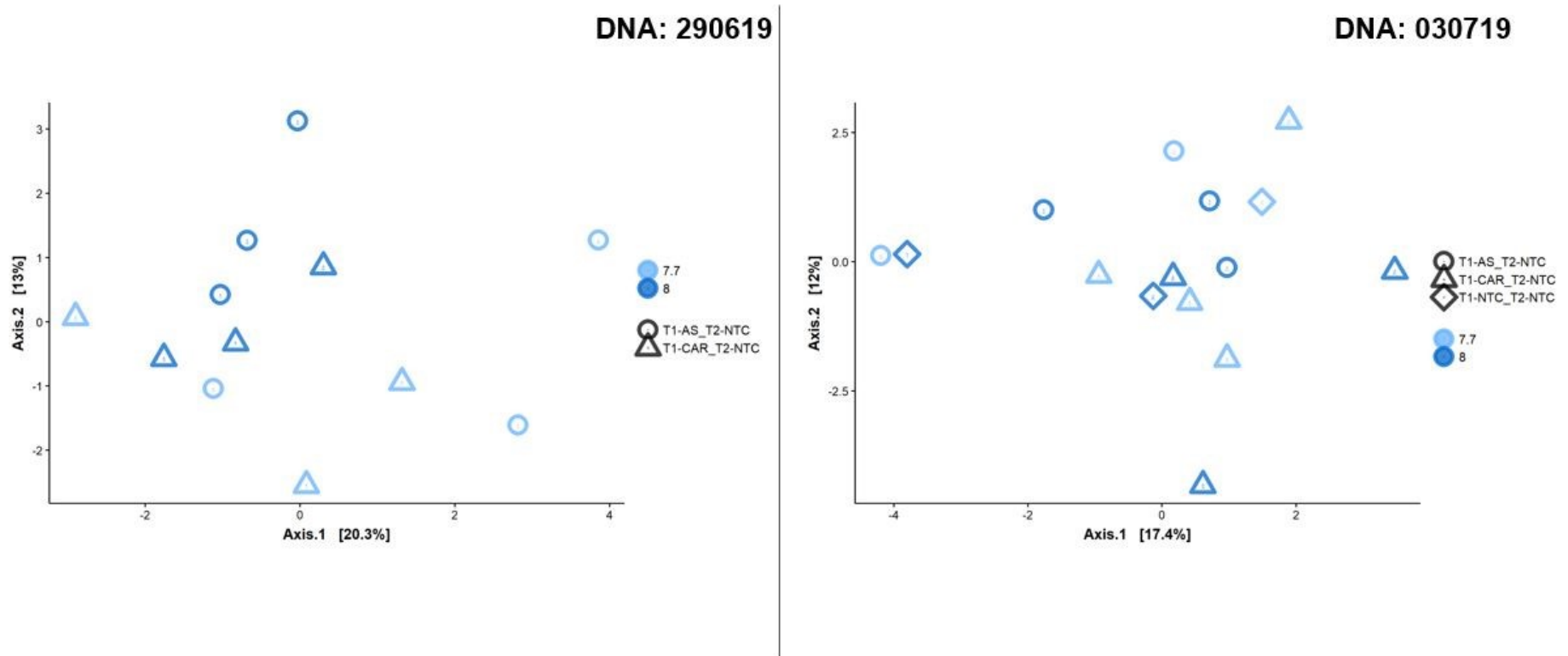
Supplementary Figure 9a. PCoA ordinations of the PhILR log-transformed Total [DNA] data matrix for the AS/OC and CC/OC treatments (270619 and 010719). Symbols indicate the amendment addition (Ashed Sand (AS); CaCO$_3$(CC); Organic Carbon (OC); where T1 and T2 represent Treatment One and Treatment Two per each sample, and the color of each symbol describes pH (8.0; 7.7).

Supplementary Figure 9b. PCoA ordinations of the PhILR log-transformed Total [DNA] data matrix for the AS/NTC and CC/NTC treatment controls (290619 and 030719). Symbols indicate the amendment addition (Ashed Sand (AS); CaCO₃ (CC); Organic Carbon (OC); No Treatment Control (NTC)), where T1 and T2 represent Treatment One and Treatment Two per each sample, and the color of each symbol describes pH (8.0; 7.7).

