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# Effects of land-derived anthropogenic stressors on temperate mangrove forests

*A thesis*

*submitted in fulfilment*

*of the requirements for the degree*

*of*

**Doctor of Philosophy in Biological Sciences**

*at*

**The University of Waikato**

*by*

**Timothy Thomson**



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## *Abstract*

Coastal ecosystems worldwide are facing unprecedented challenges due to ever increasing human activities, resulting in increased land-derived stressors that can threaten the health and integrity of these systems. Mangrove forests are unique ecosystems that play a crucial role in filtering nutrients, retaining pollutants, and sequestering carbon. However, to what extent they are impacted by an intensification of human pressures is unclear and empirical evidence of landscape-scale effects of a holistic view of the ecosystem is scarce. This thesis investigates the effects of human land use on several aspects of temperate mangrove ecosystems and aims to identify potential vulnerabilities of ecosystem processes to provide a holistic view on the impacts that humans are having on these important ecosystems.

By working across a broad gradient of human land use, the first research chapter establishes a connection between human land use intensity in catchments and regionally generalisable trends in mangrove forest structure and physicochemical parameters in sediments. Highlighting the integrated nature of anthropogenic impacts, this chapter emphasises the need for holistic evaluations in assessing responses of complex natural systems to change.

The second research chapter investigates environmental controls on organic matter degradation in mangrove forests. Results from this study emphasise the importance of considering multiple factors influencing degradation processes, that vary with the quality of the decaying organic matter. Furthermore, using the human land use gradient from the previous chapter, this study identifies an impact of human land use through eutrophication on the rate of labile organic matter loss from mangrove sediments.

The third research chapter provides insights into bacterial communities in temperate mangrove sediments, revealing the persistent effects of nutrient enrichment even after the biochemical signal is lost. The study highlights the spatial resolution needed to detect impacts on microbial communities, warning that functional changes may persist beyond observable eutrophication effects.

The synthesis of these chapters provides a holistic view of the effects of anthropogenic impacts on temperate mangrove ecosystems across various scales. While some components of the system may benefit from moderate amounts of nutrient enrichment, others can deteriorate, ultimately diminishing overall service provisioning of the system. To understand and quantify



these effects, this thesis calls for a nuanced understanding of disturbance effects on mangrove ecosystems, emphasising their complexity and the interconnectedness of their components. It adds a valuable information about the functional resilience of mangrove forests in the face of human pressures and highlights the importance of an ecosystem-based perspective when assessing the effects of external stressors on these important ecosystems.

## *Preface*

The main body of this thesis comprises three research chapters (Chapters 2-4), which are published, or currently in preparation for publication in peer reviewed scientific journals. I was responsible for the conception of the research, the field work, laboratory, and data analysis, and writing of the manuscripts. Unless otherwise referenced, the information in this thesis was produced from my own ideas, and all work presented was carried out under the guidance and supervision of my scientific supervisors.

Chapter 2 was published in Science of the Total Environment, under the title “Effects of catchment land use on temperate mangrove forests”.

Thomson, T., Ellis, J. I., Fusi, M., Prinz, N., Lundquist, C. J., Bury, S. J., et al. (2024). *Effects of catchment land use on temperate mangrove forests*. Sci. Total Environ. 940, 173579. doi:10.1016/J.SCITOTENV.2024.173579.

Chapters 3 and 4 are being prepared for submission.

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To my family for love.

To Natalie, for always.

...and to my child, whoever you may be, for giving me the strength and determination to see this through, and for showing me what really matters in life.



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N = nitrogen; ORP = oxygen reduction potential;  $\text{NH}_4$  = ammonium concentration;  $\text{NO}_x$  = nitrate/nitrite concentration; DOC = dissolved organic carbon; Chl  $\alpha$ :phaeo = chlorophyll  $\alpha$  to phaeophytin ratio.

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**They stand alone** where no other trees can survive; not outcasts, but pioneers breaking new ground at the margins of our land. Bathed twice daily by the sea, and rooted in waterlogged soils that are virtually devoid of oxygen, they are marvels of nature, flourishing in conditions that are intolerable to all other flowering trees, and forming dense, luxuriant forests.

*-John Walsby, NZ Geographic*



# 1 | General introduction

## 1.1 Background

### 1.1.1 Land-derived human stressors on coastal ecosystems

The constant increase in human population has come with a need for space and resources, which has put a significant strain on natural ecosystems worldwide (Seto et al., 2012b; Steffen et al., 2015). This need has led to large-scale changes in how land is being utilised, often causing long-term reductions of biological productivity and ecological integrity (Olsson et al., 2019). Such land degradation and its impacts are a critical issue identified by the IPCC (Olsson et al., 2019). Human practices on land also have a direct impact on coastal ecosystems, exacerbating the direct adverse effects of land degradation through secondary effects downstream (Kennish, 2002; Thrush et al., 2021; Gladstone-Gallagher et al., 2022). Accelerated migration patterns of people into low-lying coastal zones concentrate human pressures on coastal ecosystems, with hotspots in developing countries with poor or no regulations in place (Seto et al., 2012a; Neumann et al., 2015; Merkens et al., 2016). But even in developed countries our human footprint on the coast and ocean is growing and there is a pressing need to better understand and manage the effects of land-derived stressors on all aspects of marine ecosystems.

Anthropogenic effects on coastal systems range from physical removal for space, resources, or aesthetic reasons, to increased sediment delivery, pollution, and nutrient availability (Alongi, 2002; Polidoro et al., 2010). Each of these stressors comes with its own challenges for coastal habitats and depending on the type of habitat, the effects may vary in their strength and direction (Thrush et al., 2021). Furthermore, depending on the intensity of the stressor, it may have opposing effects or be non-linearly related with the outcome variable. Increased rates of sedimentation for example could be enough to smother seagrass beds or mussel reefs but benefit mangrove forest expansion and adaptation to sea level rise (Thrush et al., 2004; Saintilan et al., 2020). Similarly, limited nutrient input may increase plant productivity, but high concentrations can lead to increased vulnerability to extreme events (Alongi, 2018). Globally, anthropogenic stressors are degrading coastal ecosystems at an alarming rate (Duarte, 2009; Li et al., 2018). In particular, coastal eutrophication from increased nutrient availability is a globally increasing threat to near-shore marine habitats, leading to trophic



shifts, increased greenhouse gas emissions, and oxygen minimum zones (Rabalais et al., 2009, 2010; Doney, 2010). Coastal ecosystems, and particularly semi-enclosed systems such as fjords and estuaries, face heightened vulnerability to eutrophication, due to their proximity to the sources of these stressors and poor tidal mixing with offshore waters leading to extended slow turnover times (Thrush et al., 2021; Jiang et al., 2023; Krause et al., 2023). Therefore, a focus on coastal ecosystems, when dealing with land-derived influences, should be of the highest priority.

The difficulties arising from catchment-level stressors intensify with a warming planet, leading to the amplification of specific challenges and the establishment of positive feedback loops with other local pressures (Spivak et al., 2019). Increased storm events for example will lead to heightened land-sea connectivity, which generally intensifies sedimentation and associated nutrient delivery to coasts (Seneviratne et al., 2012). Elevated temperatures in turn will accelerate metabolic processes that have the potential to shift the existing stoichiometric balance and exacerbate effects of land-derived stressors (Craine et al., 2010; Ouyang et al., 2023).

### 1.1.2 Mangrove forests

Mangrove forests are highly productive ecosystems that cover vast amounts of coastline in tropical and subtropical regions (Twilley et al., 2019). Their high productivity coupled with slow decomposition rates due to anoxic sediments, has been associated with an accumulation of carbon-rich organic matter (Duarte and Cebrián, 1996). Hence, mangroves play an important role in decreasing carbon dioxide concentrations from the atmosphere and thereby mitigating climate change (Duarte et al., 2004, 2013). By changing the speed and flow of water currents and attenuating wave energy, mangroves protect coastlines from erosion and storm surges (Koch et al., 2009; Menéndez et al., 2020), and simultaneously retain land-derived sediment particles and pollutants within their dense root structures (Morell and Corredor, 1993; Valiela and Cole, 2002; Xie et al., 2023). As primary ecosystem engineers, they provide shelter and substrate for economically and ecologically important species, often in the form of nursery grounds, and their high rates of productivity fuel energy transfer within and between coastal ecosystems (Figure 1.1, Odum and Heald, 1975; Ward et al., 2017, 2020; Saavedra-Hortua et al., 2020). The global monetary value of services provided by mangrove forests and salt marshes has been estimated to be 194,000 US\$ ha<sup>-1</sup> year<sup>-1</sup> (Costanza et al., 1997; de Groot et al., 2012). While temperate mangroves contribute less to this figure because of lower

productivity and different utilisation, their value to coastal communities, biodiversity, and carbon storage remains considerable (Morrisey et al., 2010; Dencer-Brown et al., 2018).

Due to their position of growing on the interface between the land and the sea, mangrove forests are exposed to a number of land-derived (e.g. soil run-off, deforestation, coastal development, pollution) and ocean-derived (rising sea level, increased storms) pressures (Valiela et al., 2001; Schaffelke et al., 2005; Duke et al., 2007; Lovelock et al., 2015; Saintilan et al., 2020). The land-derived influx of sediments, and nutrients are an important supply to keep up with fluctuating sea levels and to sustain high growth rates, however, excessive amounts of either have the potential to severely unbalance the system (Figure 1.1, Lovelock et al., 2009; Sidik et al., 2016). Generally, mangrove forests develop in nutrient-limited environments, in which the stoichiometric relationship of the limiting nutrients is finely balanced, making use of efficient recycling and retention processes to sustain growth (Schaffelke et al., 2005; Reef et al., 2010). External nutrient additions can offset that balance, shifting metabolic pathways and communities, ultimately leading to regime shifts or unhealthy systems in which the provision of ecosystem services is impaired (Figure 1.1, Taylor and Townsend, 2010).

A variety of studies highlight the variability of nutrient regimes in mangrove sediments and their influence on tree species distribution, forest structure, and within tree nutrient cycling (Feller, 1995; Feller et al., 2003; Lovelock and Feller, 2003). Tree growth tends to increase when the limiting nutrient is supplied (Lovelock et al., 2004), but contrasting information exists on the allocation of above ground to below ground growth (Hayes et al., 2017). Some mangrove forests, especially in temperate latitudes have expanded in response to increased supply of fine sediment and limiting nutrients, which provides favourable conditions for seedling establishment and growth (Figure 1.1, Ellis et al., 2004; Lovelock et al., 2007). However, nutrient enrichment can have negative effects by decreasing the recycling efficiency of nutrients in mangrove trees with implications for nutrients leaching from the system (Feller, 1995). High levels of nutrients can lead to mangrove mortality by increasing the sensitivity to additional stressors such as high salinity or droughts (Lovelock et al., 2009; Lovelock et al., 2017). The majority of studies on nutrient effects on mangrove forests have been performed in relative isolation of stressors and across few sites, limiting our understanding of complex interactions and potential feedback loops between ecosystem components.

### 1.1.3 Environmental controls on carbon storage

The capacity of mangrove forests to out-scale other ecosystems' carbon storage is in many parts due to the unique environment present in their sediments (Donato et al., 2011; Alongi, 2020). Mangrove sediments are often naturally rich in organic carbon but low in available nutrients and anoxic (Reef et al., 2010); conditions which lead to low remineralisation rates of organic material and therefore, high amounts of carbon that are stored underground (Bouillon et al., 2008; Duarte et al., 2013; Alongi, 2014). The resulting gradual accumulation of belowground carbon leads to disproportionately large contributions of sediment carbon, which exceeds that of tropical rainforests, making mangroves one of the most efficient ecosystems at sequestering carbon (Duarte and Cebrián, 1996; Donato et al., 2011). However, the effects of anthropogenic stressors on processes controlling the carbon cycle in mangrove sediments are not well understood.

Carbon enters the soil through allochthonous or autochthonous pathways, most of which is rapidly degraded and respired back into the atmosphere in the form of CO<sub>2</sub>, by a complex network of interactions between macro- and micro-detritivorous organisms (Holguin et al., 2001; Kristensen, 2007; Alongi, 2012). A fraction of this carbon escapes the fate of remineralisation through a combination of selective preservation of biochemically unreactive compounds (recalcitrance), and mineral protection (Mayer, 1994; Burdige, 2007; Hemingway et al., 2019). Another option for carbon sequestration in highly productive but oxygen limited sediments, is the rapid burial of organic material through high sedimentation rates (Chmura et al., 2003). In this scenario, the degradation pathways, constrained by substantial energy limitations and anoxic conditions, effectively decrease the rate at which carbon undergoes remineralisation (Pérez et al., 2018). The rate of organic matter degradation as a regulatory mechanism to maximise carbon storage in mangrove sediments is one process that is likely to be strongly influenced by the environmental setting, and hence vulnerable to the effects of anthropogenic stressors (Figure 1.1). It is therefore of great importance to understand the drivers of organic matter degradation in mangrove forests and how they may be affected by human impacts.

## Chapter 2



## Chapter 4

6

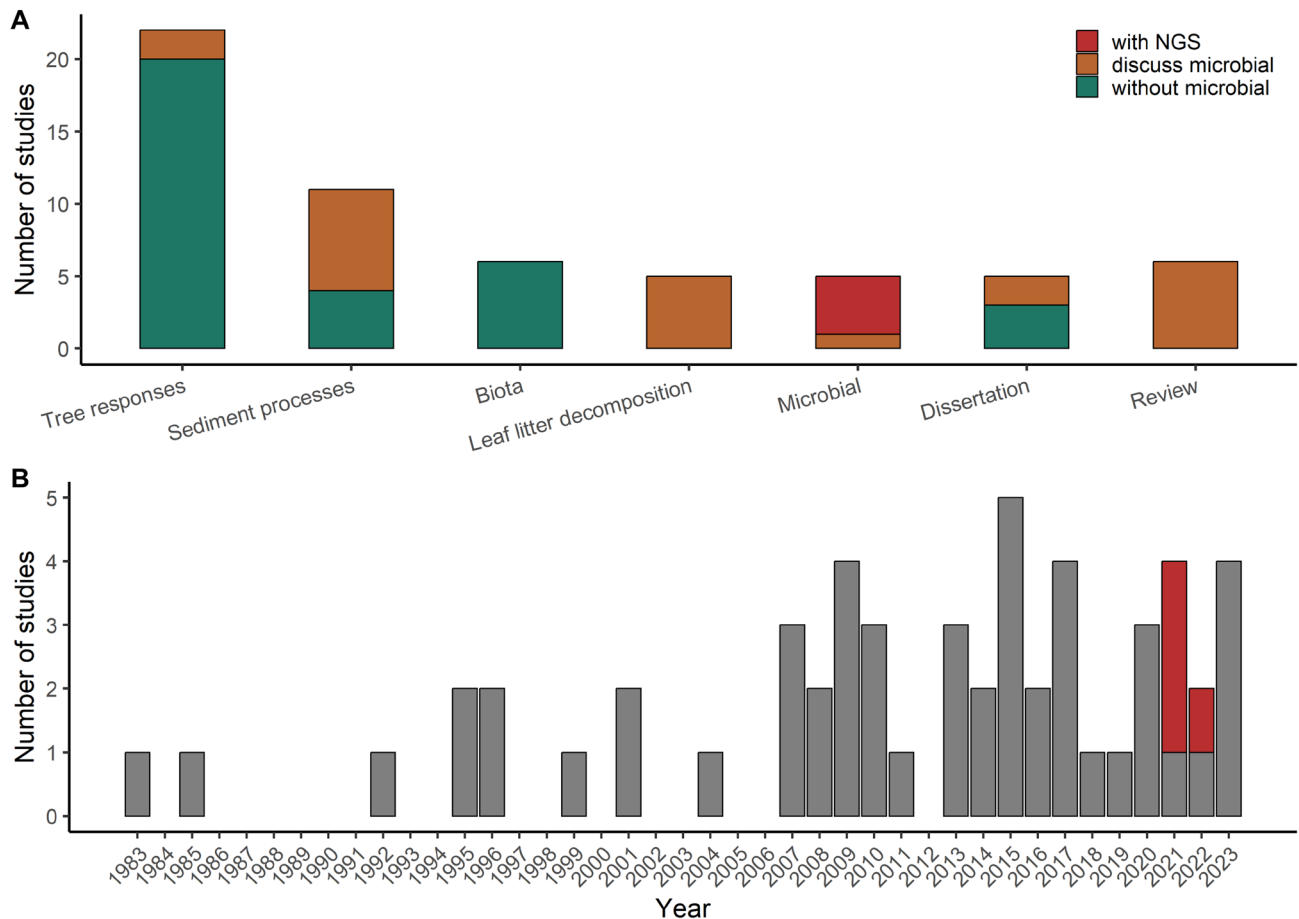


### 1.1.4 The role of microbial communities in mangrove sediments

One component of mangrove forests that has been widely overlooked in regard to the effects of anthropogenic impacts, are the microbes within their sediments. Microbial communities are one of the main regulators of nutrient (including carbon) cycling in soil ecosystems (Figure 1.1, Van Der Heijden et al., 2008; Reef et al., 2010; Cavicchioli et al., 2019; Delgado-Baquerizo et al., 2020). And with microbial processes driving the rate and the efficiency of organic matter degradation in soils, their role in the carbon cycle of mangrove systems is of central importance (Holguin et al., 2001). In terrestrial biomes, the effect of increased amounts of biologically reactive nitrogen due to the increasing use of fossil fuels and fertilisers over the past century has been found to stimulate plant growth but also affect soil microbial biomass and activity, and thus the recycling of soil carbon and nutrients (Treseder, 2008; Janssens et al., 2010). However, these processes have received limited attention in mangrove systems, in which important factors such as water availability, sedimentary conditions, dispersal limitation, and microbial performance rates differ substantially from terrestrial systems.

Currently, there is limited knowledge on how mangrove sedimentary microbial communities respond to nutrient enrichment (Figure 1.2). A range of studies present contrasting arguments about the impacts of eutrophication on microbially driven processes, which is mainly due to the specific nature of each eutrophication event. Nutrient input from shrimp farming for instance comes with a primed community of microbes already feasting on the organic matter present (Sousa et al., 2006). Similarly, enriched waste from urban settings comes with high concentrations of pollutants and organic waste (Sanders et al., 2014; Conrad et al., 2023). These contrasting modes of enrichment introduce too many confounding factors to assess the mechanistic effects of nutrient enrichment on present microbial communities. To avoid these confounding factors, the focus of the following literature review is exclusively on manipulative fertilisation experiments. The majority of studies (n=22) investigating the effects of nutrient enrichment in mangrove environments focussed on fertilisation effects on mangrove tree responses, such as their growth, physiological responses, shoot and root development, mortality, and tree expansion (Figure 1.2; Supplementary table A1.1). Six studies were conducted to assess the effects of fertilisation on biota such as herbivores and macrofauna, yet the lack of experiments on microbial responses and sediment processes to nutrient enrichment is evident in the time before 2000. From then on, the literature provides more insights into the effects of nutrient enrichment on gas fluxes from the sediments (n=12), and nitrogen transformations (ammonification, denitrification, nitrification). Studies focussing on leaf litter decomposition (n=5) with nutrient enrichment have increased since 2015, most

likely with the rising interest in carbon sequestration by mangroves. Out of six reviews on nutrient dynamics in mangrove forest environments, all discuss the role of microbial processing and their importance to the functioning of the system, however, always speculatively and not based on empirical data from mangroves. One study from 2015 recorded the responses of microbial enzyme activities to nutrient enrichment, and only in 2021 next generation sequencing (NGS,  $n=4$ ) was started to be utilised for a community-based approach. Moreover, only one of these studies investigated this *in situ* (Palacios et al., 2021).



**Figure 1.2** Literature review of existing studies on fertilisation experiments in mangrove forests. Microbial studies, and particularly those using next generation sequencing (NGS) are highlighted. The review was conducted using Scopus and Google (Scholar) search engines using the strings “nutrient AND enrichment AND mangrove”, and “16s AND rna AND mangrove AND nutrient AND soil OR sediment”.

Due to their short generation times, it is expected that sediment microorganisms will react faster than trees to such changes, with implications for chemical conditions, nutrient cycling pathways, and carbon sequestration rates of the whole system (Bardgett et al., 2008). Few papers have reported changes in nitrogen cycling genes and taxa, and variations in microbial biomass in response to artificial enrichment, however, the detection of associated ecosystem

processes has been difficult (Craig et al., 2021; Dai et al., 2021; Erazo and Bowman, 2021). In unvegetated intertidal habitats, nutrient delivery led to bursts of microbial autotrophic growth which sustained increased carbon turnover and remineralisation of 'old' refractory carbon (Riekenberg et al., 2018, 2020). This highlights a mechanism which may threaten the already stored carbon stocks in mangrove systems exposed to eutrophic settings. However, more research is needed to observe this relationship in vegetated habitats in which the flow of nutrients is complicated by the dominant macrophyte species.

### 1.1.5 Mangroves (Mānawa) in a New Zealand context

In New Zealand, the only species of mangroves present is *Avicennia marina* subsp. *australasica*. This species is native to New Zealand, and its cover is increasing in many estuaries of the North Island, predominantly due to the effects of increased sedimentation and associated nutrients caused by rapid land development after early European settlement (Dencer-Brown et al., 2018; Horstman et al., 2018). This rapid expansion and subsequent changes to the estuarine space (often perceived as loss of recreational space) have led to mangroves being a highly controversial topic in New Zealand, leading to the managed (or compassionate) removal of trees in many places (Dencer-Brown et al., 2019; Le Heron et al., 2022). This controversy partly arises due to the lack of knowledge around ecosystem services related to the temperate nature of the local systems, which are often considered to be less than those of their tropical counterparts (Morrisey et al., 2010). Temperate mangroves only cover a small fraction of the total mangrove area worldwide (between 0.25% and 1.50%; Twilley et al., 1992; Morrisey et al., 2010), however, they still play a crucial role in sustaining important ecosystem functions (Morrisey et al., 2010). Moreover, there is potential and growing interest for their utilisation as blue carbon ecosystems, presenting an opportunity for local carbon offsetting (Suyadi et al., 2020; Ross et al., 2023). Therefore, it is critical to evaluate ecological processes in temperate mangrove forests in their own right, to maximise the protection and use of these unique habitats.

## 1.2 Knowledge gaps

Despite its undeniable importance, research on the effects of nutrient enrichment on the sediments of mangrove ecosystems is scarce, especially of *in situ* approaches and in combination with microbial community analyses. A comprehensive approach is needed to identify relevant anthropogenic stressors and their effects on ecosystem health, carbon and

nutrient cycling and associated microbial processes. Based on the current state of research I have identified major knowledge gaps that will be addressed in this thesis:

**1) Landscape-scale effects of anthropogenic land use on mangrove forests.** While multiple studies have assessed the effects of single anthropogenic stressors on mangrove forests, or the effects of multiple stressors on a small number of sites, a comprehensive understanding of the cumulative landscape-scale effects of human land use on mangrove systems is missing. Filling this gap is important to evaluate the effects that human activities on land have on mangrove forests, with a holistic understanding of the ecosystem, rather than on its isolated components.

**2) Environmental controls of organic matter degradation in mangrove forests.** Degradation of organic material in mangrove forests has been studied in relation to temperature and experimental nutrient input, however, which environmental factors exert the most influence on this important process is unclear. Evidence from terrestrial work suggests a multitude of interacting factors to be important, instead of individual control mechanisms. Furthermore, organic matter of varying recalcitrance may be differently affected by these controls. As above (knowledge gap 1), it is important to understand these drivers on a scale relevant to regional decision makers rather than to single out specific controls in single systems.

**3) Spatial and temporal understanding of eutrophication effects on microbial communities in mangrove forests.** Very few studies have assessed the effects of nutrient enrichment on the microbial community in mangrove sediments, and there is even less empirical research testing these effects *in situ*. Creating mesocosm studies or even transporting sediments to a laboratory for incubations can answer important questions, however, it threatens (and usually destroys) the delicate spatial organisation present in sediments. This structure is a result of the environmental conditions and extremely important for species interactions and the flow of nutrients and energy through the communities. Furthermore, the complexity of natural systems and the dynamism induced on a temporal scale are likely to affect and obscure the results. However complicated to acquire and 'noisy' this data may be, it provides essential real-life information that is crucial for informed decision-making, model checking, and to gain valuable insights into the complexities of our natural world.

### 1.3 Thesis organisation, hypotheses, and objectives

This thesis aims to identify land-derived stressors on temperate mangrove forests and their effects on carbon and nutrient cycling within the ecosystem, with a focus on microbially driven



pathways of degradation. My overall objective was to identify variables within the mangrove ecosystem that responded to the amount of human land use in the catchments (Chapter 2) and determine specific drivers of organic matter degradation (Chapter 3). Lastly, I aimed to investigate the effects of experimental nutrient enrichment on the bacterial community and associated biogeochemical cycles in temperate mangrove forests (Chapter 4).