Chapter 4: Experiment 3

Chapter 5: The Effects of Ocean Acidification on Microbial Nutrient Cycling and Productivity in Coastal Marine Sediments: A Summary

Ocean acidification remains one of the most prominent and severe ecological issues affecting global oceans to date (Mason, 2010). The chronic nature of OA ensures that this will be a continual and long-term problem worldwide (IGBP, 2013; Doney et al., 2009; Stocker et al., 2013). Current studies clearly demonstrate the negative trajectory of the health of our oceans under all increasing atmospheric CO_2 emission scenarios (Crummett, 2020; Currie et al., 2017; Nelson et al., 2020; Sala et al., 2016; Webster et al., 2013). This study provides a modicum of *hope* however, in the sense that some parts of the marine ecosystem appear to be more naturally resistant to lower pH than previously predicted. The nature of these experiments began their inception with the intention of answering, on a fundamental level, how inherently resistant are microbial communities in coastal/nearshore sediments to the effects of OA? We applied a series of mesocosm experiments, each with increasing levels of biological and system complexity, under various IPCC specific acidified conditions, across two very different sediment types. The aim was to identify the measurable threshold at which the benthic microbial communities respond structurally or compositionally to sustained low pH conditions.

We selected sediments from active and dynamic coastal regions (intertidal and sub-tidal) that supported microbial communities that were naturally exposed to major diel fluxes in pH. We found that microbial communities from both intertidal and subtidal sediments were surprisingly resistant to lower pH conditions. This resistance was likely either the result of natural selection pressures provided by the dynamic environment from which they come, and/or from the geochemical resilience (natural buffering) of the environment itself. This is an interesting concept which plays on the dichotomy between whether the individual organism itself is resistant to the perturbation, or if it is the environment that is working to buffer the introduced impact. To put this in context, benthic sediments are exceptionally good at sequestering carbon given their composition, DIC and corresponding alkalinity, which essentially allows for them to buffer excess CO_2 at a very different capacity than that of the pelagic environment (Yaakov, 1973; Middelburg et al., 2020). This means that they capable of maintaining a far more constant and homeostatic environment for the sedimentary microbes

than other less CO_2 absorptive systems (Ben-Yaakov, 1973; Morse et al., 2006b). Whereby, the microbial communities themselves remain directly unaffected by changes in overlying water column pH as their immediate surrounding environment is mitigating the effects of OA. Thus, allowing them to maintain normal functional processes and activity. We observed this phenomenon in the first (Chapter 2.) and second (Chapter 3.) experiments, where the communities demonstrated apparent fortitude under varying low pH exposure. However, decoupling environmental resilience from biological resistance remains a significant ecological challenge (Levin & Lubchenco, 2008).

If one were to repeat these studies, it would be prudent to examine the saturation point of intertidal/subtidal sediments to excess pCO_2, as it pertains to the Revelle factor (Revelle & Suess, 1957). Here, the continual uptake of atmospheric CO_2 shifts the system chemistry in favor of HCO_3^- and away from CO_3^{2-} effectively reducing the overall buffering capacity of the sediment porewater. If one were to lower the pH along a gradient outside of the predicted and tested values applied in this study, it is likely the sediments would reach a point of saturation where they are no longer able to buffer pCO_2. Under these new conditions, the microbial communities are likely to demonstrate quantifiably less physiological plasticity. There are two compelling questions that might emerge. The first, is at what point the environment (i.e., the sediments) destabilizes under reduced low pH. This could potentially trigger a trophic cascading effect that impacts all biological taxa. The second, is how independently resistant are these microbial communities after the sedimentary buffer is removed? One method of decoupling environmental resilience versus biological resistance would be to investigate the regulatory gene response of the sediment bacterial community when exposed to the environmental perturbation using contemporary metatranscriptomic techniques. This information would be useful in identifying from a bottom-up approach, how the marine ecosystem will respond beyond the predicted impending changes in oceanic climate.

Intriguingly, though the majority of the microbial communities in this study did not structurally or compositionally change under low pH exposure, a select few sensitive taxa were identified. These fringe (i.e., low abundance, high response) taxa (Alteromonadaceae, Oceanospirillales) were shown to consistently respond across all three experiments despite major differences in the individual treatments. The commonality between all three

experiments suggests that these responsive members of the community are possibly sentinel taxa and may be suitable for monitoring future ecosystem health or changes driven by OA. A post hoc analysis (i.e., nucleotide sequence comparison) of the overlapping taxa from each experiment, identified that though they are derived from the same genus, they are not the same bacterial species. This could suggest that the impact of OA is not species specific but is affecting different microorganisms on a broader scale. However, significantly more research is required to better understand these particular taxa, their global distribution, and their functional role within the community. It is also critical to determine the potential functional impact of removing or reducing these taxa from the total community under projected low pH conditions. In the first experiment we identified these low tolerant taxa were likely essential nitrifying species that if absent had the potential to disrupt ecosystem processes (i.e., primary productivity). As discussed before, AOA and AOB are the only taxa that perform the first step of nitrification (i.e., ammonia oxidation) in marine sediments, making them invaluable in the nitrogen cycling process. Decreases in their abundances could result in an increase in the flux of fixed nitrogen back into the water column, subsequently increasing the risk eutrophication effects. This could be further investigated through the application of metatranscriptomic analyses and the possible ramifications on the functionality of ecosystem processes.

In this series of experiments, we specifically selected microbial communities from dynamic and variable environments, and that the normal diel cycling shifts in pH naturally oscillate outside the projected end of the century values for 2100. The coastal ecosystem is therefore consistent in its *inconsistency* where it is predictably dynamic, and the microbial communities anticipate these frequent and sometimes extreme fluxes. We determined that physically manipulating sediments proved to have a far greater impact on microbial composition and structure than acidification. What we did to the microbial community during the destructive sampling process in the first experiment pushed the bacteria outside their normal tolerance range creating a "disturbance" shift that would mask any imposed by the lower pH treatments in the first few weeks of the experiment. The community and specific taxa elicited a distinct structural response to the disturbance. Suggesting that any perturbation that falls outside their physiological pre adaptive intertidal "wheelhouse" will evoke a biological response. When an external stressor is imposed that is not pre-adapted by the community, the natural tolerability of the community is destabilized. Disturbances of

short duration may produce a stress like response from the bacteria followed by near or total recovery post disturbance. Whereas a medium to long term exposure to a perturbation is more likely to drive the community from a state of sensitivity to a point of either selective adaptation or fatality.

What we observe in this study, is that the bulk of the community was able to adapt to sustained low pH over long periods of time. From a global standpoint, this suggests that the microbial communities in benthic sediments, will adapt to the rate of pH decline caused by ocean acidification with adequate time. Though it should be noted that a system that is continuously anthropogenically stressed, despite its ability to resist the immediate disturbance, will become increasingly destabilized with time. Meaning a system that is able to withstand environmental change at the beginning may collapse over time when a tipping point is reached. Therefore, a lack of biological response from the microbial community (initially) is not inherently a good thing, as it could indicate that the community is shifting towards a more destabilized state. The frequency and intensity of disturbance events is also an important contributing factor when determining the rate at which community could decline. Ecologists classify global changes such as OA as "press" disturbances or long-term disturbance events (Bender, Case, & Gilpin, 1984)(bender et al., 1984) that are predicted to frequent or ongoing as predicted by the IPCC. Although we measured structural community stability in each of our experiments, it cannot be determined how well this stability will be maintained over long periods of time under continual press events. Additionally, as discovered in the final experiment, ocean acidification as an ecological stressor, rarely works in isolation. However, a typically, a nearshore or coastal environment is exposed to a multitude of ecosystem impacts (i.e., eutrophication induced acidification) which often compound to further decrease seawater pH. These added regional or local stressors may even exert a greater influence on the ecosystem than the predicted global anthropogenic disturbances.

We identified in Chapter 4, that eutrophication induced acidification, rather than ocean acidification was the more pronounced of the two perturbations. As coastal systems are the intermediary between land, and the open ocean they are governed by both oceanic and terrestrial input. These environments, as previously stated, are exceptionally resilient because of this dual impact. This final experiment was aimed at investigating this in greater

detail by examining an additional injection of organic nutrients to simulate both OA and eutrophication impacts on the sediment communities. The enrichment of calcium carbonate into the sediment surface was used to address its potential buffering effects on community structure and composition under various eutrophication and OA treatments, alluding to its use as a possible mitigation strategy. This scenario is also somewhat representative of an oyster raft farm located in an estuary, where the introduction of organic material from the oysters and the natural breakdown of the outer shell composition fertilizes the benthic sediments with both nutrients and calcium carbonate.

In this situation, the microbial degradation of the organic matter consumes O_2 and releases CO_2. However, the continual seeding of calcium carbonate by the resident shellfish may also help to buffer and subsequently neutralize the change in pH caused by the eutrophication process. Our results indicated that from a microbial compositional and structural level, this seems to be the case. The combination of both organic carbon and calcium carbonate neutralizes one another. This leads us to suggest that marine sediments that have naturally occurring high levels of calcium carbonate may be less impacted by the driving forces of OA or eutrophication on seawater pH. Under these conditions these $CaCO_3$ enriched sediments have a physical advantage over other sedimentary environments that are not naturally rich in calcium carbonate. However, we do not condone the use of calcium carbonate loading as an effective mitigation strategy against acidification. This approach is costly, logistically complicated and does not produce powerful enough results for mitigation of reduced water column pH. Though apparently effective on a small laboratory mesocosm scale, it is unlikely this will translate up to larger model in an actual natural environment. The potential of this approach will need considerably more research in the future.