I hypothesise that human land use intensities in the catchment will considerably alter the environmental conditions in mangrove leading to enriched conditions in trees and sediments. Furthermore, I expect these changes to affect the rate of organic matter degradation, increasing the loss of carbon from the system. Finally, I hypothesise the bacterial community to react to nutrient enrichment which will be apparent from changes in microbial biomass, changes in the taxonomic profile, and increased losses of greenhouse gasses and dissolved carbon from the sediments.

A crucial aspect of ecological studies is the inference of general patterns from isolated experiments (Thrush et al., 2000). It is impossible to constrain all ecological variation in field observations and experiments, but the simplification of incubations, lab-based experiments, and numerical models can never accurately depict the complexities induced by environmental conditions (Snelgrove et al., 2018). Nevertheless, we must do our best to combine our mechanistic understanding of processes from laboratory experiments and translate it into the intricacies of the real world. We can attempt this generalisation by working along gradients of factors that will most likely modify the effect of interest, to build generality from field observations and experiments (Thrush et al., 2000). However, the detailed investigation of specific processes is also needed to form a basis of knowledge from which to formulate our hypotheses. In the specific example of this thesis, I utilise a broad human land use gradient in the catchments to answer questions of anthropogenic impact and ecosystem processes on a landscape-scale, providing generality to the results. However, to answer the mechanistic question of nutrient impacts on microbial communities and processes, I use the semi-controlled approach of a manipulative field experiment to isolate the effects of nutrients, without confounding factors such as increased organic matter or pollutants.

This thesis comprises three research chapters that are based on observational and experimental field studies. The specific aims, objectives, and summary of each chapter are described below:

### 1.3.1 Chapter 2

In this chapter, I aimed to identify the impacts of human land use in catchments on temperate mangrove forests in New Zealand. The study was designed at a scale that enabled the identification of generalisable relationships between anthropogenic impacts and environmental variables, that transcend site or estuary specific effects. To achieve this, I selected 30 different estuaries in a relatively narrow latitudinal window, along a gradient of human land use in the catchments. The land use was quantified using geospatial tools and a range of variables were collected relating to forest structure, sedimentary, and porewater variables. Understanding how anthropogenic activities on land affect mangrove forests is of global importance. However, the scarcity of data on large-scale impacts from agricultural and urban land use often impedes effective coastal management. With this research I aim to provide a set of priority areas for coastal management based on sensitive indicators of human pressures on temperate mangrove forests.

### 1.3.2 Chapter 3

The ability to protect organic matter from decomposition is an ecosystem property. My objective of this chapter was to identify a set of environmental variables that control the rate of organic matter degradation in temperate mangrove forests. By using the gradient of human land use described in Chapter 2, I further aim to identify effects of anthropogenic pressures on this important ecosystem function. Here, I used standardised assays to identify environmental controls of organic matter decomposition in temperate mangrove forests. I used different substrate qualities (an agar-based substrate, green tea, and red tea) with increasing recalcitrance of the material, which were incubated for varying lengths of time to differentiate controls on early- and later-stage decomposition. By including temperature measurements, I aimed to identify the effects of climatic drivers on decomposition as well as local environmental factors. Results from Chapter 3 enable me to link catchment land use effects with organic matter degradation rates which highlights potential vulnerabilities of carbon sequestration processes to anthropogenic impacts.

### 1.3.3 Chapter 4

For this chapter my objectives were to observe changes in the bacterial communities of mangrove forest sediments in response to manipulative nutrient enrichment, with consideration given to small-scale spatial variability. Furthermore, I aim to quantify ecosystem processes represented by gas and solute fluxes from the sediment surface and link these to changes in the bacterial community. I conducted a manipulative field experiment to investigate the effects of nutrient enrichment on the bacterial community and associated biogeochemical cycles in temperate mangrove forest sediments. Plots were experimentally enriched with a complex fertiliser (N:P:K) for 4.5 months, which was followed by an extensive sampling campaign. High-throughput amplicon sequencing (16S rRNA) was utilised to examine bacterial community composition changes in surface and subsurface sediment layers of a characteristic New Zealand mangrove forest. Detailed measurements of environmental variables from the sediment and porewater were taken to identify associations with the bacterial community and how they changed with enrichment. Ecosystem processes were measured as solute and gaseous fluxes from the sediment surface to the overlying water column (submerged, at high tide) and the atmosphere (emerged, at low tide). Chapter 4 underpins the importance of considering microbial community changes when assessing the effects of eutrophication on mangrove forests and highlights their prolonged role in carbon and nutrient cycling after the effects have worn off.

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**Their light green leaves,** clearly setting them apart from the backdrop, mangroves are easily spotted from the sea. At low tide, the innumerable pneumatophores trap sediments and pieces of organic material. Together with restricting conditions below ground this leads to high sequestration rates and facilitates carbon storage.





## 2 | Effects of catchment land use on temperate mangrove forests

Thomson, T., Ellis, J. I., Fusi, M., Prinz, N., Lundquist, C. J., Bury, S. J., et al. (2024). *Effects of catchment land use on temperate mangrove forests*. *Sci. Total Environ.* 940, 173579. doi:10.1016/J.SCITOTENV.2024.173579.

### 2.1 Introduction

The conversion and subsequent degradation of land through anthropogenic activities is a major global issue, contributing to climate change and weakening the resilience of ecosystems to disturbances (Seto et al., 2012b; Steffen et al., 2015; Olsson et al., 2019). Land degradation may occur through the transformation of native forests and scrublands to agricultural land, short-cycle forestry, and urbanisation, and is defined as the “long-term reduction or loss of at least one of the following: biological productivity, ecological integrity, or value to humans” (Olsson et al., 2019). In addition, adjacent ecosystems may suffer from land management decisions upstream (Galloway and Cowling, 2002; Rabalais et al., 2009; Levin and Bris, 2015; Landrigan et al., 2020; Gladstone-Gallagher et al., 2022). It is well understood that anthropogenic land use in catchments reduces riverine water quality, which inevitably impacts coastal and marine ecosystems (Lotze et al., 2006; Clapcott et al., 2010; Billen et al., 2013; Diop and Scheren, 2016; Bierschenk et al., 2017). These impacts have been steadily increasing due to intensification of coastal urbanisation from human migration towards to the coast and associated agricultural intensification (Small and Nicholls, 2003; Seto et al., 2012a; Neumann et al., 2015; Merkens et al., 2016).

The historical impacts of human settlement and land use changes on estuaries have been clearly demonstrated from dated sediment cores (Macreadie et al., 2012; Conrad et al., 2017; Hapsari et al., 2020). Intensified land usage for human purposes increases sedimentation rates, pollutants, and nutrient concentrations in adjacent coastal areas, often with detrimental effects on marine life and ecosystem functioning (Ellis et al., 2002, 2017; Douglas et al., 2018; Cendrero et al., 2020; Conrad et al., 2023). However, how these stressors interact with mangrove forests, a prominent ecosystem type in tropical, subtropical, and some temperate estuaries, remains uncertain and difficult to study because of multiple potential feedback loops between tree physiology and sediment biogeochemistry. Mangrove forests are listed as highly threatened by human land use (Alongi, 2002; Li et al., 2021), while at the same time being proposed as natural filter systems for land-derived pollutants (PUMPSEA, 2008; Yang

et al., 2008). These contrasting evaluations highlight the need for a clear understanding of the effects of anthropogenic stressors on the ecology of these important ecosystems (UNEP, 2014; Pearson et al., 2021).

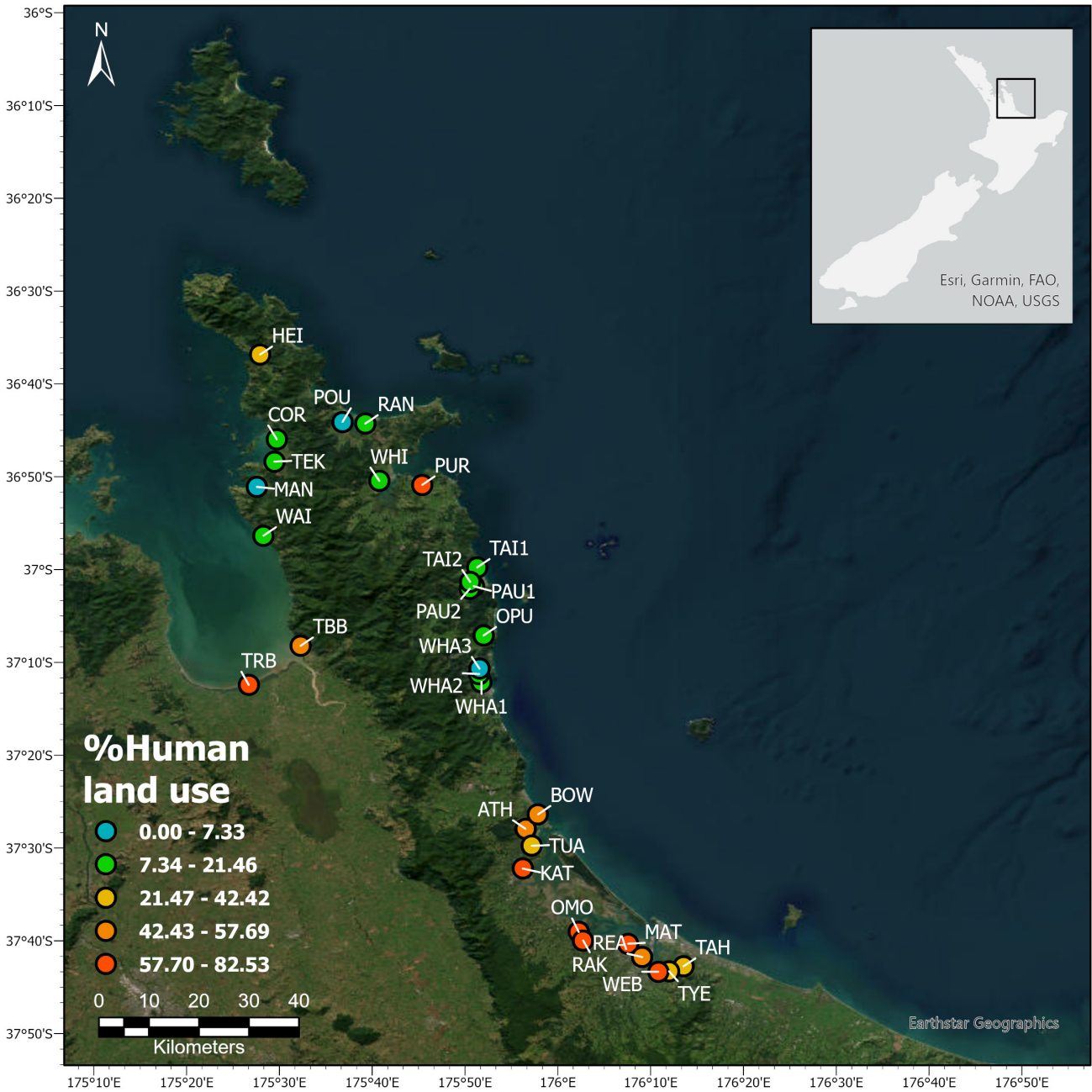
A range of studies have investigated individual effects of sedimentation, pollution, and nutrient enrichment on either tree physiology, forest biodiversity, sedimentary conditions, or the impact on benthic invertebrate communities in mangrove forests. High rates of sediment delivery through catchment land use, can lead to high sediment accretion rates in mangrove forests, facilitating the seaward expansion of these systems (Ellis et al., 2004; Yang et al., 2013; Suyadi et al., 2019). Within the forest, high rates of sedimentation may promote an increase in carbon sequestration through burial and assist sea level rise adaptation through surface elevation gains (Lovelock et al., 2013; Alongi, 2014; Rogers et al., 2019; Swales et al., 2019). The supply of limiting nutrients through eutrophication has been linked to increases in plant growth and reproduction (Feller, 1995; Lovelock et al., 2004; Feller et al., 2009; Huang et al., 2023). Thus, nutrient enrichment can contribute to the expansion of the system (Lovelock et al., 2007; Dangremond et al., 2020), but has also been hypothesised to weaken the resilience of trees to environmental stressors such as droughts and extreme weather due to a lack of resource allocation to roots (Mckee, 1995; Lovelock et al., 2009, 2017). Foliar stable isotope values of nitrogen have been shown to be a good indicator of anthropogenic land use with higher  $\delta^{15}\text{N}$  values representing the elevated values found in urban sewage and livestock effluent (Jones et al., 2018; Souza et al., 2018; Tanu et al., 2020). Macrofaunal densities and diversity have also been proposed as indicators of anthropogenic impacts on mangrove forests, with crab biomass increasing with low levels of organic pollution, but vulnerable species and species diversity possibly declining as stressors increase (Cannicci et al., 2009; Fusi et al., 2016). Organic matter dynamics seem to vary greatly depending on the geomorphic setting as well as the nature of the land use upstream, with high inflows of allochthonous materials often contributing to higher amounts of accumulated carbon in the sediments (Sanders et al., 2014; Lee, 2016; Rovai et al., 2018; Jennerjahn, 2021). Conversely, high nutrient availability may lead to reduced C:N ratios of autochthonous organic matter, which can act as a vector for faster microbial decomposition, reducing the systems potential for carbon storage (Nordhaus et al., 2017; Chynel et al., 2022). Greenhouse gas emissions from mangrove sediments and adjacent systems increase with intensified human land use in catchments, indicating higher respiration rates of heterotrophic organisms (Reading et al., 2020; Barroso et al., 2022). This increase in respiration leads to a reduction in available oxygen and subsequent acidification of the sediments (Reef et al., 2010).

The large spatial heterogeneity of catchments, coastal morphologies, and regional land use practices makes it difficult to extrapolate these impacts into a context relevant to regional management decisions (Gladstone-Gallagher et al., 2019). While the individual effects of anthropogenic stressors have commonly been studied in relative isolation and at a localised scale (plot, site, or estuary scale), only a few studies have focused on identifying patterns at landscape-scales (Lee, 2016). Understanding the effects of land use changes on mangrove forests in a variable, landscape-scale context is important for predicting the responses of these coastal systems to human pressures and for ensuring positive outcomes from restoration efforts (Xie et al., 2023).

In Aotearoa New Zealand, like in many parts of the world, intensive land use change has occurred recently (over the last 100-200 years), and relatively low population densities have kept estuarine water quality in an oligotrophic state (McLay, 1976; Plew et al., 2018, 2020; Dudley et al., 2020). Furthermore, the estuaries themselves have received comparatively little modifications over the years, reducing confounding effects with land-derived stressors (Plew et al., 2020). An intensification of agricultural and urban development in some catchments, however, has provided a gradient of land use within a relatively small geographical range (Tay et al., 2012; Dymond et al., 2013; Jones et al., 2022). Forestry is a mayor anthropogenic impact in some catchments, however the multidecadal rotation cycles reduce the impacts in estuaries to highly infrequent events after harvest. Therefore, their effect on the estuarine conditions is expected to be similar to native forest. Mangrove systems in New Zealand are monocultural stands of *Avicennia marina* subsp. *australasica* (Morrisey et al., 2010). *Avicennia* is a genus that is widely distributed globally due to its tolerance to a range of conditions. In New Zealand, this one species has taken over every mangrove niche from upstream to intertidal, as there are no other mangrove species to compete with. The temperate climatic conditions in northern New Zealand limit growth and productivity, potentially making these systems more sensitive to environmental stressors (Morrisey et al., 2010). These features of New Zealand estuaries make them well-placed for a focal study on the direct impacts of catchment land use on temperate estuarine mangrove forests.

To understand the impacts of human land use in catchments on temperate mangrove ecosystems, we designed this study along a broad gradient of human land use within a relatively narrow latitudinal window to mitigate potential confounding influences stemming from climatic variation. A dual focus on forest and sedimentary variables was chosen to capture a more holistic view of changes in the ecosystem. More specifically, we quantify forest architecture and reproduction, as well as sedimentary characteristics including

sediment texture, carbon, nutrient content, and porewater chemistry and correlate these with anthropogenic land use in the catchments. The principal objective of this study was to ascertain whether relationships established in previous studies at smaller spatial scales (i.e. sites, estuaries), hold at broader regional scales. A priori we predict that generalisable patterns dominate over context-dependent attributes of individual catchments and estuaries and aim to identify sensitive indicators of human pressure in mangrove forests at landscape-scales.



**Figure 2.1** Map of the study area on the east coast of the North Island of Aotearoa New Zealand. The locations of the 30 study sites and the human land use intensity (percentage of agricultural and urban land cover) in their respective catchments.



## 2.2 Methods

### 2.2.1 Study sites

This study was conducted at 30 sites in estuaries of the Bay of Plenty and the eastern Waikato region of New Zealand, during the Southern Hemisphere summer of 2022. Some sites were co-located within larger estuaries; however, they were generally influenced by exposure to sub-catchments and streams, which created unique conditions at each site (Figure 2.1; Table 2.1). The sites were located on the eastern coast of New Zealand's North Island and spanned a relatively narrow latitudinal range, with only 140 km between the northernmost and southernmost sites. This range was chosen to minimise climatic differences, such as temperature and rainfall, between the sites. The catchments of these estuaries are subject to varying types and intensities of anthropogenic land use (Table 2.1), which creates a natural gradient of contrasting environments allowing us to test whether land is correlated with forest and sediment characteristics. The mangrove forests in the different estuaries display a wide range of characteristics from extensive coverage of the estuary (up to 50% of the total area) to marginal growth along shorelines tucked away in stream heads and ranging from tall growth forms (>4 m) to small and stunted individuals (<0.7 m) (Supplementary Table A2.1). Site locations were chosen largely according to accessibility and the minimum area of the stand exceeding 0.01 km<sup>2</sup>. Each site was accessed from the seaward fringe of the mangrove stand and was located 15 m into the interior of the forest, perpendicular to the fringe line and at least 15 m away from any channels and streams.

### 2.2.2 Surveys and sample collection

Surveys were conducted to assess mangrove forest architectural characteristics (e.g., tree size, and mangrove reproduction) and ecological soil/sedimentary characteristics (e.g., pH, crab burrow densities). Ten randomly selected trees were measured for height and diameter at breast height (DBH; smaller individuals were measured just below the first major splitting of branches), and tree density was estimated as the percentage of canopy cover at each site. Pneumatophore-, propagule-, seedling-, and crab burrow-density (categorised by burrow diameter: <2 cm and >2 cm) were counted from three randomly placed quadrats (25 cm x 25 cm for pneumatophore density, 50 cm x 50 cm for all others). In situ measurements of porewater salinity, pH, and oxygen reduction potential (ORP) were obtained from a dug-out hole using a hand-held multi-parameter probe (YSI ProQuatro, Xylem, Washington DC,

USA). Sediment samples were collected using a 60 mL polyethylene syringe ( $\varnothing$  3 cm) with a cut-off tip. At each site, nine cores of surface sediments (0-2 cm) were collected. Three cores were then combined into one composite sample to account for small-scale local variability, resulting in three pooled samples per site. This collection method was repeated for the porewater samples. Leaf samples for foliar analysis were collected from three randomly selected trees in the sampling area. One apical branch, with at least ten leaves was sampled per tree. All samples were kept in the dark and on ice and placed in a freezer after sampling was completed (within 3 h maximum). Porewater was extracted by centrifugation (Douglas et al., 2016) as soon as possible after sampling and the supernatant was filtered through 0.7  $\mu$ m filters (GF/F Whatman, Buckinghamshire, UK) before being separated into nutrient and dissolved organic carbon (DOC) samples. Nutrient samples were frozen at -20°C while DOC samples were protected from light and cooled at 4°C until analysis.

### 2.2.3 Analytical methods

Sediment grain size (GS) was measured using a Malvern Mastersizer 3000 (Malvern Panalytical Ltd., Malvern, UK) after removing the organic matter with 10% hydrogen peroxide over three weeks. Particles larger than 4 mm were removed using a sieve, and Calgon (Sodium Hexametaphosphate 20% solution) was added to disperse the particles (Singer et al., 1988). Mud content was defined as the relative contribution of silt/clay particles with diameters < 63  $\mu$ m (Udden, 1914; Wentworth, 1922). Water content was measured as the mass lost after drying the sediment for 24 h at 60°C and was expressed as a percentage. Sediment density was calculated as the mass of the dry sample divided by its volume and expressed as g m<sup>-3</sup>. The loss on ignition method was used to determine the total organic matter (TOM) content of the sediments (in %). Between 6-8 g of sediment were dried (at 60°C for 24 h) and subsequently combusted at 550°C for 4 h. Sediment chlorophyll *a* (chl *a*) and phaeophytin (phaeo) content were extracted in 90% buffered acetone and their fluorescence measured before and after acidification with 0.1 N HCl on a 10-AU fluorometer (Turner Designs, San Jose, USA) (Arar and Collins, 1997). Prior to pigment analysis, the sediment was freeze-dried using a Lyovapor L-200 (Büchi, Flawil, Switzerland) at -50°C with a pressure of 2 mbar.

Dissolved nutrients from the porewater samples were analysed on a Quikchem 8500 Series 2 flow injection analyser (LACHAT instruments, Loveland, USA) following standard methods for nitrate/nitrite (NO<sub>x</sub>), ammonium (NH<sub>4</sub><sup>+</sup>), and orthophosphate (PO<sub>4</sub><sup>3-</sup>) analyses (QuikChem® Methods:31-107-04-1-A, 31-107-06-1-B, and 31-115-01-1-H, respectively). Porewater DOC was analysed using an Aurora 1030 Wet Oxidation TOC analyser (OI Analytics, College

Station, USA) following a modified protocol for non-purgeable organic carbon (NPOC) to guarantee the complete oxidation of organic carbon from a complex saline sample matrix (sample volume: 1 mL; oxidiser volume: 8 mL; dissolved inorganic carbon purge: 4 min; DOC detection: 8 min).

Dried, homogenised sediment and mangrove samples were weighed into tin boats and combusted in a Flash 2000 elemental analyser (EA) linked to an isotope ratio mass spectrometer (IRMS) detector via an open split Conflo interface (all Thermo-Fisher Scientific, Bremen, Germany) at the Environmental and Ecological Stable Isotope Facility, National Institute of Water and Atmospheric Research Ltd., Wellington. The ISODAT software (Thermo-Fisher Scientific, Bremen, Germany) calculated  $\delta^{15}\text{N}$  values against an atmospheric air international standard. Values of  $\delta^{13}\text{C}$  were calibrated against a  $\text{CO}_2$  reference gas, relative to Pee Dee Belemnite (PDB) limestone standard and were then corrected for  $^{17}\text{O}$ . Stable isotope ratios were expressed as delta values ( $\delta$ ) in per mil units (‰), which represent the ratios of heavy to light isotopes within a sample ( $R_{\text{sample}}$ ), relative to the ratio in an international standard ( $R_{\text{standard}}$ ) as:

$$\delta = \left( \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right) \times 1000 \quad \text{Eq. (2.1)}$$

All estimates of variance were calculated to 1 standard deviation (SD). Carbon isotope data were corrected via a two-point normalisation process (Paul et al., 2007) using NIST 8573 (USGS40 L-glutamic acid; certified  $\delta^{13}\text{C} = -26.39 \pm 0.09$  ‰) and NIST 8542 (IAEA-CH-6 Sucrose; certified  $\delta^{13}\text{C} = -10.45 \pm 0.07$  ‰). Nitrogen isotope data were corrected using the same two-point normalisation process using NIST 8573 (USGS40 L-glutamic acid; certified  $\delta^{15}\text{N} = -4.52 \pm 0.12$  ‰) and IAEA-N-2 (ammonium sulphate; certified  $\delta^{15}\text{N} = +20.41 \pm 0.20$  ‰). At the start of each run, %C and %N values were calculated relative to a laboratory reference standard of DL-Leucine (DL-2-Amino-4-methylpentanoic acid,  $\text{C}_6\text{H}_{13}\text{NO}_2$ , Lot 127H1084, Sigma-Aldrich Pty Ltd., Sydney, Australia), which was also run every ten samples to monitor analytical precision and drift. Additional international standards NIST 8574 (USGS41 L-glutamic acid; certified  $\delta^{13}\text{C} = +37.63 \pm 0.10$  ‰ and  $\delta^{15}\text{N} = +47.57 \pm 0.22$  ‰), NIST 8547 (IAEA-N1 ammonium sulphate; certified  $\delta^{15}\text{N} = +0.43 \pm 0.04$  ‰) were run daily to check isotopic accuracy. Repeat analysis of standards produced data accurate to within 0.25% for %C, 0.15% for %N, and 0.25 ‰ for  $\delta^{13}\text{C}$  and 0.10 ‰ for  $\delta^{15}\text{N}$ ; and a precision of better than 0.25% for %C, 0.15% for %N, and 0.25 ‰ for  $\delta^{13}\text{C}$  and 0.15 ‰ for  $\delta^{15}\text{N}$ .

## 2.2.4 Calculation of catchment land cover

The catchment land cover was calculated using land cover classes from the Land Cover Database Version 5.0 (LCDB5, Landcare Research, <http://www.lris.scinfo.org.nz>). All rivers and streams entering the estuaries and sub-estuaries at each site defined the catchment boundaries and were identified using the New Zealand River Network (REC2, feature layer, <https://data-niwa.opendata.arcgis.com/datasets/NIWA::nz-river-names-rec2-featurelayer/explore>) and were used to trace the watershed specific to each site. Following Dudley et al. (2020), urban land cover was calculated as the summed area of four LCDB5 classes (1: Built-up Area (settlement); 2: Urban Parkland/Open Space; 5: Transport Infrastructure; and 6: Surface Mine or Dump). Agricultural land cover was summed over four classes (30: Short-rotation Cropland; 33: Orchards, Vineyards, or Other Perennial Crops; 40: High Producing Exotic Grassland; 41: Low Producing Grassland), and forestry land use over two classes (64: Forest – Harvested; 71: Exotic Forest). The native land cover was calculated as the summed area of eight LCDB5 classes (45: Herbaceous Freshwater Vegetation; 46: Herbaceous Saline Vegetation; 47: Flaxland; 50: Fernland; 52: Manuka and/or Kanuka; 54: Broadleaved Indigenous Hardwoods; 68: Deciduous Hardwoods; 69: Indigenous Forest). A description of all LCDB5 classes is provided in the Supplementary Material (Supplementary table A2.5). The summed areas of each class were divided by the total area of each catchment to obtain the relative land use cover of each class. After preliminary data analysis, %-human land use cover (combined agricultural and urban areas) in the catchment was chosen as a measure of land use intensity, as it performed better than the individual land use types (Agricultural, Urban, Forestry, Native). However, individual contributions of agricultural and urban land use were retained to test for interaction effects (see section 2.5). Despite being a human-induced land use, the effect of the 'Forestry' category was similar to the native catchment cover, probably due to the long-term rotation period of harvested pine forest, which leads to relatively high land stabilisation and low soil and nutrient erosion from this land use type. Hence, forestry was not included in the %-human metric. Moving forward, human land use in the catchment will be referred to as "%Human."

## 2.2.5 Data analysis

All analyses were performed using R v4.2.1 (Core Team, 2021). Several collinear variables (tree height, and sediment water content, median grain size, C, N, and  $\delta^{15}\text{N}$ ) were excluded from the analysis (Pearson's correlation coefficient,  $R^2 > 0.75$ ). Most variables were collected



in triplicate from each site, while some variables were measured only once per site. To better control for within-site variability, triplicate measures were retained throughout the analysis. Three missing values of DOC (at site TUA), and one value each of salinity, pH, and ORP (at site WAI) were imputed using a random forest based method with the 'missForest()' command from the missForest package v1.5 (Stekhoven and Bühlmann, 2012). After visual exploration of the data, variables with a right skew (propagule density, seedling density, crab burrow (<2 cm) density, crab burrow (>2 cm) density, chl  $\alpha$  : phaeo ratio, DOC,  $\text{NO}_x$ ,  $\text{PO}_4^{3-}$ ) were  $\log_{10}(x+1)$  transformed to better fit the assumptions of the analyses. Variables that were presented as proportions (TOM, mud content, %Agricultural, %Urban, %Human) were logit-transformed to meet the assumptions of a normal distribution (Warton and Hui, 2011). A total of 26 environmental variables were selected and classified into two groups: mangrove forest variables ( $n = 12$ ) and sediment variables ( $n = 14$ ).

To test the effect of human land use in the catchment on the set of environmental variables, a multivariate linear model with %Human as the continuous predictor variable was constructed using the function `manyglm()` from the R package `mvabund` (Wang et al., 2012). Correlations between environmental variables in the response matrix were allowed (`cor.type = "shrink"`). The model-based structure of this method enables a further test for univariate relationships between the predictor variable and individual responses by implementing a step-down resampling process which provides p-values that are adjusted for multiple testing (Wang et al., 2012). Principal component analysis (PCA) was performed on the set of significant variables to visualise the relationship between %Human and the multivariate response variables (package: `factoextra` v1.0.7; Kassambara and Mundt, 2020). A second multivariate model was constructed with %Urban and %Agricultural as predictor variables, and the interaction between them, to identify the individual contributions of each land use type. Testing for the interaction as well as the individual contributions of each land use type allowed for the identification of synergistic or antagonistic effects between the two anthropogenic land use types. As in the first model, the univariate responses of each environmental variable were assessed. The function `best.r.sq()` from the `mvabund` package provided an  $R^2$  value for each predictor variable to indicate its importance in constraining variance within the model (Wang et al., 2012).

## 2.3 Results

### 2.3.1 Human land use in catchments

The catchment size varied greatly between sites, with a median of 26.9 km<sup>2</sup> (range of 2 – 3631 km<sup>2</sup>). Two sites (TBB and TRB) skewed this distribution, as they superseded the next largest catchments by over a factor of ten (Table 2.1). The human land use in each catchment ranged from minimal agricultural and urban contribution (POU and MAN: 0% and 5.5% human land use, respectively) to highly developed and utilised land (MAT and WEB: 82.5% and 81.3% human land use, respectively) (Table 2.1). Forestry was a large contributor to some of the more northern catchments (up to 77.3%), however, large parts of this area were classified as exotic forest (afforested exotic species, as opposed to harvested, bare area) and with that appeared similar to native forest cover (Table 2.1).

**Table 2.1** Site abbreviations, locations, and catchment area land use type. Human land use is defined as the sum of urban and agricultural land uses. Dominant land cover was assigned based on the land use contributions (Dominant land cover = highest single land use contribution).

| Site code | Estuary       | Lat.   | Long.  | Catchment area (km <sup>2</sup> ) | %land use cover in catchment |       |              |       | Dominant land cover |
|-----------|---------------|--------|--------|-----------------------------------|------------------------------|-------|--------------|-------|---------------------|
|           |               |        |        |                                   | Natural                      | Urban | Agricultural | Human |                     |
| HEI       | Whangaahei    | -36.61 | 175.47 | 10.3                              | 66.7                         | 0.1   | 29.6         | 29.7  | Native              |
| POU       | Whangapoua    | -36.73 | 175.61 | 2.0                               | 94.2                         | 0.0   | 0.0          | 0.0   | Native              |
| RAN       | Matarangi     | -36.74 | 175.65 | 20.4                              | 20.3                         | 0.7   | 15.2         | 15.9  | Forestry            |
| COR       | Coromandel    | -36.77 | 175.50 | 20.7                              | 70.1                         | 6.6   | 14.8         | 21.5  | Native              |
| TEK       | Te Kouma      | -36.81 | 175.49 | 11.3                              | 29.5                         | 0.5   | 16.8         | 17.3  | Forestry            |
| WHI       | Whitianga     | -36.84 | 175.68 | 91.6                              | 78.4                         | 0.4   | 11.9         | 12.3  | Native              |
| PUR       | Purangi       | -36.85 | 175.76 | 17.2                              | 15.1                         | 0.0   | 68.5         | 68.5  | Agriculture         |
| MAN       | Manaia        | -36.85 | 175.46 | 57.5                              | 87.8                         | 0.2   | 5.2          | 5.5   | Native              |
| WAI       | Waikawau      | -36.94 | 175.47 | 33.8                              | 84.1                         | 0.1   | 14.9         | 15.0  | Native              |
| TAI1      | Tairua 1      | -37.00 | 175.86 | 9.4                               | 74.5                         | 3.7   | 14.7         | 18.5  | Native              |
| TAI2      | Tairua 2      | -37.02 | 175.84 | 256.4                             | 68.0                         | 0.2   | 14.7         | 14.9  | Native              |
| PAU1      | Pauanui 1     | -37.03 | 175.85 | 254.8                             | 68.0                         | 0.2   | 14.7         | 14.9  | Native              |
| PAU2      | Pauanui 2     | -37.03 | 175.84 | 244.3                             | 68.2                         | 0.1   | 14.9         | 15.0  | Native              |
| OPU       | Opoutere      | -37.12 | 175.87 | 80.7                              | 41.9                         | 0.1   | 11.3         | 11.4  | Forestry            |
| TBB       | Thames 1      | -37.14 | 175.54 | 2121.9                            | 32.5                         | 1.3   | 55.1         | 56.3  | Agriculture         |
| WHA3      | Whangamata 3  | -37.18 | 175.86 | 11.2                              | 10.1                         | 0.0   | 7.3          | 7.3   | Forestry            |
| WHA2      | Whangamata 2  | -37.19 | 175.86 | 3.1                               | 8.2                          | 5.3   | 11.1         | 16.4  | Forestry            |
| WHA1      | Whangamata 1  | -37.20 | 175.86 | 27.0                              | 61.9                         | 4.3   | 13.9         | 18.2  | Native              |
| TRB       | Thames 2      | -37.21 | 175.44 | 3630.8                            | 21.0                         | 1.1   | 70.4         | 71.6  | Agriculture         |
| BOW       | Bowentown     | -37.44 | 175.96 | 26.9                              | 10.7                         | 1.0   | 45.2         | 46.3  | Agriculture         |
| ATH       | Athenree      | -37.47 | 175.94 | 5.6                               | 28.8                         | 0.0   | 49.3         | 49.3  | Agriculture         |
| TUA       | Tuapiro       | -37.50 | 175.95 | 59.9                              | 59.0                         | 0.1   | 37.9         | 38.0  | Native              |
| KAT       | Katikati      | -37.54 | 175.94 | 32.1                              | 23.0                         | 4.9   | 64.6         | 69.5  | Agriculture         |
| OMO       | Omokoroa      | -37.65 | 176.04 | 6.3                               | 3.0                          | 3.6   | 76.5         | 80.1  | Agriculture         |
| RAK       | Waikaraka     | -37.67 | 176.05 | 26.7                              | 23.0                         | 0.2   | 70.2         | 70.4  | Agriculture         |
| MAT       | Matua         | -37.67 | 176.13 | 8.2                               | 4.1                          | 61.8  | 20.7         | 82.5  | Urban               |
| REA       | Waikareao     | -37.70 | 176.15 | 73.8                              | 32.2                         | 14.0  | 43.7         | 57.7  | Agriculture         |
| TAH       | Tahuwhakatiki | -37.71 | 176.23 | 36.7                              | 41.1                         | 1.3   | 41.1         | 42.4  | Agriculture         |
| TYE       | Tye Park      | -37.72 | 176.20 | 67.8                              | 42.5                         | 1.4   | 40.4         | 41.9  | Native              |
| WEB       | Welcome Bay   | -37.72 | 176.18 | 14.8                              | 10.8                         | 14.6  | 66.6         | 81.3  | Agriculture         |

### 2.3.2 Relationships between environmental variables

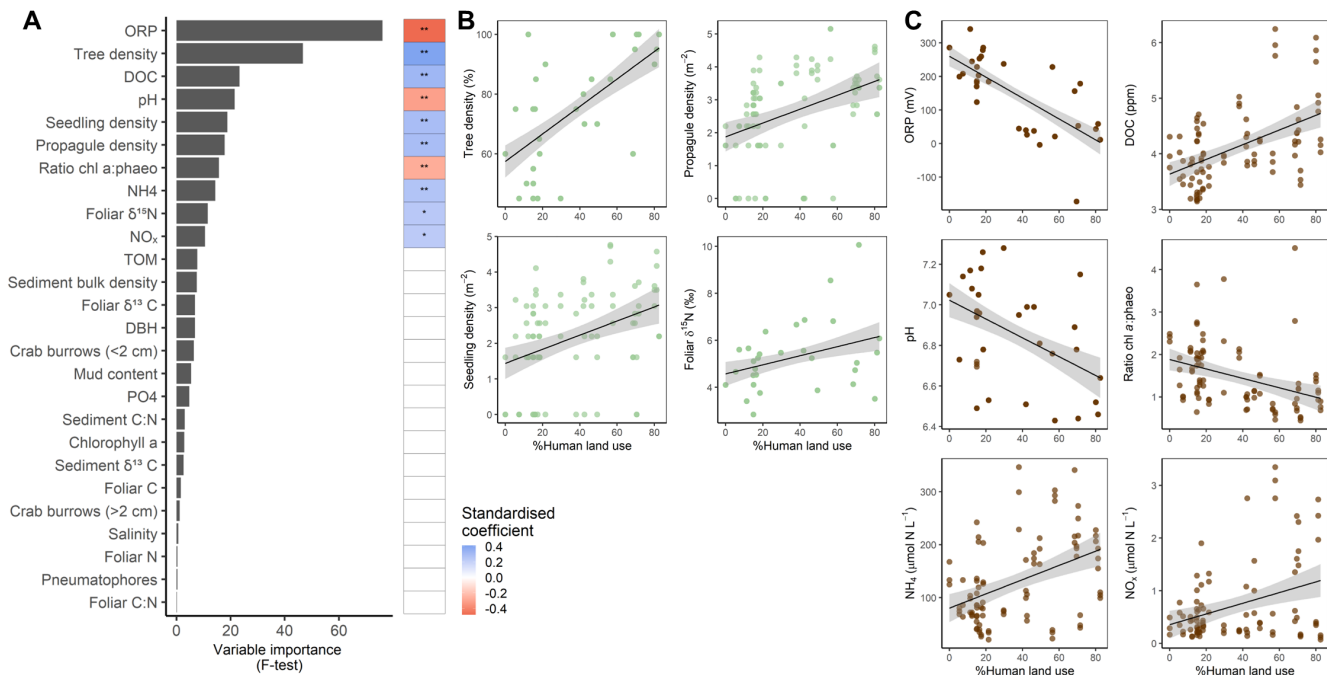
The mean values (and standard deviations, where available) of all environmental variables are listed in Supplementary Tables A2.1 and A2.2 (mangrove forest, and sedimentary variables, respectively). The TOM was a good indicator of sediment texture with strong positive correlations to water (Pearson's correlation  $r = 0.78$ ;  $p$ -value  $< 0.001$ ) and mud content ( $r = 0.67$ ;  $p$ -value  $< 0.001$ ) and a negative relationship with bulk density of the sediment ( $r = -0.73$ ;  $p$ -value  $< 0.001$ ) (Supplementary Figure A2.1). It was also a good proxy for the organic properties of the sediment with high correlations with sediment carbon and nitrogen content ( $r = 0.76$ ;  $p$ -value  $< 0.001$ , and  $r = 0.79$ ;  $p$ -value  $< 0.001$  respectively). The concentrations of porewater nutrient species were positively correlated with each other, the strongest relationship being between DOC and  $\text{NH}_4^+$  ( $r = 0.74$ ;  $p$ -value  $< 0.001$ ). Other chemical indicators of sediment condition such as pH and ORP were negatively correlated with the various nutrient variables (Supplementary Figure A2.1). Interestingly, there were no strong relationships between the porewater nutrient concentrations (DOC,  $\text{NO}_x$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ) and those in the sediment (sediment C, sediment N).

### 2.3.3 Land use effects on environmental variables

Multivariate regression revealed a significant effect of %Human in the catchments on the set of environmental variables in the mangrove forest (Table 2.2). Results from the univariate comparisons identified ten out of 26 environmental variables that were significantly correlated with %Human (Figure 2.2A, Supplementary Table A2.3) of which four were forest variables and the remaining six were sedimentary variables (Figure 2.2B, C). From the set of forest variables, tree density showed the strongest correlation to %Human (Figure 2.2A; Supplementary Table A2.3) which increased by a factor of 0.41 (standardised coefficient) with each percent of human land use. This relationship was mirrored by seedling (std. coef. = 0.29) and propagule densities (std. coef. = 0.29). Foliar  $\delta^{15}\text{N}$  values also increased significantly with %Human (std. coef. = 0.24). The linear relationships of these variables with %Human are displayed in figure 2.2B. ORP was the overall best-fitting response variable, with a strong negative correlation with %Human (std. coef. = -0.48). Similarly, the pH of the sediments decreased significantly (std. coef. = -0.31) with increasing %Human. The ratio of chl  $\alpha$  to phaeo (std. coef. = -0.27) was also negatively correlated with %Human. A positive relationship with %Human was observed in the porewater variables of DOC (std. coef. = 0.32),  $\text{NH}_4^+$  (std. coef. = 0.26;), and  $\text{NO}_x$  (std. coef. = 0.23) (Figure 2.2C). The relationships between the significant variables are visualised in the PCA plot (Figure 2.3). The %Human gradient separates the



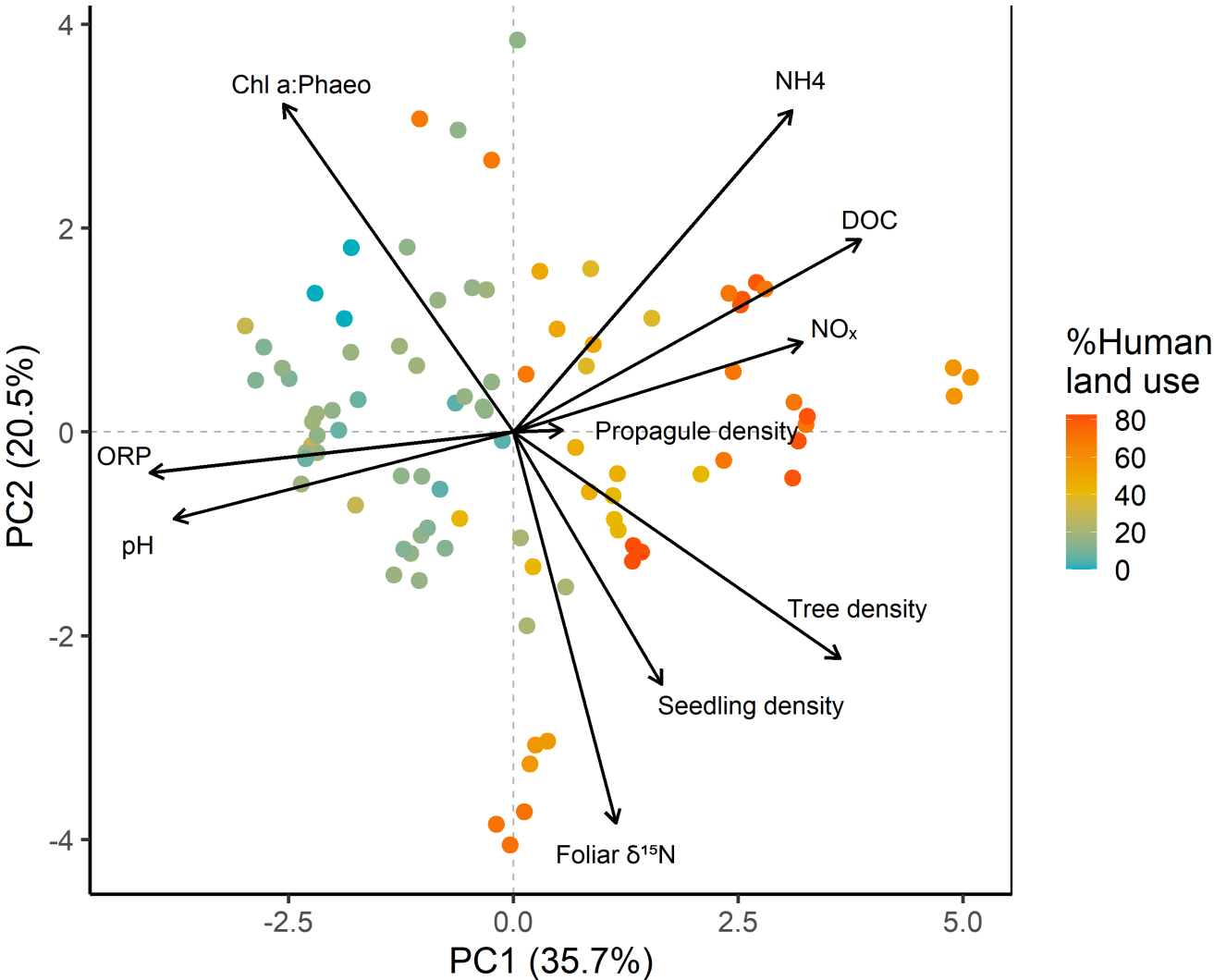
sites well, mainly along the first PCA axis. Generally, porewater variables (ORP, pH, and  $\text{NH}_4^+$ , DOC,  $\text{NO}_x$ ) influenced the distribution of data along the first axis (35.7% variance explained), while forest related variables (tree density, seedling density, and foliar  $\delta^{15}\text{N}$ ) separated the data along the second axis (20.5% variance explained). The ratio of chlorophyll  $\alpha$  to phaeophytin was negatively associated with forest density metrics.



**Figure 2.2** Responses of environmental variables in mangrove forests to human land use in the catchments. **A** Individual contributions of the environmental variables to the explained variance of model 1. The bar graphs show the importance (F-value) of each environmental variable for the model outcome. The heatmap shows the standardised coefficients of each variable and their direction of change along each factor (red = negative, blue = positive). Significant univariate relationships are indicated by asterisks (\* < 0.05; \*\* < 0.01). Univariate linear responses of the significant forest, **B** and sediment, **C** variables to %Human land use in the catchments. Correlation is indicated by a smoother function using a simple linear model formula “ $y \sim x$ ” and the shaded area represents the 95% confidence interval. Variables were ordered by decreasing strength of the standardised coefficient (i.e. effect size). ORP = oxygen reduction potential (mV); DOC = dissolved organic carbon (ppm);  $\text{NH}_4$  = ammonium concentration ( $\mu\text{mol N L}^{-1}$ );  $\text{NO}_x$  = nitrate/nitrite concentration ( $\mu\text{mol N L}^{-1}$ ); TOM = total organic matter (%); DBH = diameter at breast height (cm);  $\text{PO}_4$  = phosphate concentration ( $\mu\text{mol P L}^{-1}$ ).

The multivariate model on the contributions of different human land use factors revealed a significant interaction term (i.e., %Urban  $\times$  %Agricultural; Table 2.2). The combination of both land use types (%Urban  $\times$  %Agricultural) had an additive effect on DOC and  $\text{NO}_x$  concentrations in porewaters, while it acted antagonistically towards ORP, tree density, salinity, the ratio of chlorophyll  $\alpha$  to phaeophytin, and pH (Figure 2.4, Supplementary Table A2.4). The %Agricultural land use was the strongest individual contributor to the model fit ( $R^2 = 0.17$ )

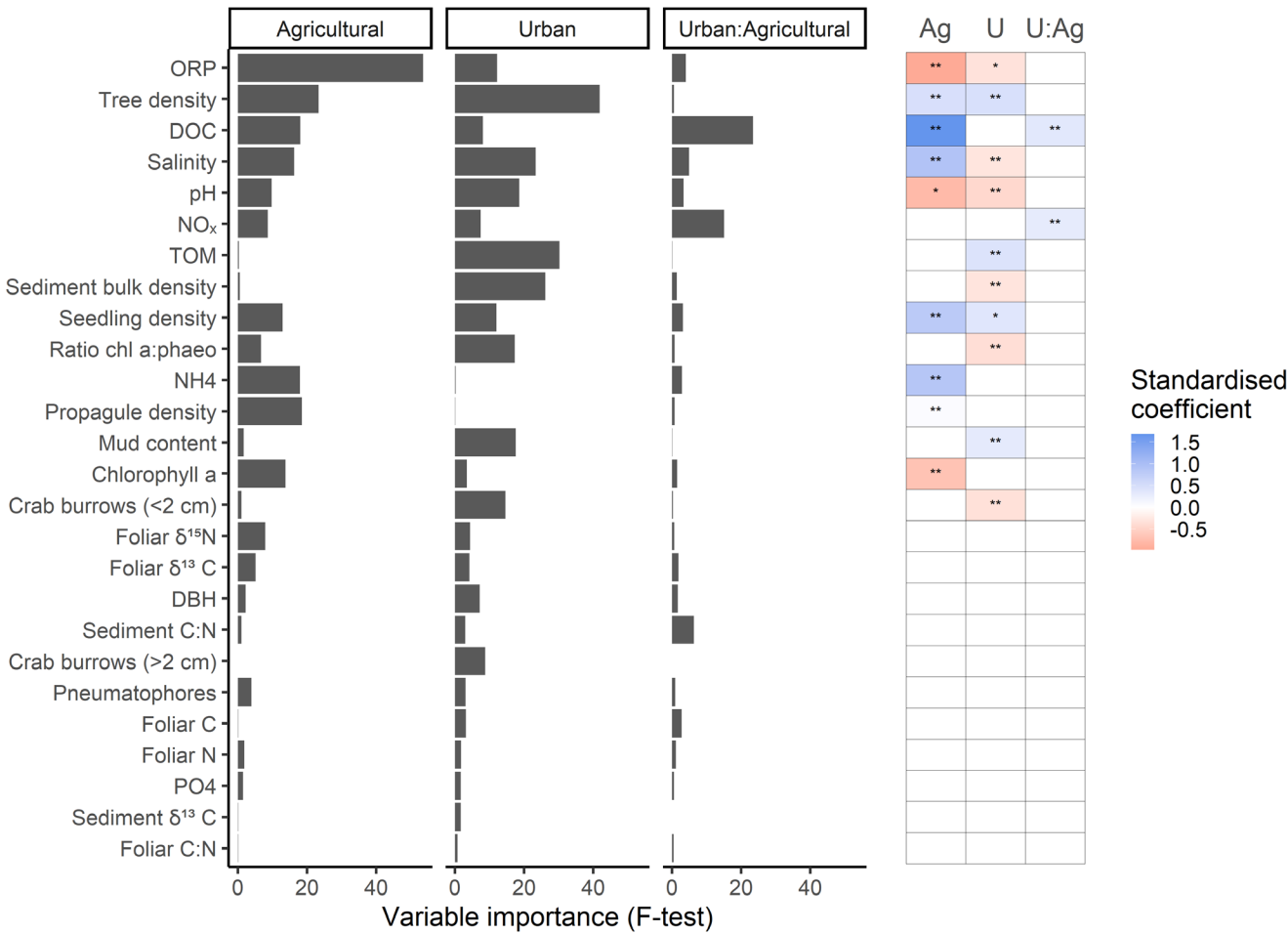
over %Urban ( $R^2 = 0.10$ ; Table 2.2). The variable ORP retained the strongest relationship but was more important in agriculturally dominated catchments, whereas tree density was more strongly related to urban land use in the catchment (Figure 2.4; Supplementary Table A2.4). Higher agricultural land use also promoted DOC,  $\text{NH}_4^+$ , and propagule density while being negatively related to chlorophyll  $\alpha$ . Urban land use was positively associated with TOM and mud content, and negatively associated with bulk density and small crab burrows (<2 cm).