Across each of the 3 experiments we address only the structural or compositional response of the sediment microbial communities to different pH conditions and amendment treatments dictated only by the DNA (total community) and cDNA (active community). This information is useful to detect a response to a disturbance and to identify which taxa remain metabolically active under low pH regimes. However, it is not informative for understanding any potential functional response to a disturbance. Though we did not observe major changes in community structure within the first and second experiments we acknowledge that on a transcriptional level (mRNA), the members of the community are almost certainly responding.

Chapter 5: Summary Chapter

This metabolic sensitivity is lost by using only 16S rRNA/rDNA analyses to interpret community wide response. The natural progression is to implement both metagenomic and metatranscriptomic approaches to examine in greater depth the functional impact of ocean acidification on microbial nutrient cycling and productivity in coastal marine sediments.

References

Ben-Yaakov, S. (1973). pH BUFFERING OF PORE WATER OF RECENT ANOXIC MARINE SEDIMENTS. *Limnology and Oceanography*, *18*(1), 86–94. https://doi.org/10.4319/lo.1973.18.1.0086

Bender, E. A., Case, T. J., & Gilpin, M. E. (1984). Perturbation Experiments in Community Ecology: Theory and Practice. *Ecology*, *65*(1), 1–13. https://doi.org/https://doi.org/10.2307/1939452

Crummett, L. T. (2020). Acidification decreases microbial community diversity in the Salish Sea, a region with naturally high pCO2. *PLoS ONE*, *15*(10 October), 1–17. https://doi.org/10.1371/journal.pone.0241183

Currie, A. R., Tait, K., Parry, H., de Francisco-Mora, B., Hicks, N., Mark Osborn, A., … Stahl, H. (2017). Marine microbial gene abundance and community composition in response to ocean acidification and elevated temperature in two contrasting coastal marine sediments. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.01599

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean Acidification: The Other CO$_2$ Problem. *Annual Review of Marine Science*. https://doi.org/10.1146/annurev.marine.010908.163834

Igbp, I. O. C. (n.d.). SCOR (2013) Ocean Acidification Summary for Policymakers–Third Symposium on the Ocean in a High-CO2 World. *International Geosphere-Biosphere Programme. Stockolm, Sweden*.

Levin, S. A., & Lubchenco, J. (2008). Resilience, robustness, and marine ecosystem-based management. *Bioscience*, *58*(1), 27–32.

Mason, G. (2010). *Poisoning and Acidification of the Earth's Oceans*. Nova Science Publishers.

Middelburg, J. J., Soetaert, K., & Hagens, M. (2020). Ocean Alkalinity, Buffering and Biogeochemical Processes. *Reviews of Geophysics*, *58*(3). https://doi.org/10.1029/2019RG000681

Morse, J. W., Andersson, A. J., & Mackenzie, F. T. (2006). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO2 and "ocean acidification": Role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, *70*(23), 5814–5830.

Nelson, K. S., Baltar, F., Lamare, M. D., & Morales, S. E. (2020). Ocean acidification affects microbial community and invertebrate settlement on biofilms. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-60023-4

Revelle, R., & Suess, H. E. (1957). Carbon Dioxide Exchange Between Atmosphere and Ocean and the Question of an Increase of Atmospheric CO2 during the Past Decades. *Tellus*, *9*(1), 18–27. https://doi.org/10.3402/tellusa.v9i1.9075

Sala, M. M., Aparicio, F. L., Balague, V., Boras, J. A., Borrull, E., Cardelus, C., … Marrase, C. (2016). Contrasting effects of ocean acidification on the microbial food web under different trophic conditions. *Ices Journal of Marine Science*, *73*(3), 670–679.

https://doi.org/10.1093/icesjms/fsv130

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., … Midgley, P. M. (2013). Climate change 2013: the physical science basis. Intergovernmental panel on climate change, working group I contribution to the IPCC fifth assessment report (AR5). *New York*.

Webster, N. S., Negri, A. P., Flores, F., Humphrey, C., Soo, R., Botté, E. S., … Uthicke, S. (2013). Near-future ocean acidification causes differences in microbial associations within diverse coral reef taxa. *Environmental Microbiology Reports*, *5*(2), 243–251. https://doi.org/10.1111/1758-2229.12006