**Figure 2.3** PCA plot on the significant environmental variables in mangrove forests, showing the distribution of sites across a human land use gradient in the catchments. The plot was constructed using only the variables that returned significant from the multivariate analysis (Figure 2.2). The arrows indicate the effect that each of these variables has on the distribution of the sites. Chl  $\alpha$ : Phaeo = ratio chlorophyll  $\alpha$ : phaeophytin; ORP = oxygen reduction potential (mV);  $\text{NH}_4$  = ammonium concentration ( $\mu\text{mol N L}^{-1}$ ); DOC = dissolved organic carbon (ppm);  $\text{NO}_x$  = nitrate/nitrite concentration ( $\mu\text{mol N L}^{-1}$ ).

**Table 2.2** Results from the multivariate regression analysis. Model 1 was constructed using %Human land use as explanatory variable. Model 2 was constructed using %Urban and %Agricultural land use as explanatory variables with an interaction between them. %Human land use was defined as the sum of %Urban and %Agricultural land use.

| Model   | Factor               | F-test | p-value |
|---------|----------------------|--------|---------|
| Model 1 | Human                | 217.10 | 0.002   |
|         |                      |        |         |
| Model 2 | Agricultural         | 181.89 | 0.002   |
|         | Urban                | 179.54 | 0.002   |
|         | Urban × Agricultural | 58.59  | 0.002   |

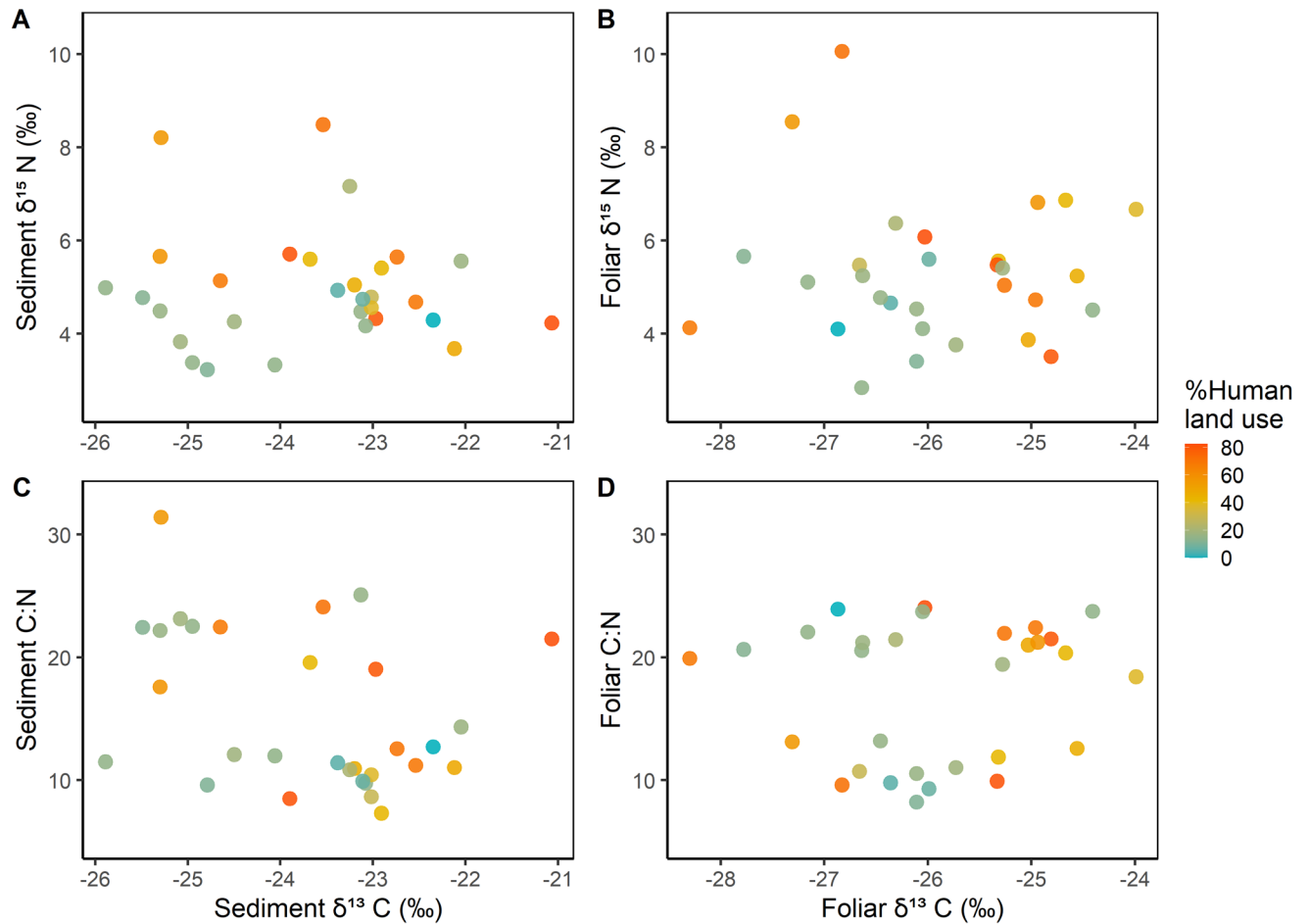


**Figure 2.4** The univariate results showing the individual contributions of the environmental variables to the explained variance of the model, separated into human land use categories (Agricultural (Ag) and Urban (U)). The bar graphs show the importance (F-value) of each environmental variable for the model outcome. The heatmap shows the standardised coefficients of each variable and their direction of change along each factor (red = negative, blue = positive). Significant univariate relationships are indicated by asterisks (\* < 0.05; \*\* < 0.01). ORP = oxygen reduction potential (mV); DOC = dissolved organic carbon (ppm); Ratio chl  $\alpha$ : phaeo = ratio chlorophyll  $\alpha$ : phaeophytin; NH<sub>4</sub> = ammonium concentration ( $\mu\text{mol N L}^{-1}$ ); NO<sub>x</sub> = nitrate/nitrite concentration ( $\mu\text{mol N L}^{-1}$ ); TOM = total organic matter (%); DBH = diameter at breast height (cm); PO<sub>4</sub> = phosphate concentration ( $\mu\text{mol P L}^{-1}$ ).

### 2.3.4 Isotopic composition of sediment and plant material

Values of  $\delta^{13}\text{C}$  in the sediments ranged from -25.9 to -21.1 ‰ and showed no clear relationship with %Human ( $F_{1,88} = 2.621$ ; p-value = 0.578) (Figure 2.5). Foliar  $\delta^{13}\text{C}$  values were lower, ranging between -28.3 and -24.0 ‰ (Figure 2.5) and as in the sediments, %Human had no significant influence on the  $\delta^{13}\text{C}$  value in the leaves. Sediment and foliar  $\delta^{15}\text{N}$  values ranged between 3.2 - 8.5 ‰, and 2.8 – 10.1 ‰, respectively, and were strongly correlated with each other (Pearson's  $r$  correlation = 0.85; Supplementary Figure A2.1). Values of  $\delta^{15}\text{N}$  increased significantly with %Human (Figure 2.2). Overall, higher human land use in the catchments influenced the nitrogen isotope values in both the sediments and the leaves, while the variation in  $\delta^{13}\text{C}$  values was too high to establish significant trends (Figure 2.5A, B). The C:N mass ratio of the sediment samples had a wide range of values between 7.3 and 32.4 but showed no significant correlation with %Human ( $F_{1,88} = 3.06$ ; p-value = 0.56). There was a clear separation between sites with high C:N mass ratios (17 – 31) and sites with low values (5 – 15), however, this split could not be explained by any of the variables measured in this study (Figure 2.5C, D). In the foliar samples, the C:N mass ratios (8.2 – 24.1) exhibited a similar phenomenon with a clear split between high and low values at different sites. However, the sites with high foliar C:N ratio did not necessarily have high sediment C:N ratio, decoupling the sedimentary stoichiometry from that of the plants.





**Figure 2.5** Bi-plots of stable isotope values and carbon to nitrogen mass ratios of sediments (A and C) and mangrove leaves (B and D) coloured by %Human land use. Sediment C:N = sediment carbon to nitrogen ratio; Foliar C:N = foliar carbon to nitrogen ratio.

## 2.4 Discussion

The results from this study show that the amount of human land use in the catchments has a direct effect in estuarine mangrove forests on several forest architectural and sedimentary variables. Porewater chemistry was more affected than sediment-associated variables, while tree density and reproduction were the most responsive forest variables. The fact that both plant- and sediment-associated variables responded to the land use gradient in the catchments supports the need for a holistic view on the effects of land-derived stressors on coastal vegetated habitats. The novelty of this study is the scale at which it was conducted, enabling us to make more generalised assumptions about patterns of mangrove forest ecology and biogeochemistry in relation to human pressures, that transcend site-specific responses. This provides essential information for ecosystem models of human pressures as well as for coastal management applications and successful restoration efforts. The two major themes that

can be identified as showing responses to human land use in the catchments were eutrophication and forest expansion and reproduction. The role of specific variables in response to human stressors are discussed below.

### **2.4.1 Evidence of eutrophication from human land use**

There was a clear trend of increased eutrophication in the mangrove sediments with increasing human land use in the catchments, which was characterised by changes in the ORP, pH, and nutrient concentrations of porewaters. Nutrient enrichment is one of the most obvious and expected effects of human land use in catchments which nevertheless, can be hard to capture at this scale (Alongi and McKinnon, 2005). Global estimates attribute about half of the riverine nitrogen input into coastal areas to anthropogenic sources (Galloway et al., 2003; Seitzinger et al., 2005; Howarth, 2008) and several studies have shown a positive relationship between human land use and nutrient concentrations in mangrove sediments (Reading et al., 2020; Passos et al., 2022; Sharma et al., 2022; Conrad et al., 2023). The significant indicators of porewater eutrophication from this study were DOC,  $\text{NH}_4^+$ , and  $\text{NO}_x^-$ , but all measured nutrients were generally positively correlated, indicating an overall enrichment of the system, rather than increased contributions of specific nutrient species (Supplementary Figure A2.1). One interpretation of this may be that the nutrient concentrations had not yet reached a level at which the capacity of the system to assimilate and recycle them was saturated. Therefore, nutrient uptake by plants and conversions between nutrient species in the sediments were still taking place. However, there is little to no information on saturation points of mangrove sediments to nutrient concentrations in the current literature. The higher  $\delta^{15}\text{N}$  values of sediments and leaves associated with human land use confirmed the higher contribution of anthropogenic nitrogen into the mangrove system, as increased  $\delta^{15}\text{N}$  values have been reported as one of the most consistent indicators of anthropogenic impacts on coastal systems (Gritcan et al., 2016; Branoff, 2017; Tanu et al., 2020; Barroso et al., 2022; Conrad et al., 2023). High nitrogen inputs, excessive fertiliser use, anthropogenic deposition, and sewage effluents can all contribute to higher values of  $\delta^{15}\text{N}$  in plant tissues (Komor and Anderson Jr., 1993; Harrington et al., 1998; Fry et al., 2003), while the acquisition of nitrogen in a natural and undisturbed systems generally results in lower values (Handley and Scrimgeour, 1997; Costanzo et al., 2001). The positive trend between %Human and  $\delta^{15}\text{N}$  values was strongly driven by two sites, which drain an extensive area of farmland, and which are the only sites with foliar and sedimentary  $\delta^{15}\text{N}$  values  $> 8\text{‰}$  (Supplementary Table A2.1 and A2.2). Overall, almost all sites in our study had higher than expected  $\delta^{15}\text{N}$  values for pristine

mangrove forests and tree leaves (natural range: -5 to 3 ‰) (Peterson and Fry, 1987; Fry et al., 2000; Costanzo et al., 2001). Instead, values fell in the range of medium to highly impacted mangrove forests in New Zealand (reported range: 5 – 10 ‰) (Gritcan et al., 2016). High collinearity between  $\delta^{15}\text{N}$  values of sediments and mangrove leaves ( $R^2 = 0.85$ ) indicated strong connectivity between the two components of the ecosystem, suggesting land-derived eutrophication to be the main vector of nutrient input into the system.

Values of ORP and pH were strongly negatively correlated with human land use and therefore, also with nutrient concentrations. A reduction in ORP may be associated with a nutrient-induced boost of microbial activity in the sediments leading to a subsequent depletion of oxygen in all but the uppermost layer of the sediment (Rabalais et al., 2010). Such a shift in ORP could have a strong effect on the nutrient cycling capacity of the sediments. Nitrification, for example, is an aerobic process that may be inhibited in strongly reducing conditions, leading to an accumulation and efflux of  $\text{NH}_4^+$  into the overlying waterbody, exacerbating the present eutrophic conditions (Rabalais et al., 2010). A negative correlation was observed between ORP and  $\text{NH}_4^+$  concentrations ( $R^2 = 0.53$ ) across all sites. However, it is not possible to say if this relationship was due to an increase in  $\text{NH}_4^+$  efflux or a legacy of the enrichment due to human land use. Simultaneously, low ORP may slow down organic matter degradation since a shift from aerobic to anaerobic metabolism yields less energy (Twilley et al., 2019). While a retardation of the rate of organic matter breakdown through oxygen limitation and acidification could increase carbon storage potential (Zhang et al., 2020), it may simultaneously adversely affect other processes that contribute to sequestration such as tree productivity, microbial biomass, and microbial diversity (Malik et al., 2018; Twilley et al., 2019). The decreased pH, hence, acidification of the sediments exposed to increased human pressures may be due to an increase of the benthic metabolism or the high concentrations of nitrogen in the porewaters (Fauzi et al., 2014). Low pH in the sediments potentially decreased the availability of important cations (e.g.,  $\text{Ca}^+$ ,  $\text{Mg}^+$ , and  $\text{Na}^+$ ) which have been shown to be important factors regulating tree growth and resilience to stress in terrestrial systems (Shortle and Smith, 1988; McLaughlin et al., 1989). This may be enhanced by the waterlogged state of mangrove sediments (Slessarev et al., 2016; Shi et al., 2017). Simultaneously, oxygenation of the sediments around mangrove roots to combat, for example, sulphide toxicity would further enhance acidification of the sediments (Reef et al., 2010). Despite the negative relationship between human land use and pH, the lowest values of pH did not fall below 6.4, which may be due to the high buffering capacity of seawater and coastal sediments (De Wit et al., 2001; Cyronak et al., 2018; Drylie et al., 2019).

### 2.4.2 Human land use did not lead to changes in carbon sources

Values of  $\delta^{13}\text{C}$  in the sediments were higher than those in the mangrove leaves, albeit with some overlap of values between -24 to -26 ‰ (Figure 2.5A, B). This is to be expected, as the mangrove sediments comprise a mixture of organic carbon with a high amount of microphytobenthic and allochthonous marine contributions, which generally have higher values of  $\delta^{13}\text{C}$  (Lamb et al., 2006). Sediment  $\delta^{13}\text{C}$  values have been used as indicators of different carbon inputs into systems, with microbial (microphytobenthic), macro-algae, and seagrasses having higher  $\delta^{13}\text{C}$  values than mangrove tree and terrestrial carbon inputs (Lamb et al., 2006). The high variation in  $\delta^{13}\text{C}$  values of mangrove leaves could not be explained by any of the environmental variables in this study and highlights the need for further research into carbon fractionation in mangrove plants (Kelleway et al., 2018; Velázquez-Ochoa et al., 2022). There was no significant relationship between %Human and  $\delta^{13}\text{C}$  values, however the high variability in  $\delta^{13}\text{C}$  values throughout the sampling sites suggested that the underlying drivers were not captured in this study. The geomorphic environment has been shown to play an important role in shaping the carbon dynamics in mangrove forests through differences in sediment type and mode of delivery (Rovai et al., 2018; Breithaupt and Steinmuller, 2022). Investigating this relationship may yield some answers to the variance displayed by the  $\delta^{13}\text{C}$  values in this study. Our results tentatively indicate that there may be a higher contribution of marine-derived carbon into the mangrove stands closer to urban centres. However, it is not possible to tell from this study if this is directly connected to anthropogenic stressors, since it could be due to higher amounts of seagrass and macroalgal detritus being washed into the forests. The observed variation in the foliar and the sedimentary C:N mass ratios cannot be explained by any of the environmental variables recorded in this study. The age of the tree/leaf as well as the position where it was sampled from can have an influence on the C:N mass ratio of the sample (Rundgren et al., 2003; Niinemets et al., 2007; Chai et al., 2015). The sediments in mangrove forests are spatially heterogeneous with pockets of fresh and decaying allochthonous and autochthonous material which all contain varying mass ratios of C:N (Adame et al., 2010; Song et al., 2023). These results suggest a higher resolution sampling approach to understand the relationships between litter quality and environmental influences as well as the sedimentary composition and quality in mangrove forests.

### 2.4.3 Human influence drives factors of forest expansion through growth and recruitment

The strong positive correlation between human land use and tree density was likely due to the increased delivery of nutrients from the more impacted catchments. Mangrove forests



are generally nutrient-limited (Reef et al., 2010) and so it is unsurprising to see more growth in forests where this limitation has been lifted. Tree height and DBH did not vary significantly along the land use gradient. However, multiple studies have shown that the number of shoots increased with nutrient delivery, suggesting that the expansion is lateral instead of vertical (Feller, 1995; Lovelock et al., 2006; Alongi, 2011). Simultaneously, factors such as wind exposure or the temperate climate may limit the upwards growth of the trees (Morrissey et al., 2010; Knapp and Hadley, 2012). Even though the expansion of mangrove forest area has not been recorded in this study, numerous spatial analyses from the area have identified high rates of mangrove expansion in estuaries close to urban centres and in places with high agricultural land use (Feller et al., 2010; Yang et al., 2013; Swales et al., 2016; Suyadi et al., 2019). High rates of sediment delivery have been shown to be the driving factor behind this expansion, through the provision of inhabitable space by sediment accretion at the seaward fringe (Kirwan and Megonigal, 2013; Swales et al., 2019). However, another New Zealand study suggests an additive effect of sedimentation and nutrient concentrations, further accelerating the growth of new forest areas (Lovelock et al., 2007). Propagule and seedling densities in this study were also positively correlated with human land use. Curiously, these densities were not strongly correlated with tree density ( $R^2 = 0.08$  and  $0.36$ , respectively), suggesting other potential mechanisms influencing recruitment success, such as periods of calm conditions for root development (Balke et al., 2011). Nutrient concentrations have been shown to both positively and negatively affect the establishment of propagules and the growth of seedlings of various mangrove species (Duarte et al., 1998; Almahasheer et al., 2016).

#### **2.4.4 How does anthropogenic impact affect ecosystem function and services in mangrove forests?**

Mangrove forests are likely one of the ecosystems best adapted to take on anthropogenic stressors such as urban run-off and agricultural effluents, since the trees respond with growth to nutrient inputs, and sediment accumulation rates lead to burial of heavy metals and other contaminants (Lewis et al., 2011; Bayen, 2012; Maiti and Chowdhury, 2013). This ability to absorb considerable amounts of nutrients from the environment has been suggested as a service, ameliorating conditions for more vulnerable adjacent ecosystems such as seagrass beds and mussel reefs (Tam and Wong, 1995; Yang et al., 2008; Jitthaisong et al., 2012). However, it is important to consider the “unseen” effects of these stressors on the health of the mangrove ecosystem and more research is needed to understand the fine interplay of

processes and possible feedback loops in response to human impacts.

Anthropogenically induced nutrient enrichment, such as demonstrated here, may favour the growth of above ground biomass over roots to maximise carbon acquisition (Reef et al. 2010). This increased allocation of energy into leaves and shoots combined with higher litter quality through reduced nutrient resorption (Feller et al., 1999; reviewed in Reef et al., 2010) has the potential to change the carbon dynamics of the system through accelerated decomposition, leading to reduced rates of carbon sequestration (Keuskamp et al., 2015). The reduced root growth has been hypothesised to have detrimental effects on tree resilience to extreme weather events such as droughts and storms (Lovelock et al., 2009, 2013). Furthermore, a reduction in root growth may decrease oxygen transported into the sediments, leading to exacerbated anoxia and potential sulphide toxicity, which in turn may have adverse effects on sediment biodiversity and ecosystem health (Lamers et al., 2013).

Elevated nutrients may increase the survival of propagules and facilitate the faster establishment of seedlings (Lovelock et al., 2007; Gillis et al., 2019). This mechanism, especially when coupled with sediment delivery through erosion, could lead to an expansion of mangrove area and increase ecosystem services associated with this ecosystem. However, the expansion of mangrove area in estuaries can lead to the loss of other valuable habitats. For example, studies have shown that experimental nutrient enrichment increased the competitive advantage of mangrove seedlings (*A. germinans*) over salt marsh plants (Simpson et al., 2013; Dangremond et al., 2020). And while not an issue in New Zealand, high nutrient availability has been shown to favour invasive mangrove species with a lower ecosystem service capacity (Huang et al., 2023).

Macrobenthic communities have been shown to be fairly resistant to sewage inputs and urban development upstream (Cannicci et al., 2009; Fusi et al., 2016). Crab biomass increased with urban proximity in east Africa, which was related to the amount of organic matter delivery at that site providing more easily accessible food sources (Cannicci et al., 2009). However, in a following study, the authors observed behavioural changes in the animals exposed to increased sewage and organic matter availability, that affected the turnover rate of organic material as well as the bioturbation activity (Bartolini et al., 2009, 2011). While our study shows that human land use in the catchments can trigger changes in macrobenthic communities (reduced number of small crab burrows with increasing %Urban), deeper insights into community dynamics in response to human impacts were beyond the scope of this work.

Shifts in the chemical composition of the sediments (such as nutrient enrichment and chang-

es in ORP and pH, as demonstrated) will have deterministic effects on the microbial community composition (Bahram et al., 2018). However, the effects of eutrophication on microbial communities are difficult to distinguish and depend on the setting in which they were tested. Some studies have found an increase in microbial biomass, diversity, and/or microbial respiration after experimental nutrient enrichment (Bulsecu et al., 2020; Koceja et al., 2021), while another study reports no changes in the microbial community composition of salt marsh sediments after significant long-term enrichment (Bowen et al., 2011). Finally, a meta-analysis of nutrient effects on microbial biomass found an overall decline in biomass across 82 field studies (Treseder, 2008). These contrasting results highlight the need for more research efforts to understand the effects of eutrophication on the microbial community composition and diversity in coastal vegetated ecosystems. Soil pH has been shown to strongly determine the diversity of microorganisms in terrestrial soils, where lower pH was negatively correlated with diversity and richness of microbial communities (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). Furthermore, environmental, and anthropogenic stress has been associated with decreases in microbial diversity and network stability (Hernandez et al., 2021; Yang et al., 2022). The diversity of microbial communities in sediments is directly linked to trait diversity and functional redundancy and given the key role that microbes play in nutrient cycling and the health of the ecosystem; a loss of microbial diversity may significantly weaken the resilience of temperate mangrove forests (Wagg et al., 2014, 2019; Delgado-Baquerizo et al., 2015).

#### **2.4.5 Implications for management of temperate mangrove forests**

The observed mechanisms linking mangrove forest expansion with catchment land use in this study, highlight the need for targeted management actions. The encroachment of mangroves into adjacent ecosystems, both landward and seaward, poses challenges, with potential to diminish the services provided by adjacent habitats (Ellis et al., 2004). While mangroves contribute to carbon storage and provide other valuable services, this study prompts a reflection on the associated costs of these services and emphasises the importance of maintaining a diversity of healthy ecosystems, rather than a singular dominance of mangroves in estuaries. For example, the encroachment of mangroves into salt marsh habitats may cause declines in habitat availability for migratory birds (Kelleway et al., 2017), and loss of adjacent sandflats will have implications for provisional services such as fisheries and cultural practices (Ellis et al., 2004; Guy et al., 2021).

Mangroves are native to northern New Zealand but rapid expansion of their extent within estuaries has prompted the intentional removal of mangroves in an attempt to manage and restore estuaries for ecological and recreational purposes (Lundquist et al., 2014; Horstmann et al., 2018; Le Heron et al., 2022). These efforts are costly and have had only limited success, often not returning the envisioned pristine conditions and sometimes resulting in a further deterioration of the ecological state of the estuary (Dencer-Brown et al., 2018; Stokes et al., 2023). Several studies have attributed the mangrove expansion to increased sediment delivery into estuaries, due to urbanisation and forest clearance in adjacent catchments (Horstmann et al., 2018; Suyadi et al., 2019). Our study discusses that nutrient delivery from catchment land use may aid this process. These findings clearly show that a catchment-scale management strategy is needed to address the issue of excessive mangrove growth in estuaries of New Zealand.

Furthermore, the study reveals the current stressed state of the systems due to high nutrient levels and changes in sediment chemistry, emphasising the urgency of addressing these issues. With catchment use intensifying and natural buffers diminishing upstream, mangroves emerge as the “last and only line of defence” against coastal degradation. A possible management strategy would involve retaining a diversity of habitats to reduce human impact, including the rewilding of rivers, protection of peatlands and other freshwater wetlands, and safeguarding the saltmarsh–mangrove–seagrass ecotone. In essence, this study advocates for an integrated approach to catchment management that considers the broader ecological context (Gladstone-Gallagher et al., 2022) to preserve the resilience and functionality of temperate mangrove forests in New Zealand.

## 2.5 Conclusion

This study underscores the critical implications of catchment land use on environmental variables in temperate mangrove forests in New Zealand. Several environmental variables were identified that responded to the coarse predictor of human land use in the catchment, indicating effects of eutrophication, acidification, and mangrove forest expansion. This research supports the evidence that human land use in the catchments increases the availability of nutrients in estuarine ecosystems on a landscape-scale and suggests that these changes affect other chemical processes in the sediments. Furthermore, the reported effects of human land use were detected across a wide range of estuarine settings, overcoming local variation indicating that they may be good indicators of anthropogenic stressors on temperate man-

grove forests. And while New Zealand's estuaries may still be relatively healthy compared to more densely populated global regions, it is important to note that the land use in the catchment definitively impacts coastal systems and brings about changes in the ecology and biogeochemistry, which may have consequences on the long-term health of these systems. Slight changes in critical environmental parameters can change ecological baselines such as microbial communities, which may have cascading effects on ecosystem health and functioning. This in turn may lead to altered benefits from ecosystem services to humans. The dual response of forest and sedimentary variables shows that mangrove forests and other estuarine habitats need holistic integrated management approaches that consider the interconnectedness of species and their environmental factors.



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**Poised on the interface** between the land and the sea, mangroves have adapted to a life in the extremes. Salt glands and a waxy coating on the leaves regulate salt levels and minimize water loss, while pneumatophores provide efficient gas exchange. Viviparous propagules give seedlings an early advantage in the challenging intertidal environment. Collectively, these adaptations enhance the resilience of mangroves in harsh coastal conditions.





## 3 | Eutrophication enhances labile organic matter decomposition in mangrove ecosystems

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### 3.1 Introduction

Mangrove forests are among the most efficient ecosystems at sequestering carbon (Donato et al., 2011) due to a combination of high productivity, high sediment retention, and low decomposition rates leading to rapid carbon accumulation and large carbon stocks (Reef et al., 2010; Mcleod et al., 2011; Trevathan-Tackett et al., 2017; Alongi, 2020). Decomposition rates are low due to the high sediment water content and small particle size which leads to anoxic conditions just below the sediment surface inhibiting the faster oxygenic decomposition processes and leading to sulphate-reduction and fermentation as the main pathways of carbon decomposition (Alongi, 1998; Kristensen et al., 2008). Decomposition rates are also influenced by organic matter quality which can be variable in mangrove forests ranging from highly labile compounds associated with microphytobenthic (MPB) production to refractory leaf litter and wood production (Kristensen et al., 2008; Arndt et al., 2013; Friesen et al., 2018). The capacity of a system to sequester carbon relies on the balance between carbon inputs and losses and is therefore intrinsically linked to the rate at which sedimentary/soil organic carbon (SOC) degrades (Davidson and Janssens, 2006). However, understanding the environmental factors influencing decomposition is an active field of research and empirical data is variable, indicating a high complexity of potential feedbacks and interactions of global and local factors (Friedlingstein et al., 2006; Schmidt et al., 2011; Viscarra Rossel et al., 2019).

It is now commonly understood, that a multitude of factors such as climate, organic matter quality, and other environmental variables control SOC degradation and that even labile carbon can be protected from decomposition through physical protection, stabilisation, resource limitation, and/or inhibition (Schmidt et al., 2011; Doetterl et al., 2015; Hemingway et al., 2019). Temperature has been determined as major driver of SOC decomposition on a global scale, as higher temperatures generally increase the metabolic rate of organisms until a certain point (Davidson and Janssens, 2006; Allison et al., 2010; Trevathan-Tackett et al., 2021). It is therefore unsurprising that high seasonal variability of organic matter degradation

has been observed, particularly in temperate environments (reviewed in Arndt et al., 2013). Several studies have also highlighted the importance of leaf litter stoichiometry (litter quality), and available nitrogen on degradation rates, with low C:N ratios being more beneficial for degradation (Parton et al., 2007; reviewed by Kristensen et al., 2008; Nordhaus et al., 2017; Djukic et al., 2018; Luo et al., 2020). Sun et al. (2019) found high rates of aromatic compounds and complex aggregate structure to provide protection to SOC in mangrove sediments, while others observed that the shore elevation affected decomposition of mangrove litter, probably due to variations in leaching rates and microbial dispersal (Middleton and Mckee, 2001; Thomson et al., 2022b). The results from these studies suggest that the factors controlling carbon decomposition in mangrove forests are temporally and spatially variable. This variability combined with the dynamic nature of different coastal morphologies, makes it extremely difficult to accurately predict biological processes on a large scale and how they may be impacted by environmental change (Feller et al., 2010; Arndt et al., 2013; Rovai et al., 2018; Twilley et al., 2019).

Coastal zones are particularly exposed to land-derived stressors that are globally exacerbated by anthropogenic activities (Downing et al., 1999; Doney, 2010; Macreadie et al., 2012). With human land use in catchments ever increasing, stressors such as nutrient enrichment and sedimentation are becoming more pronounced with sometimes devastating effects on coastal vegetated habitats (Cloern, 2001; Duke et al., 2007; Pendleton et al., 2012; Steffen et al., 2015). While the effects of nutrient enrichment on the trees is well studied (see Feller et al., 2009 and refs. within), it is unclear how the increased nutrient availability will impact the persistence of organic material in mangrove sediments. Several studies have suggested an increase in leaf-over-root production, lower C/N ratio of plant tissues, and reduced nutrient retention rates from senescent leaves (Mckee, 1995; Naidoo, 2009; Reef et al., 2010; Gritcan et al., 2016). These factors could likely contribute to an accelerated SOC degradation in impacted sediments. High rates of sedimentation, however, may restrict oxygen penetration into the sediments, or reduce the production of MPB, reducing the pool of available labile organic material. These effects may lead to a reduction in degradation rate, possibly enhancing the carbon storage potential of these systems.

Several studies have assessed the effects of individual stressors such as temperature, nutrient availability, or grain size on the decomposition rate of organic matter in mangrove forests (Gladstone-Gallagher et al., 2014; Mueller et al., 2018; Jessen et al., 2021). However, these studies have generally focussed on interspecific differences or single stressors to compare degradation rates, thereby neglecting the complexity of interacting factors that may regulate

this process (Middleton and McKee, 2001; Nordhaus et al., 2017). On the other hand, studies that focus on global drivers of change in decomposition rates can be biased by large scale patterns that understate the importance of local conditions (Djukic et al., 2018; Mueller et al., 2018). And lastly, very few studies attempt to identify a direct link between anthropogenic stressors and SOC degradation in mangrove forests (Spivak et al., 2019; Santos-Andrade et al., 2021). To address these shortcomings, we applied a field-based approach along a broad land use gradient that was confined to a narrow latitudinal range to constrain climatic variation and that spanned thirty monospecific *Avicennia marina* subsp. *australasica* forests. This design enables us to identify regional environmental drivers of organic matter degradation and simultaneously assess the effects of anthropogenic activities at landscape-scale on this important process.

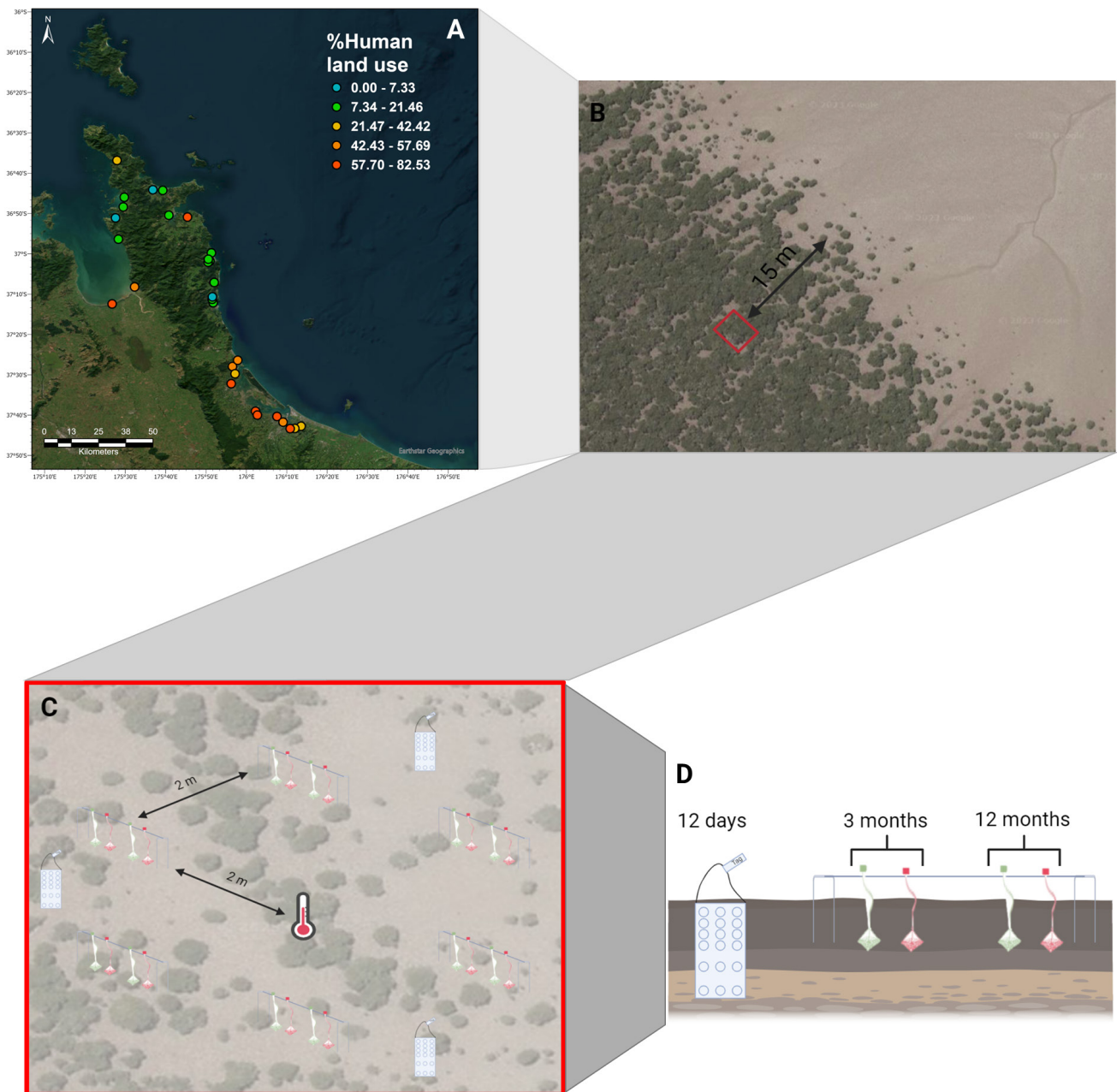
This study was designed to identify environmental controls of organic matter decomposition in temperate mangrove forests across a gradient of human catchment land use intensity. Previously we showed that catchment land use influenced mangrove canopy density and sediment nutrient concentrations and chemistry (Chapter 2). The goal here was to determine how these changes affected SOC degradation rates. We used two standardised assays to assess the degradation of organic material of differing qualities in mangrove sediments over time: (1) the tea bag index (TBI; Keuskamp et al., 2013), and (2) the rapid organic matter assay (ROMA; O'Meara et al., 2017). Although results from these methods need to be interpreted with some reservations about how accurately they reflect the decomposition of local litter as well as the formation of SOC, ultimately leading to carbon sequestration, they are useful tools to compare degradation rates across a wide range of sites and settings (Prescott, 2010; Cotrufo et al., 2013; Joly et al., 2023). Based on the literature presented above, we hypothesize here, that nutrient concentrations will have a strong impact on organic matter decomposition and that this impact will be reflected along the gradient of land use intensity.

## 3.2 Methods

### 3.2.1 Study design

This study was initiated in the austral summer of 2022 in estuaries along the east coast of the North Island of Aotearoa New Zealand (Figure 3.1A). Sites were selected along a land use gradient that ranged from 0 to >80% combined agricultural and urban development in the catchments. To minimize climatic variability along this gradient, sampling sites were located in a narrow latitudinal window spanning only 140 km from north to south. A detailed description of sites and sampling procedures is given in Chapter 2 and only a brief overview is provided

here. Each study site was located 15 m into the interior of the forest perpendicular to the seaward fringe line (Figure 3.1B). At each site, one temperature logger and 12 Lipton green and rooibos (red) tea bags were buried approximately 5 cm below the sediment surface, in January 2022 (Figure 3.1C).



**Figure 3.1** Study design showing: **A** the distribution of the sites along the east coast of New Zealand along a land use gradient (**A**, adapted from Chapter 2) and **B** the location of each sit relative to the fringe-line in the interior of the forest. At each site a temperature logger and six “sets” of four tea bags that were arranged around the logger, about 2 m apart and three ROMA plates **C**. Tea bags were deployed in January 2022, half retrieved after 3 months and the remainder after 12 months. ROMA plates were deployed for 12 days to co-incide with the first tea bags sampling **D**.



Temperature in the sediment at each site was continuously logged (sampling frequency 0.5 h, sampling resolution 0.1 °C; EnvLogger T2.4, electricblue, Portugal) for the duration of an initial 3-month incubation (January-April 2022). Four tea bags (2 green and 2 red) were grouped together in a set (replicate) and buried about 0.2 m apart. Spacing between the 6 sets was about 2 m (Figure 3.1C). In early April 2022 (autumn) three ROMA plates were buried at each site. Six of each colour tea bags (one from each set), the ROMA plates, and temperature loggers were retrieved during the first sampling period in mid-April 2022, and the remaining six tea bags of each colour were retrieved in April 2023. The tea bags had been incubated for a period of 92 days and 372 days, and the ROMA plates for 12 days (Figure 3.1D). Temperature data was downloaded from the loggers immediately after retrieval and were not redeployed.

### 3.2.2 Decomposition assays

#### 3.2.2.1 ROMA plates

ROMA plates were prepared as described in O'Meara et al. (2017). Briefly, these are acrylic plates (180 x 90 mm) with 18 wells of 0.9 mL volume each. The wells are arranged in three columns and are separated along the vertical profile at 1, 3, 5, 7, 10, and 15 cm from the top of the plate. Each well was filled with a 0.026 g C mL<sup>-1</sup> mixture of food grade agar, microcrystalline cellulose (CAS 9004-34-6; Thermofisher), and powdered bran flakes (Edmond's brand). A 25 µm nylon mesh screen was used to exclude macro- and meiofauna from accessing the wells. The volume of agar-mix that was lost from each well over the duration of the incubation was used to calculate the carbon degradation rate (g C day<sup>-1</sup>) over time. We used the integrated carbon degradation at the depths that corresponded to the depth of the tea bags (5 ± 2 cm; i.e. average values of wells located at 3, 5, and 7 cm beneath the sediment surface) by measuring the volume of agar lost from the wells. A decomposition rate could be calculated by:

$$k_{ROMA} = \frac{(V_{initial} - V_{final}) * 0.029}{t} \quad \text{Eq. (3.1)}$$

Where  $k_{ROMA}$  is the carbon degradation rate in g of Carbon per day,  $V_{initial}$  is the initial agar volume (0.9 mL),  $V_{Final}$  is the volume of agar after incubation, and  $t$  is the time (in days) of the incubation. The value of 0.029 (g C mL<sup>-1</sup>) is the concentration of carbon of the agar substrate. A detailed protocol of the ROMA plate preparation and analysis is given in the supplementary materials (Supplementary Protocol 1).

### 3.2.2.2 Tea bag index

Following the method outlined by Keuskamp et al. (2013), we buried two types of tea (Lipton green tea (EAN:87 22700 05552 5) and Lipton rooibos (red) tea (EAN:87 22700 18843 8)) in surface sediments of mangrove forests to assess the rate at which they decompose. Green tea represents a more labile material source, (representative of leaves and fine roots) and is expected to decompose faster, whereas the rooibos tea (from here on called 'red tea') is made up of more recalcitrant material with expected slower rates of decomposition, such as woody plant components (Keuskamp et al., 2013). As a modification to the original tea bag index, we incubated additional bags for 12 months, as some studies have found long-term incubation data to be better related to environmental factors (Marley et al., 2019). A description of the methods used has been provided in the Supplementary Materials (Supplementary Protocol 2). We used the time series data of each tea type to calculate decomposition rates by fitting a single-term exponential decay model to the data at each site, using a nonlinear least squares regression with the Levenberg-Marquardt algorithm as the measure of estimation (Levenberg, 1944). The model was fitted using the 'nlsLM()' command from the minpack.lm package v1.2-3 (Elzhov et al., 2023) and defined as:

$$y = y_0 e^{-kt} \quad \text{Eq. (3.2)}$$

where  $y$  is the percent mass remaining at a time point,  $y_0$  is the initial mass at  $t_0$ , and  $t$  is the time elapsed since the beginning of the incubation (in days). For the comparison of degradation methods used in this study, the degradation rates were multiplied by the carbon content of each tea as presented in the original methods paper (Keuskamp et al. 2015). Therefore, all degradation rates are presented as loss of g C day<sup>-1</sup>.

To further facilitate comparisons with studies following the TBI protocol to calculate the decomposition rate ( $k$ ) and stabilisation factor ( $S$ ), these values are presented in the supplementary materials (Supplementary Table A3.1).

### 3.2.3 Environmental variables

Environmental variables were measured only once in mid-April 2022. A detailed description of the sampling procedure for environmental variables is given in Chapter 2. Briefly, in situ measurements of porewater salinity, pH, and oxygen reduction potential (ORP) were taken from a dug-out hole with a handheld multimeter probe (YSI ProQuatro, Xylem, Washington DC). Crab burrow density was counted from three randomly placed quadrats (0.5 cm<sup>2</sup>).

Burrow sizes were distinguished between large (>2 cm) and small (<2 cm) and their density was calculated per m<sup>2</sup>. Samples for sediment parameters were collected using a 60 mL polyethylene syringe (Ø 3 cm) with a cut-off tip. At each site, nine cores of surface sediments (0-2 cm) were taken from a random location near the buried tea bags. Three cores were then combined into one composite sample to account for small-scale local variability resulting in three pooled samples. This collection method was repeated for the porewater samples. Leaves were sampled from three random trees in the sampling area. Three shoots were collected from each tree if they had ten or more apical green leaves. All samples were kept in the dark and on ice and placed in a freezer after sampling was completed (after 3 h max). Porewater was extracted by centrifugation as soon as possible after sampling and the supernatant was filtered through 0.7 µm filters (GF/F Whatman) before being separated into nutrient and dissolved organic carbon (DOC) samples. Nutrient samples were frozen at -20°C while DOC samples were protected from light and cooled at 4°C until analysis.

Human land use was defined as the sum of all agricultural and urban related land use types in each catchment. Land use cover was calculated from the Land Cover Database Version 5.0 (LCDB5, Landcare research, <http://www.lris.scinfo.org.nz>). For a detailed description of land cover classification and methodology, see Chapter 2. Human land use in the catchments is expressed as '%Human land use' throughout the manuscript.

### **3.2.4 Laboratory analyses**

Sediment median grain size (GS) was measured using a Malvern Mastersizer 3000 (Malvern Panalytical Ltd., Malvern, UK) after removing organic matter with 10% hydrogen peroxide for at least three weeks. Particles larger than 4 mm were removed with a sieve, and calgon (Sodium Hexametaphosphate 20% Solution) was added to disperse the particles (Singer et al., 1988). The mud content of each sample was defined as the relative contribution of silt particles with diameters of < 63 µm and the clay content as particles < 2 µm (Udden, 1914; Wentworth, 1922). The water content was measured as the mass lost after drying the sediment for 24 h at 60°C and was expressed as % water. Sediment density was calculated as the mass of the dry sample divided by its volume and expressed as g m<sup>-3</sup>. Sediment chlorophyll *a* (chl *a*) and phaeophytin (phaeo) content were extracted in 90% buffered acetone and its fluorescence measured before and after acidification (0.1 N HCl) on a Turner Designs 10-AU fluorometer (Arar and Collins, 1997). The loss-on-ignition (LOI) method was used to determine the total organic matter content (TOM) of the sediment samples. Sediment and leaf samples

were analysed for total organic carbon (%C) and total nitrogen (%N) as well as the isotopic composition of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively). Prior to the analysis the samples were freeze-dried using a Lyovapor L-200 from Büchi (Switzerland, settings: pressure 2mbar, temperature  $-50^\circ\text{C}$ ), ground and acidified (sediments only). Samples were combusted in a Flash 2000 elemental analyzer (Thermo-Fisher Scientific, Bremen, Germany), and  $\delta^{15}\text{N}$  values were calculated against an atmospheric air international standard. Values of  $\delta^{13}\text{C}$  were calibrated against a  $\text{CO}_2$  reference gas, relative to Pee Dee Belemnite (PDB) limestone standard and was then corrected for  $^{17}\text{O}$ . Elemental mass is expressed in percent (%), and stable isotope ratios were expressed as delta values ( $\delta$ ) in per mil units (‰).

Dissolved nutrients from porewater samples were analysed on a LACHAT Quickchem 8500 series 2 flow injection analyser following standard methods for nitrate/nitrite ( $\text{NO}_x$ ), ammonium ( $\text{NH}_4^+$ ), and ortho-phosphate ( $\text{PO}_4^{3-}$ ) analyses (QuikChem® Methods: 31-107-04-1-A, 31-107-06-1-B, and 31-115-01-1-H, respectively). Dissolved organic carbon (DOC) was analysed from porewater samples on an Aurora 1030 Wet Oxidation TOC analyser (OI Analytics, Texas, USA) following a modified protocol for non-purgeable organic carbon (NPOC) to guarantee complete oxidation of OC from a complex saline sample matrix (sample volume: 1 mL; oxidizer volume: 8 mL; DIC purge: 4 min; DOC detection: 8 min).

### 3.2.5 Statistics

Some explanatory variables (bulk density, water content, DOC,  $\text{NO}_x$ ,  $\text{PO}_4^{3-}$ , crab burrows (<2 cm), and crab burrows (>2 cm)) were log+1 transformed to eliminate the impact of a few high values while others (TOM, mud content, ratio chl  $\alpha$ :phaeo, tree density, %Human) were logit-transformed as suggested for proportionate data (Warton & Hui, 2011). Three missing values (variable: DOC from Tuapiro) and six missing response values (variable: green tea (after 12 months) from Manaia) were imputed using the `missForest()` command from the `missForest v1.5 package` (Stekhoven and Bühlmann, 2012). To capture the daily temperature variation at each site, the daily temperature measured from each logger was aggregated into quantiles (Min, 5<sup>th</sup>, 10<sup>th</sup>, 25<sup>th</sup>, Median, 75<sup>th</sup>, 90<sup>th</sup>, 95<sup>th</sup>, Max). Individual linear models were used to determine which temperature quantile explained the most variation ( $R^2$ ) in the degradation metrics (Supplementary Figure A3.1). From the results, the most influential quantile (25<sup>th</sup>) was carried forward into the model selection process.

Overall, 32 variables environmental variables were compiled. To avoid overfitting, which can have a negative effect on the model performance and interpretability through collinear



variables (Huang et al 2011), the Pearson's correlations of the environmental matrix was assessed. From variable pairs that exceeded a correlation score of  $>0.75$ , one variable was removed prior to the analysis. This is commonly done to reduce high dimensional datasets, especially if the variables are ecologically linked or they represent similar conditions or processes. For example, water content was removed due to its high inverse correlation with bulk density, which makes sense, as less dense sediment can hold more water in the interstitial spaces. The variance inflation factors (VIF) of each variable were subsequently calculated to confirm a low remaining level of collinearity ( $VIF < 10$ ). It is important to note that the excluded variables may remain important drivers of degradation in mangrove sediments and that they are being considered when assessing the results of the statistical analysis.

From the set of response variables, a subset was chosen to represent the degradation processes of varying litter quality over different time scales. The ROMA plates represent the most labile fraction of available material and its decomposition over a few days. The decomposition rate ( $k$ ), calculated from the exponential decay function (Eq. 3.2) from the time series of green and red tea, represents the labile and recalcitrant fractions of plant material, respectively, that are exposed to degradation. Thus, three metrics were carried forward into the analyses as response variables and will be henceforth referenced as  $k_{\text{ROMA}}$ ,  $k_{\text{green}}$ , and  $k_{\text{red}}$ .

To identify the individually most influential environmental variables, the marginal effects of each predictor variable on the various response variables were assessed using the `add1()` command from the `stats` package v 4.2.1 in R. This was done after checking the influence of the factor 'Site' as a random variable using a linear mixed-effects model (by the variance term in the summary output) which had no effect. The multiple  $R^2$  values of the summary output were recorded as a measure of the strength of the marginal relationship between the dependent and explanatory variables.

To assess the effect of the environmental variables on degradation, separate Random Forest (RF) regression models were constructed, using each of the selected degradation metrics ( $k_{\text{ROMA}}$ ,  $k_{\text{green}}$ , and  $k_{\text{red}}$ ) with the environmental variables presented above as independent predictors (Breiman 2001). The RF models were built using the `randomForest` package v4.7-1.1 (Liaw & Wiener 2002) and tuned to select optimal values for model parameters `mtry` (the number of variables used in each tree node), `maxnodes` (the maximum number of terminal nodes), and `ntree` (the number of trees to grow). Bootstrapping (150 iterations) was used to incorporate uncertainty into the model performance and variable importance estimates. Model performance was assessed using the % variance explained (`%var`;  $R^2$  values estimated from the internal out-of-bag error) and the mean squared residuals (MSE). Variable

importance was calculated from the '%IncMSE' value and expressed as relative importance of that variable in respect to all other variables included in the model.

Partial dependence plots (pdp) of the random forest were utilised to establish relationships between selected variables and degradation metrics while eliminating confounding effects of other variables. To investigate how the nutrient enrichment in combination with temperature changes interacted with degradation, bi-variable partial dependence plots were created from selected variables. Partial dependence was calculated using the R package 'pdp' v. 0.8.1 (Greenwell, 2017) and uncertainty estimates were calculated using bootstrap sampling of the model predictions.

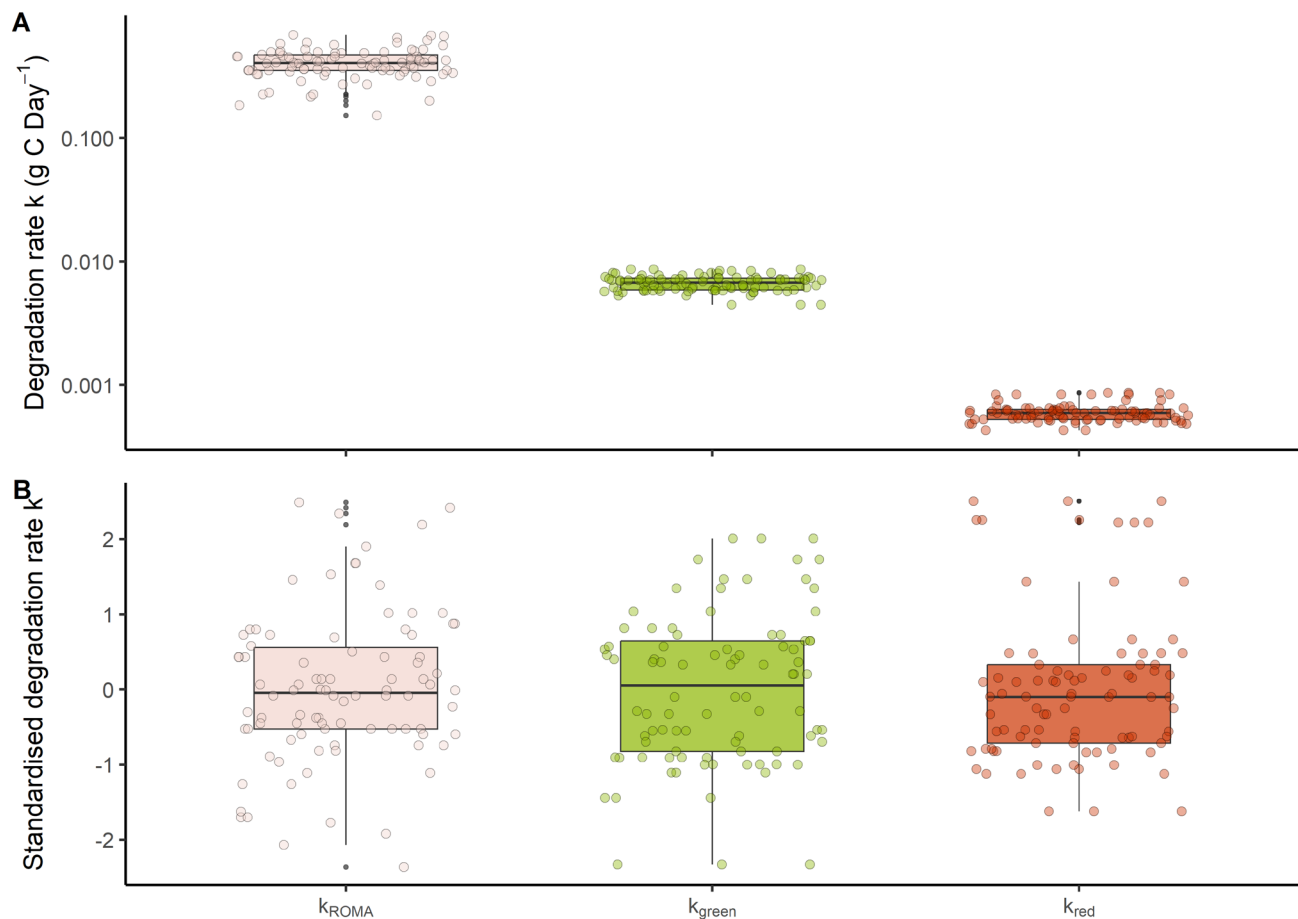
## 3.3 Results

### 3.3.1 Degradation metrics

The average degradation rate ( $\pm 1$  standard deviation) from the ROMA plates was  $0.41 \pm 0.11$  g C day<sup>-1</sup>, which was based on an average of  $16.9 \pm 5.8\%$  of the substrate volume lost after only 14 days. The green tea lost on average  $73 \pm 4\%$  of its initial mass over 3 months of incubation while the red tea only lost on average  $25 \pm 4\%$ . After 12 months the mass loss increased to  $81 \pm 3\%$  and  $36 \pm 5\%$  for the green and red tea respectively. The differences in mass loss were reflected in the decomposition rates with  $k_{\text{green}}$  ( $0.0067 \pm 0.001$  g day<sup>-1</sup>) being an order of magnitude greater than  $k_{\text{red}}$  ( $0.0006 \pm 0.0001$  g day<sup>-1</sup>). While the rate of degradation varied by several orders of magnitude between each of the degradation metrics (Figure 3.2A) the variance of each measure was similar as can be seen from the standardised degradation rates (Figure 3.2B).

### 3.3.2 Influence of environmental controls depends on substrate recalcitrance

The recalcitrance of the substrate materials (difference between  $k_{\text{ROMA}}$ ,  $k_{\text{green}}$ , and  $k_{\text{red}}$ ) had a distinct effect on the relationship between environmental variables and degradation metrics as well as the overall model fit. The most labile of the organic matter substrates, which was incubated for the shortest period (ROMA), showed the weakest relationship between degradation rate and environmental variables. This was obvious from the comparison of the marginal predictors which showed the individual effect of each variable on the degradation metrics. While large crab burrows were the best predictor of  $k_{\text{ROMA}}$  that explained about 10%



**Figure 3.2** The range and distribution of degradation rates of ROMA plates, green tea, and red tea from mangrove forests along a land use gradient in New Zealand. **A** Degradation from ROMA was almost 1.5 magnitudes faster than that of green tea, which in turn was about one magnitude higher than degradation in of red tea. **B** Standardised and centralised values of degradation showed a comparative variance in each of the response variables.

of variance, the best predictor for  $k_{green}$  ( $NO_x$ ) explained 23% and the best predictor for  $k_{red}$  (Daily temperature (25<sup>th</sup>)) explained 38% (Table 3.1). Similarly, the model fit of the random forest models improved substantially for green and red tea (Table 3.2).

Daily temperature became more important in explaining degradation rate with increasing recalcitrance of the substrate material (Table 3.1; Figure 3.3). Independently it explained only a small fraction of variance in  $k_{ROMA}$  (marginal test:  $R^2 = 0.03$ , Table 3.1; RF: relative importance =  $4.0 \pm 0.9\%$ , Figure 3.3A). Despite being a strong individual predictor of  $k_{green}$  from the marginal test ( $R^2 = 0.17$ ; Table 3.1), the importance of daily temperature (25<sup>th</sup>) was lower for the RF model (rel. importance =  $5.3 \pm 0.9\%$ ; Figure 3.3B). For the decomposition of red tea, daily temperature (25<sup>th</sup>) was by far the strongest predictor variable (marginal test:  $R^2 = 0.38$ , Table 3.1; RF:  $15.1 \pm 5.1\%$ , Figure 3.3C). Contrary to the influence of temperature on

the different substrates, porewater nutrients (specifically  $\text{NO}_x$ ) were generally more important in predicting the degradation of the more labile substrates.

### 3.3.3 Drivers of degradation in temperate mangrove forests

The set of environmental variables that best explained degradation was specific to each organic matter substrate (Supplementary Tables A3.2, A3.3, and A3.4). Given the large uncertainty in the  $k_{\text{ROMA}}$  model (Table 3.2), the exact order of variable importance was hard to define, however, SOC stood out with  $8.7 \pm 3.3\%$  relative importance to the model performance (Figure 3.3A, D). Partial dependence plots indicated that higher SOC values were associated with lower rates of  $k_{\text{ROMA}}$ , which may rather be attributed to sediment density or water content, since these variables were strongly correlated with SOC (see Supplementary Figure A3.2).

**Table 3.1** Marginal effects of the ten most important individual predictors of the degradation metrics.

| ROMA                                  | R <sup>2</sup> | Green                                 | R <sup>2</sup> | Red                                   | R <sup>2</sup> |
|---------------------------------------|----------------|---------------------------------------|----------------|---------------------------------------|----------------|
| Crab burrows (>2 cm)                  | 0.10           | $\text{NO}_x$                         | 0.23           | Daily temperature (25 <sup>th</sup> ) | 0.38           |
| SOC                                   | 0.09           | Daily temperature (25 <sup>th</sup> ) | 0.17           | Crab burrows (>2 cm)                  | 0.16           |
| Foliar C                              | 0.07           | ORP                                   | 0.15           | Pneumatophore height                  | 0.14           |
| Foliar $\delta^{15}\text{N}$          | 0.04           | SOC:N                                 | 0.13           | Crab burrows (<2 cm)                  | 0.13           |
| Crab burrows (<2 cm)                  | 0.04           | Sediment density                      | 0.11           | Foliar C:N                            | 0.09           |
| $\text{NO}_x$                         | 0.03           | Foliar $\delta^{13}\text{C}$          | 0.10           | pH                                    | 0.08           |
| ORP                                   | 0.03           | Foliar C:N                            | 0.08           | Sediment $\delta^{13}\text{C}$        | 0.06           |
| Daily temperature (25 <sup>th</sup> ) | 0.03           | Chl $a$                               | 0.06           | SOC:N                                 | 0.05           |
| Foliar N                              | 0.03           | Sediment $\delta^{13}\text{C}$        | 0.06           | Foliar C                              | 0.04           |
| Salinity                              | 0.03           | %Human land use                       | 0.06           | Chl $a$                               | 0.04           |



Mangrove foliar nitrogen ( $6.4 \pm 1.8\%$ ) and carbon ( $6.2 \pm 1.9\%$ ) content were good predictors of  $k_{\text{ROMA}}$ , as well as porewater chemistry ( $\text{NH}_4^+ = 6.1 \pm 1.6\%$ ,  $\text{ORP} = 5.9 \pm 1.8\%$ ,  $\text{NO}_x = 5.5 \pm 1.3\%$ ), and small and large crab burrow densities ( $6.1 \pm 1.2\%$  and  $5.6 \pm 1.4\%$ , respectively). Higher foliar N and lower foliar C were associated with faster degradation of ROMA substrate (Supplementary Figure A3.3). Porewater nutrient concentrations, ORP, and crab burrow density also positively influenced the degradation rate.

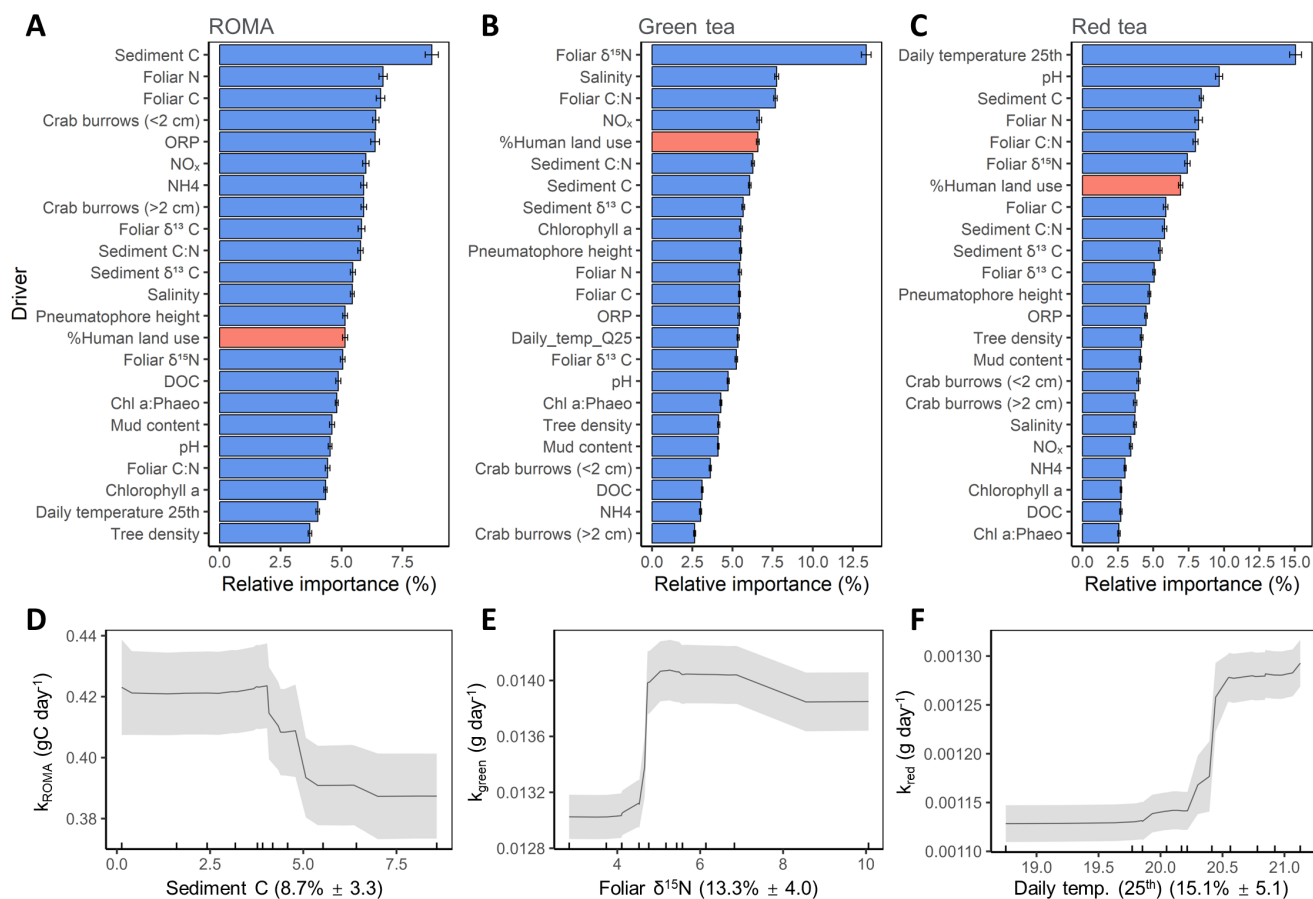
Foliar  $\delta^{15}\text{N}$  was the best predictor for  $k_{\text{green}}$  ( $13.3 \pm 3.7\%$ ), and almost twice as important as the next most important variables; salinity and foliar C:N ( $7.7 \pm 1.4\%$  and  $7.7 \pm 1.3\%$ , respectively) followed by  $\text{NO}_x$  ( $6.7 \pm 1.7\%$ ). Foliar  $\delta^{15}\text{N}$  and  $\text{NO}_x$  were both positively correlated with  $k_{\text{green}}$  (Supplementary Figure A3.4). Contrary to the expectations, foliar C:N ratio was also positively correlated with  $k_{\text{green}}$ . At a salinity of about 32, the degradation of green tea decreased. For  $k_{\text{red}}$ , daily temperature ( $25^{\text{th}}$ ) was the most important predictor, with a relative importance of  $15.1 \pm 5.1\%$ , closely followed by pH ( $9.6 \pm 3.0\%$ ) (Figure 3.3C). There was a threshold at about  $20.4^\circ\text{C}$ , where  $k_{\text{red}}$  increased sharply by 13% (from  $0.00113 \text{ g day}^{-1}$  to  $0.00128 \text{ g day}^{-1}$ ) (Figure 3.3F). Similarly, a pH increase from 7.1 to 7.2 was related with a  $k_{\text{red}}$  increase of 7 % (Supplementary Figure 3.5). Foliar C:N ratio was the next best predictor ( $7.6 \pm 1.6\%$ ) which was positively correlated with the degradation of red tea. Interestingly, sediment texture represented by mud content, ranked fairly low in all models suggesting a low explanatory power of this parameter on organic matter degradation (Figure 3.3).

### 3.3.4 Potential links between catchment land use and decomposition in mangrove forests

The amount of land in the catchment that was utilised by humans for either urban or agricultural purposes (%Human land use) was a good predictor of decomposition across all three substrate types (Figure 3.3). Its relative importance ranged from  $5.8 \pm 1.3\%$  for  $k_{\text{ROMA}}$  to  $5.9 \pm 1.3\%$  for  $k_{\text{red}}$  and  $6.1 \pm 1.2\%$  for  $k_{\text{green}}$ , however, there were less variables that exceeded its importance in the green tea model (importance rank 5), compared to the red tea (rank 7), and ROMA (rank 14) models (Figure 3.3). The partial dependence plot indicated a positive correlation

**Table 3.2** Random forest model performance metrics on the different organic matter substrates. Means  $\pm$  standard deviations were calculated using the bootstrap technique. Bootstraps were performed on 150 iterations.

|           | %var explained | Mean squared error                  |
|-----------|----------------|-------------------------------------|
| ROMA      | $40 \pm 15$    | $1\text{e}^{-2} \pm 3\text{e}^{-3}$ |
| Green tea | $92 \pm 7$     | $8\text{e}^{-7} \pm 6\text{e}^{-7}$ |
| Red tea   | $91 \pm 7$     | $1\text{e}^{-8} \pm 7\text{e}^{-9}$ |



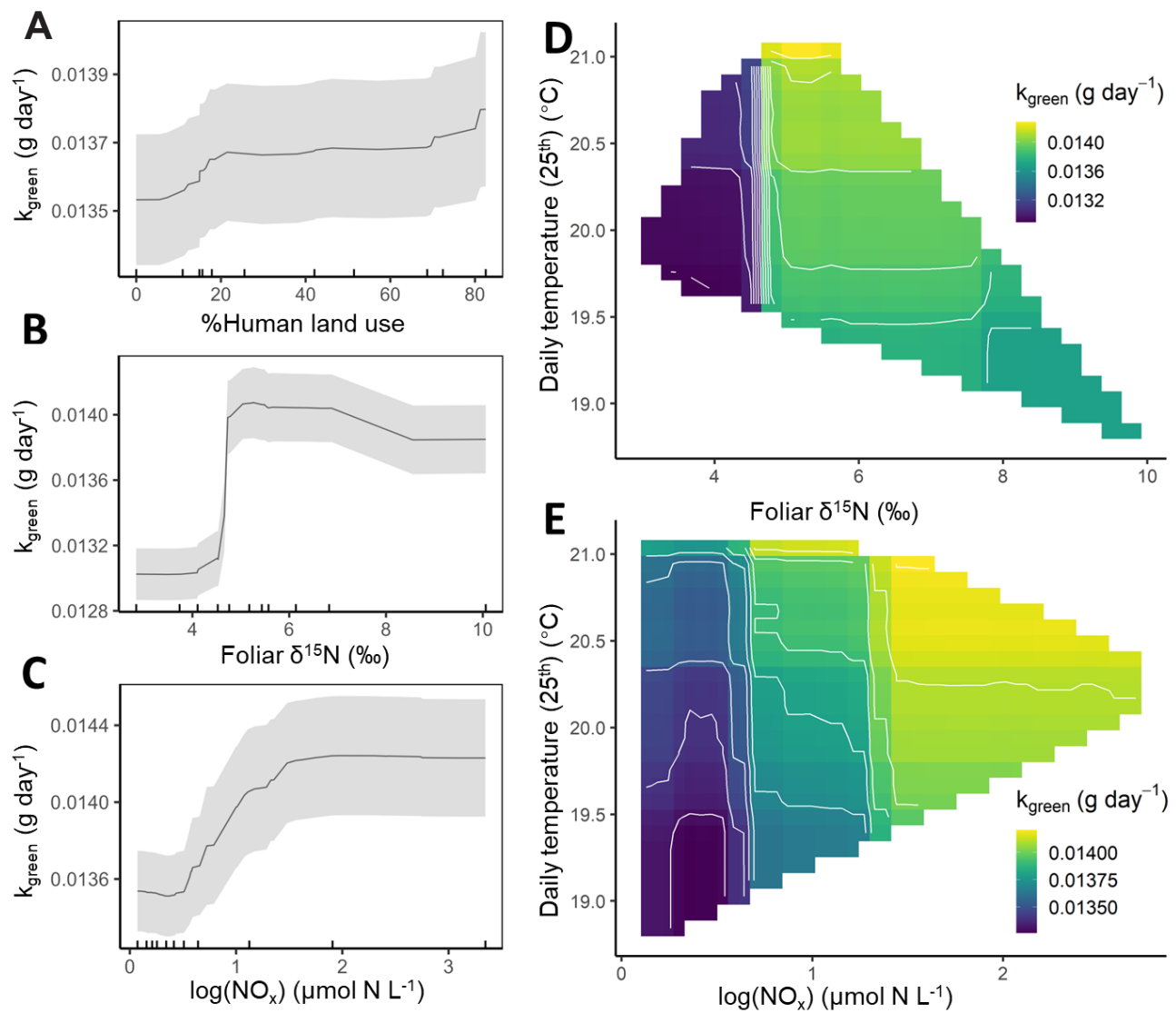
**Figure 3.3** Environmental drivers of **A** ROMA, **B** green tea, and **C** red tea degradation in temperate mangrove forests. Variable importance was based on the improvement of mean squared residuals (MSR) and displayed as relative to the variance explained by all variables. Bar charts indicate the average relative importance ( $\pm 1$  standard error) of each variable. Human land use is highlighted by red coloured bars. Partial dependence plots of the most important variable are shown below. The mean  $\pm 1$  standard deviation of the relative importance are printed below each plot in brackets. The black line indicates the mean and the ribbon the standard deviation of the partial estimate. Statistical estimates were produced using the bootstrap method with 150 iterations. C = carbon; N = nitrogen; ORP = oxygen reduction potential; NH<sub>4</sub> = ammonium concentration; NO<sub>x</sub> = nitrate/nitrite concentration; DOC = dissolved organic carbon; Chl a:phaeo = chlorophyll  $\alpha$  to phaeophytin ratio.

between %Human land use and degradation of green tea which increased continuously with human land use.

No clear correlation pattern was discernible between human land use and red tea, indicating potential interaction with other variables to elevate its importance in the random forest. Since green tea was the most susceptible to human-derived influences, we focussed our attention on the relationships of environmental factors and  $k_{green}$ .

Mangrove foliar  $\delta^{15}N$  and porewater NO<sub>x</sub> concentrations were important variables in predicting degradation of labile organic matter (green tea) in this study. Both variables

have previously been shown to be positively correlated with %Human land use (Chapter 2). Here,  $\delta^{15}\text{N}$  values above 4 ‰ were associated with higher rates of degradation while  $\text{NO}_x$  concentrations of  $1 \mu\text{mol N L}^{-1}$  and higher also related to a faster rate of degradation (Figure 3.4B, C). At  $\text{NO}_x$  concentrations of about  $5 \mu\text{mol N L}^{-1}$ , this relationship seemed to reach a plateau after which degradation did not increase with higher nitrate, however, a low number of samples with high  $\text{NO}_x$  concentrations may influence this relationship. Bi-variable partial dependence plots were investigated to assess the effects of these two variables in relationship with temperature, a major global change variable. Daily temperature ( $25^{\text{th}}$ ) was the second most important marginal predictor of  $k_{\text{green}}$  (Table 3.2) but only ranked tenth in variable importance in the random forest model (Figure 3.3). This disconnect indicates possible interaction effects of daily temperature ( $25^{\text{th}}$ ) with other predictor variables. At lower values of foliar  $\delta^{15}\text{N}$  ( $<4.5$  ‰), temperature change seemed to effect  $k_{\text{green}}$  to a lesser extent than at values above 4.5 ‰ (Figure 3.4D). This was similar for  $\text{NO}_x$  concentrations, which did not seem to influence the effects of higher temperatures on the decomposition rate of green tea much. At concentrations above a level of  $\sim 1 \mu\text{mol N L}^{-1}$  however, high temperatures seemed to affect  $k_{\text{green}}$  more strongly (Figure 3.4E). We tested these bi-variable relationships for  $k_{\text{red}}$  as well (between daily temperature ( $25^{\text{th}}$ ) and pH, Foliar  $\delta^{15}\text{N}$ , and %Human land use), however, temperature as a single predictor dominated each of these interactions (Supplementary Figure A3.6).



**Figure 3.4** Human land use induced effects on degradation of labile organic matter (green tea) in mangrove temperate forests. Partial dependence plots (pdp) of **A** %Human land use in catchments, **B** foliar  $\delta^{15}\text{N}$ , and **C** nitrate/nitrite concentrations in the porewater show the non-linear relationship between each variable and the degradation rate of green tea. The black line indicates the mean pdp estimate and the grey ribbon shows the standard deviation of 150 bootstrap iterations. In brackets below each plot are the bootstrapped average and standard deviation of the relative importance of each variable. Two variable pdp of **D** foliar  $\delta^{15}\text{N}$  and daily temperature ( $25^{\text{th}}$ ) and **E** natural logarithm of nitrate/nitrite concentrations ( $\log(\text{NO}_x+1)$ ) and daily temperature ( $25^{\text{th}}$ ).

### 3.4 Discussion

The results presented above provide clear evidence that drivers of organic matter degradation in temperate mangrove forests differ with varying molecular stability and integrity of the degrading material. While the labile materials were strongly affected by varying sedimentary conditions, degradation of the recalcitrant substrate was mainly influenced by fluctuations in lower daily temperatures. Furthermore, our results establish a link between land-based



stressors and the accelerated degradation of labile organic matter, suggesting anthropogenic nutrient enrichment impacts an important ecosystem function of mangrove forests. These relationships were reproduced across a wide range of estuaries giving confidence about the wider applicability of our results.

Not unsurprisingly, degradation of the most labile substrate (ROMA) showed the weakest relationship to the set of environmental variables (Table 3.2). The duration of this incubation was very short, which is likely to have added to the uncertainty of the model. During a short incubation, general patterns are less likely to persist over spurious instances. Nevertheless, SOC, the most important factor determining  $k_{\text{ROMA}}$ , provides some indication of processes that govern the early-stage decomposition of labile organic matter. SOC is tightly coupled to sediment texture and water content (Supplementary Figure A3.2) which largely control accessibility of organic matter to microbial decomposers (Dungait et al., 2012; Thomson et al., 2022a). At the same time, increased organic matter in the sediments is often associated with more reducing, anoxic environments (ORP was among the five most important predictors) in which degradation of organic matter is retarded (Kristensen, 2007). It is therefore reasonable that the ROMA degradation rate decreases with increasing sediment carbon content (Figure 3.3D). Density of crab burrows increases oxygenation of the sediments as well as facilitating water flow and dispersal (Needham et al., 2011; Booth et al., 2023), further increasing rates of ROMA degradation.

In contrast, the degradation rates of green and red tea were well constrained by the recorded environmental factors (Table 3.2), highlighting the importance of time in establishing clear relationships between environmental variables and degradation. Moreover, integrated measures of environmental conditions over time (temperature, Foliar  $\delta^{15}\text{N}$ ) stood out as important predictors of green and red tea decomposition. This is likely due to the variance that single measurements in time introduce and which makes them unsuited to confine a temporal process. However, the drivers associated with each tea type were quite different (Figure 3.3B, C). Green tea degradation was most strongly influenced by a set of variables that relate to enriched nutrient conditions ( $\delta^{15}\text{N}$ ,  $\text{NO}_x$ , and the ratio of C:N (in leaves and sediments)). The  $\delta^{15}\text{N}$  values in estuaries are considered good proxies for anthropogenic nutrient enrichment as they can provide an integrated landscape-scale perspective of nitrogen cycling (reviewed in Fry et al., 2003). In mangrove forests,  $\delta^{15}\text{N}$  has been shown to correlate with anthropogenic land use due to exposure to elevated nutrient concentrations (Gritcan et al., 2016; Tanu et al., 2020; Thomson et al., 2024). The integrated nature of  $\delta^{15}\text{N}$  as a proxy for high nutrient loading may explain why it was a better predictor for  $k_{\text{green}}$  than porewater or sediment concentrations of nutrients, which capture but a snapshot of the conditions in time. The most

important variables controlling  $k_{\text{green}}$  indicate that the more labile fraction of autochthonous organic matter is vulnerable to the effects of catchment human land use on coastal ecosystems. The threshold of about 4.5 ‰ of  $\delta^{15}\text{N}$  indicated by the partial dependence plot (Figure 3.3E) is just above the range reported to be the upper limit of natural values for a variety of tree leaves (-8 to 3 ‰) (Peterson and Fry, 1987), and specifically mangrove leaves (-11 to 3.8 ‰) (Costanzo et al., 2001; Fogel et al., 2008; Reis et al., 2017). Moderate to elevated  $\text{NO}_x$  concentrations ( $>1 \mu\text{mol N L}^{-1}$ ) also accelerated  $k_{\text{green}}$ , thereby supporting this thesis. Hence, the degradation rate of green tea was faster in mangrove forests that showed evidence of exposure to increased nutrient loading by anthropogenic influences.

Increased concentrations of nitrogen in various forms have been linked to higher rates of carbon efflux ( $\text{CO}_2$  and  $\text{CH}_4$ ) from mangrove sediments (Barroso et al., 2022). This is a measure of microbial heterotrophic respiration and therefore linked to degradation processes, however a direct relationship between nutrient concentrations and decomposition has not yet been observed in mangrove forests (Jessen et al., 2021). Instead, the indirect effect of eutrophication on decomposition via increasing litter quality has been proposed as a pathway in which nutrient concentrations affect decomposition (Keuskamp et al., 2015; Jessen et al., 2021). Here,  $k_{\text{green}}$  was positively correlated with mangrove foliar C:N ratio (Supplementary Figure A3.4). This is contrary to our expectations; however, the sampled leaves were not representative of the tea litter or of the senescent leaves of the forest. Increased nutrient concentrations in the porewater may be a result of organic matter decomposition through the release and remineralization of nutrients from the litter (Keuskamp et al., 2015; Zhang et al., 2018; Mamidala et al., 2023). However, the high concentrations of nutrients in the porewater at some sites (in urban settings) likely point to an external pollution source as well. In the case of coastal eutrophication, a combination of direct and indirect effects on decomposition may create a positive feedback loop that leads to a permanently enriched system in which carbon sequestration is substantially weakened (Branoff, 2017).

Degradation of the more recalcitrant red tea was mainly controlled by the daily temperature fluctuation in the sediment (Figure 3.3C, F). Temperature is well known to be an important driver of OM loss from soil and sedimentary carbon stocks (Kirschbaum, 1995; Fang et al., 2005; Trumbore and Czimczik, 2008; Schuur et al., 2009; von Lützow and Kögel-Knabner, 2009; García-Palacios et al., 2021). Microbial activity and enzymatic reactions are temperature-dependent, and a conventionally agreed doubling of SOM decomposition with every 10 °C rise in temperature ( $Q_{10}$ ) has commonly been applied in the literature (Davidson and Janssens, 2006). Therefore, the positive relationship between the decomposition rate of OM and temperature in mangrove sediments is not surprising. It has been theoretically and em-

Chapter 3

pirically reasoned that the temperature sensitivity increases with decreasing quality of the substrate that is being degraded which can be explained by the difference in activation energy required to start decomposition processes on materials of varying complexity (Fierer et al., 2005; Davidson and Janssens, 2006; Craine et al., 2010; Qin et al., 2019; Jia et al., 2020). This is corroborated by our findings which show the strongest relationship of temperature with the most recalcitrant substrate ( $k_{red}$ ). It furthermore highlights the importance of considering different pools of OM to accurately predict changes in degradation with climate or human impacts (Davidson and Janssens, 2006). Interestingly, the lower daily temperature better explained degradation rates than the average or the upper daily temperature (for  $k_{green}$  and  $k_{red}$ , Supplementary Figure A3.1). This suggests that changes in the lower temperature threshold are more likely to be driving degradation processes in temperate mangrove sediments. The degradation rate of red tea increases by about 13% (from  $\sim 0.00113$  to  $0.00128 \text{ g day}^{-1}$ ) when the daily temperature ( $25^{\text{th}}$ ) surpasses  $20.4^{\circ}\text{C}$  indicating a potential vulnerability threshold of sedimentary organic matter at that temperature. These thresholds are to be considered with care however, as the temperature was only measured for three months in summer and continuous seasonal records may affect this estimate. While %Human land use was a good predictor for  $k_{red}$ , its association with the response variable remains uncertain, as the partial dependence plot did not show a clear directional relationship (Supplementary Figure A3.5). This may be due to interactions of %Human land use with other predictors in the measured variables, however, this relationship was not further explored. Furthermore, temperature was such a dominant predictor and its control on the degradation process seemed uninfluenced by other (human land use related) variables (Supplementary Figure A3.6).

Several papers have discussed the potential to manage terrestrial forest soils and coastal sediments for them to sequester more carbon and thereby contribute to the mitigation of climate change effects on the planet (Prescott, 2010; Macreadie et al., 2017; Lehmann et al., 2020). This discussion is particularly contemporary for mangrove and salt marsh systems which are being increasingly included in private and governmental efforts towards blue carbon accreditation schemes (Wylie et al., 2016; Needelman et al., 2018; Dencer-Brown et al., 2022; Lovelock et al., 2022). While there is a lot to be learnt from studies on terrestrial systems, it is important to treat the results cautiously when aiming to transfer the knowledge to marine systems. Vital characteristics of soil profiles, such as soil moisture content, oxygen availability, redox stratification, and microbial dispersal limitations, to name only a few, fundamentally differ between these biome types creating contrasting settings for nutrient cycling and carbon storage (Spivak et al., 2019). For example, the inhibitory effects of nutrient addi-

tions in some forest soils may be dispelled by nutrient-induced microbial priming in coastal systems where microbial primary producers are abundant (Janssens et al., 2010; Riekenberg et al., 2018). Therefore, it is important to treat the management of coastal systems distinctly, targeting processes with their local environmental setting in mind. This is particularly difficult for coastal intertidal systems which exhibit a high diversity and dynamism of conditions on temporal and spatial scales. Our study provides evidence of loss of labile organic carbon due to coastal eutrophication as well as a weakening of the temperature related inhibition of OM decomposition at sites exposed to high nitrate concentrations. Since our study was performed on standardised allochthonous substrate, we cannot calculate representative rates of carbon loss through this increased decomposition to compare them to carbon fixation rates at the sites. Evidence suggests that increased eutrophication can lead to higher rates of carbon fixation by the primary producers, possibly offsetting the losses through decomposition (Feller et al., 2009; Keuskamp et al., 2015; Hayes et al., 2017). It is unclear however, how these interactions proceed over longer time scales. Nutrient rich systems tend to produce more and higher quality litter, leading to self-fertilization with direct and indirect effects on decomposition (Mamidala et al., 2023). The high carbon sequestration potential that mangrove ecosystems provide may partly be due to their ability to protect a fraction of the labile OM from decomposition, which is incorporated into the sediment matrix or the microbial loop to last for centuries (von Lützow et al., 2006). This ecosystem property can be boosted by managing terrestrial runoff to maximize ecosystem productivity while not losing the vulnerable and dynamic fraction of organic matter from the sediments.

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# pneumatophore

[nyoo-mat-uh-fohr]

noun

*"Botany. a specialized structure developed from the root in certain plants growing in swamps and marshes, serving as a respiratory organ. (dictionary.com)"*

These aerial roots of hydrophytic trees such as mangroves grow upwards from the water surface to draw oxygen into the tree's submerged root system and into the otherwise anoxic sediments below. One of nature's great inventions that besides supporting the tree's aeration also progresses their lateral expansion.





# 4

## **Legacy of nutrient enrichment on subsurface bacterial community in temperate mangrove sediments**

Timothy Thomson, Marco Fusi, Natalie Prinz, S. Craig Cary, Conrad A. Pilditch, Joanne I. Ellis

### **4.1 Introduction**

Mangrove forests are among the most productive ecosystems on the planet and have been recognised for their potential to mitigate climate change (Donato et al., 2011; Duarte et al., 2013; Alongi, 2020b). The unique combination of high productivity coupled with slow decomposition, attributed to reducing and anoxic sediments, results in the accumulation of carbon-rich organic matter within mangrove sediments (Duarte and Cebrián, 1996; Kristensen et al., 2008). The various degradation processes that affect this accumulation are controlled by diverse microbial metabolisms that are highly adapted to the constraining and dynamic conditions present in intertidal sediments (Holguin et al., 2001; Alongi, 2005; Kristensen, 2007). It is therefore of high importance for the health and functioning of these ecosystems to understand the factors that influence the microbial communities in mangrove sediments and their reactions to perturbations to the system.

It is widely understood that the environmental conditions, climate, and plant-microbe interactions shape bacterial communities in sedimentary environments (Bahram et al., 2018; Islam et al., 2020; Vasar et al., 2022; Dai et al., 2023; Philippot et al., 2023). In mangrove forests, factors such as tidal influence (Marcos et al., 2018; Thomson et al., 2022a), levels of organic enrichment (Chen et al., 2016; Zhang et al., 2019a), availability of resources (Bai et al., 2013; Tong et al., 2019), pH (Ceccon et al., 2019), bioturbation (Booth et al., 2023), and tree species (Zhuang et al., 2020; Mai et al., 2021) have been identified as drivers of microbial community composition. Steep physicochemical gradients along the vertical sediment profile create contrasting environments that shape resident microbial communities of the surface and the subsurface layers (Probandt et al., 2017; Luis et al., 2019). This variability of site-specific factors greatly compromises the transferability of observed relationships across studies. Furthermore, it is still unclear how disturbances influence the microbial communities spatially and temporally (Allison and Martiny, 2008; Langenheder and Lindström, 2019). Coastal intertidal habitats are fairly resilient to changing environmental conditions due to their dynamic nature, however, they can show sensitive responses to anthropogenic stressors

(Bernhardt and Leslie, 2013; O’Leary et al., 2017; Bernhardt et al., 2020). Given the transient and adaptable nature of microbial communities, it can be expected that they may function as sensitive indicators of change (Clark et al., 2020; Lalzar et al., 2023).

Coastal ecosystems are under enormous pressure from land-derived stressors due to ever increasing anthropogenic land use intensities and coastal modifications (Lotze et al., 2006; Thomson et al., 2024). Particularly, the exposure of aquatic ecosystems to over-enrichment of nutrients often leads to eutrophication with associated detrimental effects on present species and sometimes even the collapse of the entire ecosystem (Rabalais et al., 2009; Doney, 2010). Notably, the sediments of mangrove ecosystems, rich in organic carbon but generally low in nutrients (Lin et al., 2019), play a critical role in mediating the impacts of nutrient enrichment on ecosystem functioning, and microbial communities emerge as key regulators of nutrient cycling in these sediments (Reef et al., 2010; Dai et al., 2021). Despite their pivotal role, limited knowledge exists regarding the specific changes of microbial communities in response to nutrient enrichment (Trevathan-Tackett et al., 2019; Allard et al., 2020). Soil microorganisms, with their short generation times, are expected to react swiftly to changes induced by nutrient input (Oakes et al., 2020), influencing gas exchange and overall carbon sequestration rates (Alongi, 2020a; Shiau and Chiu, 2020). Nutrient enrichment in aquatic ecosystems, fosters the growth of opportunistic autotrophs, leading to the rapid production of labile organic carbon and subsequent proliferation of heterotrophic microorganisms and exoenzymes (Keuskamp et al., 2015; Riekenberg et al., 2020). This cascade of events accelerates nutrient remineralisation, and the eventual release of carbon back into the atmosphere as greenhouse gas.

How the microbial communities of mangrove sediments react to external nutrient input is poorly understood, partly due to the difficulties of studying these systems in a controlled environment. Few studies report on reduced bacterial diversity following enrichment and changes to the abundances of specific taxa (i. e. decreased sulphate reducers, increased nitrogen cyclers) (Craig et al., 2021; Dai et al., 2021; Erazo and Bowman, 2021; Palacios et al., 2021), whereas others observed diversity increases at affected sites (Xie et al., 2022). To date, only one manipulative fertilisation study was conducted in the field accounting for realistic levels of ecosystem variability (Palacios et al., 2021). It is important to note here, that a number of studies have investigated enrichment through runoff from anthropogenic sources, however, the added variability induced by the mode of delivery complicates the interpretation of the observed effects (organic enrichment, introduced microbial communities, etc). Microbial communities are shaped by assembly processes that are governed by ecological

interactions of stochastic and deterministic nature (Vellend, 2010). Recognising which process dominates in mangrove sediments and how it may change in response to shifting environmental conditions is important to understand and predict microbial community change to anthropogenic stressors (Nemergut et al., 2013). Moreover, a link between the present microbial communities and relevant ecosystem functions such as carbon remineralisation rate have not yet been conclusively established. While several works observed increases in carbon metabolism in eutrophied and experimentally enriched systems, the high variability in these measurements and the dynamic nature of the intertidal mangrove habitat makes reproduction of these results extremely difficult (Bernhardt and Leslie, 2013; Shiau and Chiu, 2020).

This study aims to investigate shifts in the microbial community composition in sediments of temperate mangrove forests in response to experimental nutrient inputs and its consequent impact on carbon dynamics. We particularly aim to understand some of the spatial variability induced by differences between the surface and the subsurface communities. The research seeks to address the following two questions: 1) How does the composition and abundance of microbial communities in the surface (0-2 cm) and subsurface (5-7 cm) of bulk mangrove sediments change in response to external nutrient input? 2) Does the addition of nutrients alter the emission of greenhouse gases and the exchange of dissolved carbon from mangrove sediments? Two sampling depths were chosen to account for community differences relating to light and oxygen availability, as well as the exposure to the water column. We tested the hypothesis that microbial diversity is reduced in response to nutrient enrichment, and that changes in the community are characterised by prominent nitrogen cycling taxa. Moreover, we assumed to identify an association between nutrient levels in the sediments/porewater and community composition patterns. Lastly, we expected an overall increase of carbon export from the system in response to increased microbial metabolism, and that the majority of this export was going to be in dissolved form (porewater carbon rather than gasses). Through a comprehensive exploration of these questions, we seek to enhance our understanding of the intricate interactions within mangrove sediments, particularly shedding light on the microbial dynamics crucial for carbon cycling and climate regulation.

## 4.2 Methods

### 4.2.1 Study Site

The study site was located in the northern arm of the Whangamata estuary (37°10.63' S, 175°51.68' E) on the east coast of the North Island of Aotearoa New Zealand (Figure 4.1A-C). The estuary is characterised as a barrier enclosed, drowned river valley that receives low freshwater inputs, with a tidally driven hydrology over extensive areas of low gradient intertidal flats (Hume and Herdendorf, 1988). The estuary is subject to a semi-diurnal tidal cycle with a mean spring-tide amplitude of 1.8 m (LINZ, Tide stations data base on the Web, 2024). Mean air temperature is 15.2°C with annual average rainfall of approximately 1360 mm (CliFlo: NIWA's National Climate Database on the Web, 2024). Mangrove stands of *Avicennia marina* subsp. *australasica* cover approximately 100 ha (~20% of the estuary) and are located on the shoreline of the western side of the estuary (Singleton, 2007).

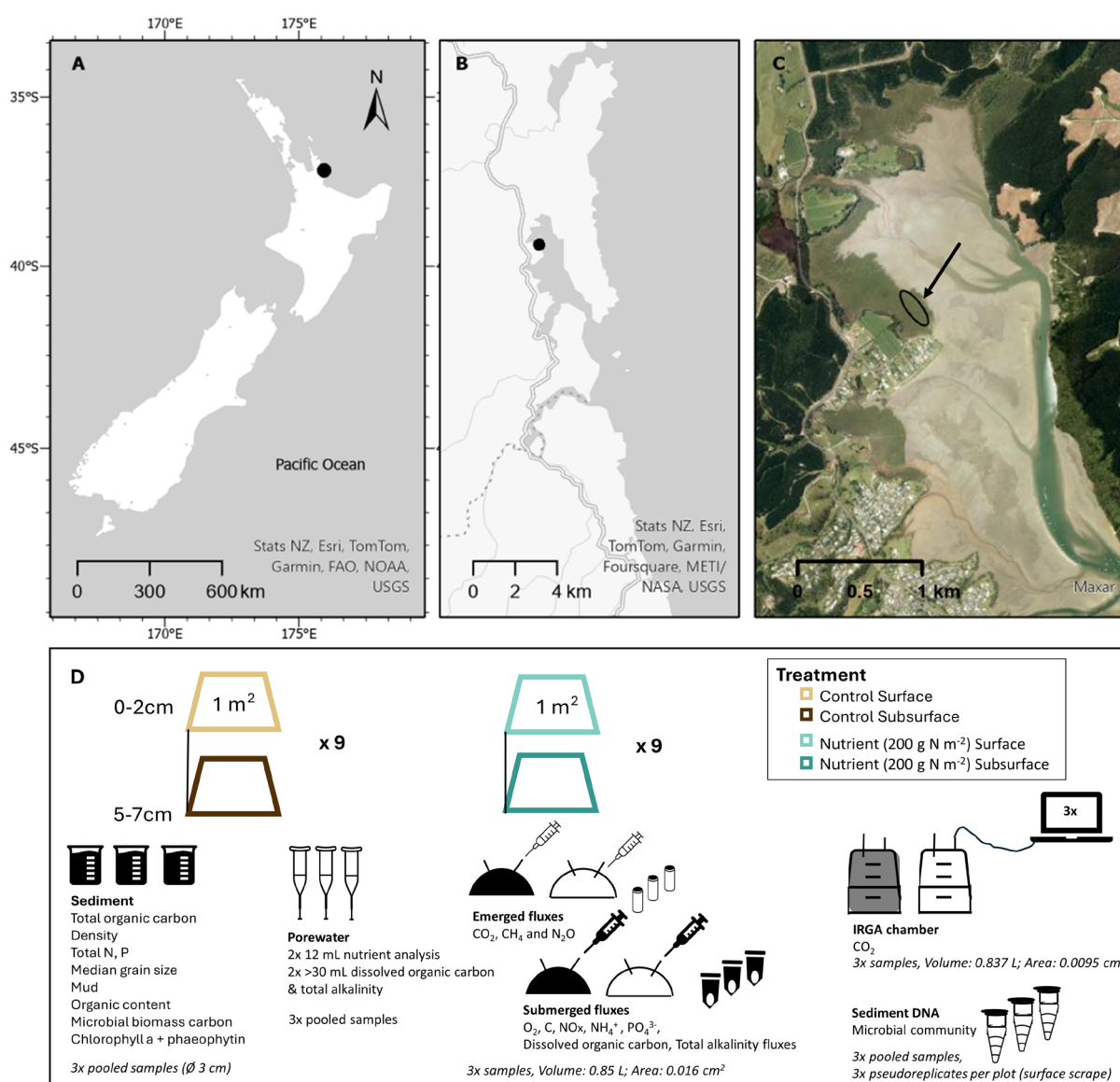
### 4.2.2 Experimental design

A fully randomised sampling design was set up in the interior of the forest (about 15-20 m back from the fringe). Care was taken to account for a homogeneous level of bioturbation across the whole sampling area as this has been shown to be a strong driver of sediment processes and microbial community assembly (Fusi et al., 2022; Kristensen et al., 2022). Plots were randomly placed under the canopy with a minimum of 10 m separating each plot. Treatment and control plots were assigned randomly using the 'sample()' function in base R (RStudio) and subsequently checked for a homogeneous distribution. Each treatment was replicated 9 times with two depths sampled, resulting in 18 plots and 36 unique samples (Figure 4.1D).

The enrichment was performed in October 2020 with a slow-release fertiliser complex (Osmocote® Exact Standard 5-6M; 15:9:12 N:P:K). The fertiliser complex was chosen over a single nutrient fertiliser to avoid inhibiting effects of limiting nutrients, as well as to mimic eutrophication events which are seldom based on a single nutrient. The duration of fertiliser release from the pellets was specified by the manufacturer to be between five to six months. An application rate of 200 g N m<sup>-2</sup> (which corresponds to 120 g P m<sup>-2</sup> and 160 g K m<sup>-2</sup>) was chosen to represent medium to high levels of enrichment that would represent concentrations found in eutrophic estuaries (Douglas et al., 2016). A series of 20 evenly spaced fertiliser



doses of equal volume were applied to each plot using corers to inject the pellets 10-15 cm into the sediment (Douglas et al. 2016). The corers used an outer sleeve to prevent the holes from collapsing and the sediment plug was replaced immediately after fertilisation to minimise physical disturbance. The experimental period was chosen to be 4.5 months, which was within the release time of the Osmocote® fertiliser, to reflect the effects of a long-term elevated nutrient regime in mangrove sediments. Several studies have used this method to successfully enrich intertidal areas for scientific purposes (Douglas et al., 2016; Gladstone-Gallagher et al., 2020).



**Figure 4.1** Map of the study area on the east coast of the North Island of Aotearoa New Zealand, in the northern arm of the Whangamata estuary (A-C). **D** Visualisation of the sampling strategy. Nine control plots and nine nutrient plots were sampled at the surface and the subsurface. Three samples per plot were pooled into one composite sample to account for small scale variability. Flux samples (submerged and emerged) were taken from benthic incubation chambers and analysed in the laboratory, except for CO<sub>2</sub> fluxes, which were measured *in situ*. Sediment samples for genetic marker analysis were collected in triplicate.

### 4.2.3 Sampling

Sampling was conducted in February 2021. Samples for sediment parameters were collected using a 60 mL polyethylene syringe ( $\varnothing$  3 cm) with cut-off tip that extended approximately 10 cm into the sediment. All cores were immediately separated into surface (0 - 2 cm) and subsurface (5 – 7 cm) partitions. Three cores were taken from a random location in the plot and the respective surface and subsurface partitions were combined into one composite sample to account for small-scale local variability. The composite sample was subsequently separated into sampling containers for distinct measurements (1) Total organic matter (TOM) + density, 2) sediment grain size, 3) Microbial Biomass Carbon, 4) Chlorophyll  $\alpha$  + phaeopigments). Samples for sediment density and water content were collected similarly but not split to retain the mass to volume ratio of the samples. Porewater samples were collected using Rhizon samplers (MacroRhizon, SoilMoisture Equipment Corp., CA, USA). For logistical reasons only ten Rhizon samplers were available. To avoid cross contamination these were split into designated treatment and control samplers. As an additional precaution, sampler and syringe were rinsed twice with deionised water and twice with sample water before each sample was taken. Subsurface samples were taken by vertically submersing the Rhizon in the sediment until only the connector and the syringe remained. Surface samples were taken by horizontally sliding the Rhizon under the surface as shallow as possible while ensuring complete coverage of the sampler by sediment. To allow for a reasonable amount of porewater to be collected, the samplers were left in position for up to 20 minutes or until a total of about 90 mL was collected. The sample was then split into 2x 12 mL for nutrient analysis and 2x >30 mL for dissolved organic carbon (DOC) and total alkalinity (TA). The DOC samples were wrapped in aluminium foil to minimise light exposure. All samples were kept in the dark and on ice and until sampling was completed (max. 3 hours after collection). Sediment, porewater nutrient, and TA samples were frozen at -20°C while DOC samples were cooled at 4°C until analysis. Samples for microbial communities were collected as individual replicates using coring devices as described above (60 mL syringe,  $\varnothing$  3 cm). Each core was separated into surface and subsurface fraction and the fractions were immediately homogenised on a clean tray with a sterile spatula before being sampled into their respective tubes. Tubes were sealed with parafilm and stored on salt ice in the dark before being transferred to -80°C freezer after the sampling was completed.

#### 4.2.4 Submerged and emerged flux sampling

Samples for submerged flux measurements were collected during the high tide period using light and dark benthic incubation chambers (Volume: 0.85 L; Area: 0.016 cm<sup>2</sup>). The chambers had two tubes attached, one open and one with a luer-lock connector. The sample was drawn from the luer-lock connector using a 60 mL acid washed syringe, after rinsing the vessel three times with approximately 20 mL of chamber water. The open tube functioned as a pressure valve, slowly equilibrating the reduced chamber pressure after sampling. This provides no problem for the first sampling as the water inside the chamber should be the same as outside the chamber. The initial water sample was drawn 20 minutes after placing the chamber to avoid sampling suspended sediment from the disturbance. For the second (final) sampling, care was taken to use only minimal amounts of chamber water to rinse the syringe to create as little pressure gradient as possible before taking the actual sample. The initial sample was carefully drawn to ~30 mL and discharged directly into the sampling container for total alkalinity (50 mL tube). Another full syringe (60 mL) was drawn for subsequent nutrient, DOC, and TA analysis and kept in a seawater bath until sample processing on shore. During the incubation period equilibrium was assumed between the chamber and the water column and diffusion was the only pathway to affect chamber solute concentrations. Since the tube was 5–7 cm long, it was assumed that no exchange took place between outside water and chamber water during the incubation period. Light and dark chambers were placed in pairs in each plot as soon as the tide reached about 30 cm water depth. Final samples were taken after one hour. To account for ambient water fluxes six ambient water samples were collected at the time of initial and again, final chamber sampling. Three sets of light and dark bottles were filled with ambient water at the time of initial samples were taken and incubated at the same depth for the same amount of time to account for processes in the water column.

Sampling of gas fluxes from the emerged sediment surface at low tide was conducted using chamber-based incubation techniques that differed for carbon dioxide (CO<sub>2</sub>) compared to methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O). An *in situ* continuous flow infrared gas analyser (LI-8100A, LI-COR, Nebraska, USA) (IRGA) was used for CO<sub>2</sub>, while three samples were drawn from the chambers over time, for CH<sub>4</sub> and N<sub>2</sub>O, which were subsequently analysed in the lab. The IRGA detects CO<sub>2</sub> (ppm) in a continuous air sample circulated from an attached custom-built cylindrical acrylic chamber designed for use in emergent intertidal environments (Drylie, 2020). The IRGA was connected to the chamber (Volume: 0.837 L; Area: 0.0095 cm<sup>2</sup>) with a base collar and an integrated thermocouple. The collared base was inserted about 5 cm into the sediment 5 minutes before the measurements were taken to minimise the disturbance

effect on the gas exchange. Before the dark measurements, the area was covered for 5 minutes to avoid legacy effects of photosynthesis on the measurements. Three light and dark measurements were taken from each plot. Operation of the IRGA followed manual instructions and the LI-8100A application was used to communicate with the instrument in the field. The data were recorded to an on-board memory logger (LI-COR Li-1400) with a logging frequency of 1 second<sup>-1</sup>. The incubation time of the sediment was chosen to be 2 minutes so as not to exceed a saturation point of the small volume chamber (Drylie, 2020). From prior pilot studies this time seemed sufficient to capture a linear trend in CO<sub>2</sub> concentrations without saturation effects, while maximising the number of replications possible by time restrictions (Drylie, 2020). Exponential regressions of CO<sub>2</sub> development over time and the coefficient of determination ( $r^2$ ) associated with calculated fluxes ( $\mu\text{mol m}^{-2} \text{h}^{-1}$ ) were extracted from the LI-COR File Viewer 8100 3.1.0. Only fluxes with an  $r^2 > 0.8$  were used for further analysis.

Gas samples for CH<sub>4</sub> and N<sub>2</sub>O evolution were taken during low tide using light and dark incubation chambers. Chambers were deployed simultaneously, and samples were taken just after closing the chambers (T0), after five minutes (T1) and after ten minutes (T2). Samples were drawn with a gas-tight syringe from small benthic chambers (Volume: 0.85 L; Area: 0.016 m<sup>2</sup>) into pre-evacuated glass exetainers (Exetainer® 4.5 mL Vial, Labco Ltd, Lampeter, UK). In some plots pneumatophores had to be cut to surface level to create enough space for the chamber to be placed. The number of trimmed pneumatophores and the number of macrofauna burrows was recorded after the measurements were completed. The exetainers were stored in the dark and measured the following week on a Quantum Cascade Laser analyser QCL (Aerodyne Research Inc., Billerica, MA, USA), which was modified for manual sample injection (Wecking et al., 2020). The initial concentration was subtracted from the final concentration to calculate the flux over time, which was then normalised by chamber volume and surface area.

#### **4.2.5 Analytical methods**

Sediment median grain size (GS) was measured using a Malvern Mastersizer 3000 (Malvern Panalytical Ltd., Malvern, UK) after removing organic matter with 10% hydrogen peroxide. Particles larger than 4 mm were removed with a sieve, and Calgon (Sodium Hexametaphosphate 20% solution) was added to aid dispersal of the particles (Singer et al. 1988). The mud content of each sample was defined as the relative contribution of silt and clay particles of diameter < 63  $\mu\text{m}$  (Udden, 1914; Wentworth, 1922). Sediment chlorophyll  $\alpha$  (chl  $\alpha$ ) and phaeophytin

(phaeo) content were extracted in 90% buffered acetone and its fluorescence measured before and after acidification (0.1N HCl) on a Turner Designs 10-AU fluorometer (Turner Designs, San Jose, USA) (Arar and Collins, 1997). Prior to pigment analysis, the sediment was freeze-dried using a Lyovapor L-200 from Büchi Flawil (Switzerland, settings: pressure 2mbar, temperature -50°C).

The loss on ignition (LOI) method was used to determine organic matter content (OM) from sediment samples. Between 6-8 g of sediment were dried (at 60°C for 24 h) and subsequently combusted following a two-step protocol of different temperature steps (300°C for 4 h, and 550°C for 4 h, respectively). The LOI was calculated for each combustion using Eq. 4.1:

$$LOI = \frac{\text{pre combustion mass} - \text{post combustion mass}}{\text{pre combustion mass}} * 100\% \quad \text{Eq. 4.1}$$

Total sediment OM was calculated by adding the LOI of each individual step. The unstable OM fraction was defined by the LOI at 300°C and the stable OM fraction is defined as the LOI at 550°C (Santos-Andrade et al., 2021). Water content was measured as the mass lost after drying the sediment for 24 h at 60°C and was expressed as a percentage. Sediment density was calculated as the mass of the dry sample divided by its volume and expressed as g m<sup>-3</sup>.

Dissolved nutrients from the porewater samples, submerged incubation chambers, and water column samples were analysed on a Quickchem 8500 series 2 flow injection analyser (LACHAT instruments, Loveland, USA) following standard methods for nitrate/nitrite (NO<sub>x</sub>), ammonium (NH<sub>4</sub><sup>+</sup>), and orthophosphate (PO<sub>4</sub><sup>3-</sup>) analyses (QuikChem® Methods:31-107-04-1-A, 31-107-06-1-B, and 31-115-01-1-H, respectively). Porewater DOC was analysed using an Aurora 1030 Wet Oxidation TOC analyser (OI Analytics, College Station, USA) following a modified protocol for non-purgeable organic carbon (NPOC) to guarantee the complete oxidation of organic carbon from a complex saline sample matrix (sample volume: 1 mL; oxidiser volume: 8 mL; dissolved inorganic carbon purge: 4 min; DOC detection: 8 min). Total alkalinity was measured using the open cell titration method (Dickson et al., 2007, SOP 3) on a TitroLine® 6000/7000 auto titrator (SI Analytics, Xylem, Germany) using a certified reference material (A. Dickson, Scripps Institution of Oceanography, La Jolla, California).

Microbial biomass carbon (MB-C) was extracted following the direct extraction method by Gregorich et al. (1990). Briefly, 5 g of sediment was weighed separately into 50 mL centrifuge tubes and 20 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> was added to each. To three subsamples, 0.5 mL of ethanol-free chloroform was added. Both the chloroform-exposed and the non-fumigated samples



were capped and shaken for 30 min. After shaking, the suspensions were allowed to settle for 10 min and the supernatants were filtered through 0.7 µm filters (GF/F Whatman, Buckinghamshire, UK). For the sub-samples with chloroform, only the top 15 mL of the supernatant was filtered to reduce the amount chloroform in the filtrate. Filtrates from soils with and without chloroform were immediately bubbled with air for 30 min to remove any residual chloroform. The DOC in all filtrates was determined as described above. Chloroform labile C was calculated as the difference between the C extracted from the chloroform fumigated and the non-fumigated sample. No conversion factor ( $k_{EC}$ ) was used to convert chloroform labile C to microbial biomass C because the range of  $k_{EC}$  values (0.41–0.58) is used in the literature and it has not been tested which is best suited for the soils used here.

#### **4.2.6 DNA extraction and library preparation**

The total DNA of  $0.20 \pm 0.04$  g of sediment was extracted using the DNeasy® PowerSoil® Pro extraction kit (QIAGEN®, Hilden, Germany) following the manufacturer's instructions. The extract quality was confirmed via gel electrophoresis and the concentration was determined using nanodrop (NanoDrop 2000, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and subsequently, each sample was standardised to 2.5 ng/µL. The V4-V5 of the 16S rRNA gene was amplified using the universal earth microbiome project primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada et al., 2016). One-step PCR of the target amplicon was performed using Nextera XT v2 index primers. Each sample was amplified in triplicate with each reaction containing 5 µL of total DNA in a final reaction volume of 20 µL.

Trimmed reads were processed following the DADA2 (v1.28; Callahan et al., 2016) workflow with the recommended parameters (except `truncLen()` which was set to 260 for forward and backward reads). The quality-controlled sequence table was filtered to remove low prevalence taxa (below 1% total abundance or in < 1% of samples) and remnants of plastid DNA (chloroplasts/mitochondria). Samples that contained < 4,000 reads were removed, and the remaining samples were rarefied to the minimum depth of 4,300 sequences per sample. Taxonomy was called against the SILVA database v138 (Quast et al., 2012). Sequences were aligned with DECIPHER 2.30.0 (Wright et al. 2016) for phylogenetic tree construction (phangorn v2.11.1, Schliep, 2011). The final abundance table contained 4,912 taxa from 103 samples with 39 individual phyla and 131 individual genera.

### 4.2.7 Statistics

All analyses were conducted in the R statistical language v4.3.1 (Core Team, 2021) using RStudio (Posit Team, 2023), and figures were prepared using the ggplot2 package v3.4.4 (Wickham, 2009). The environmental variables of the sediment and the porewater as well as the emerged and submerged fluxes were tested for differences between depths and treatments using analysis of variance (ANOVA). In cases where high values skewed the data distribution, response variables were log transformed and diagnostic plots were consulted to ensure the model assumptions (normality of residuals and homoscedasticity) were met. Tukey's honestly significant difference (TukeyHSD) test was applied to test for p-value adjusted pairwise comparisons if the interaction term was significant.

Alpha diversity values were calculated using the *phyloseq* package v1.46.0 (McMurdie and Holmes, 2013) and beta diversity was calculated using Euclidean distance on the robust centred log-ratio (rclr) transformed data frame (robust Aitchinson distance, Martino et al., 2019) using base R and the *vegan* package v2.6-4 (Oksanen et al., 2016). Phylogenetic tree construction was performed with *ape* v5.7-1 (Paradis and Schliep, 2019) and phylogenetic diversity (PD), nearest taxon index (NTI) and net relatedness index (NRI) were calculated with the *picante* package v1.8.2 (Kembel et al., 2010). Univariate tests on the various diversity metrics were performed by ANOVA and multivariate comparisons of the microbial abundance data were done with the linear model based resampling framework of the *mvabund* package v4.2.1 (Wang et al., 2012). Differential abundance analysis was performed on the non-rarefied dataset using *DESeq2* v1.42.0 (Love et al., 2014) and the differing taxa were visualised on the taxonomic tree using the *Metacoder* package v0.3.6 (Foster et al., 2017). The microbial abundance table was used to infer the mechanisms of bacterial community assembly by applying the phylogenetic bin-based null model methodology of the *iCAMP* package with the recommended settings (Ning et al., 2020). Significant environmental factors explaining variation in bacterial communities were assessed using constrained ordination (dbRDA; function *capscale()*) workflow in *vegan*. Parsimonious models for each treatment and depth combination were constructed using stepwise model selection based on AIC and significance of models and variables was subsequently tested with a permutational ANOVA (*anova.cca()*).

## 4.3 Results

### 4.3.1 Physicochemical parameters of the sediment layers

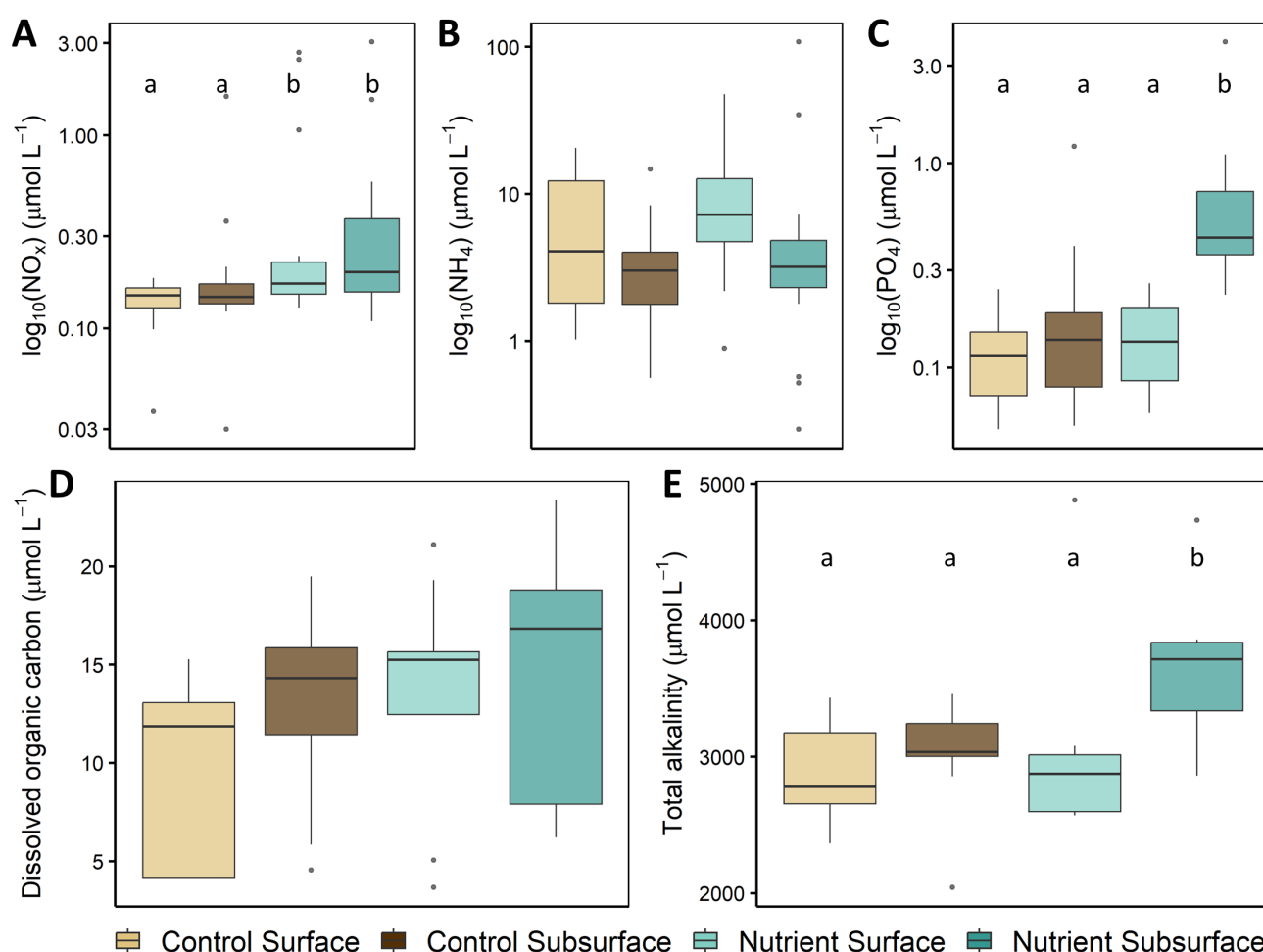
The sediment characteristics generally did not vary significantly between depth layers or treatments (Supplementary Figure A4.1A-I; Supplementary Table A4.1). While the average values of median GS, and TOM seemed to differ between the depth layers, this relationship was not significant (Supplementary Figure A4.1A, D; Supplementary Table A4.1). Similarly, the average amount of MB-C was higher in the nutrient treatments, albeit not significantly so (Supplementary Figure A4.1C; Supplementary Table A4.1). Water content of the sediments at low tide differed between treatments (Treatment;  $F\text{-test}_{(1,32)} = 4.68$ ,  $p\text{-value} = 0.03$ ) with an average of 57% and 61% for control and nutrient samples, respectively (Supplementary Figure A4.1B). This relationship may have been influenced by a slight gradient of mud content from plot 1 to plot 18 (OLS;  $r^2 = 0.23$ ,  $p\text{-value} = 0.003$ ; Supplementary Table A4.1). The pH, ORP, and salinity showed no significant differences between depths and treatments (Supplementary Figures A4.1G-I; Supplementary Table A4.1).

### 4.3.2 Porewater nutrient concentrations

Nutrient concentrations in the porewater showed a much weaker effect than anticipated after the enrichment treatment. The data were dominated by a few very high values and were characterised by a high level of variability. Nitrate/nitrite concentrations were slightly enriched in the nutrient treatment (ANOVA one factor Treatment;  $F\text{-test}_{(1,71)} = 10.75$ ,  $p\text{-value} = 0.002$ ; Figure 4.2A; Supplementary Table A4.2). The concentrations of ammonium (median (range) =  $3.56 \mu\text{mol N L}^{-1}$  (0.25 – 108.10)) were more than an order of magnitude higher than those of nitrate/nitrite (median (range) =  $0.16 \mu\text{mol N L}^{-1}$  (0.03 – 3.05)) but did not vary significantly between treatments or depths (Figure 4.2B; Supplementary Table A4.2). Phosphate concentrations (median (range) =  $0.15 \mu\text{mol N L}^{-1}$  (0.05 – 3.93)) were enriched in the subsurface layer of the nutrient treatment (Treatment  $\times$  Depth;  $F\text{-test}_{(1,68)} = 18.83$ ,  $p\text{-value} < 0.001$ ; Supplementary Table A4.2) and therefore, showed significant differences between the depth layers of the nutrient plots (TukeyHSD;  $p\text{.adj.} < 0.001$ ) and between the subsurface samples of control and nutrient samples (TukeyHSD;  $p\text{.adj.} < 0.001$ ; Figure 4.2C).

Dissolved organic carbon concentrations in the porewater were variable, but no statistically significant differences between the treatments or the depths could be identified (Treatment;  $F_{1,32} = 2.44$ ;  $p\text{-value} = 0.13$ , and Depth;  $F_{1,32} = 1.25$ ;  $p\text{-value} = 0.27$ ; Supplementary Table

A4.2). The average DOC concentration in the porewater was  $1050.83 \mu\text{mol L}^{-1}$  and mean values in the nutrient plots were slightly higher than in the control plots (Figure 4.2D). Total alkalinity however, showed significant differences between the depths and the treatments (Treatment  $\times$  Depth;  $F_{1,31} = 6.15$ ;  $p\text{-value} = 0.019$ ; Supplementary Table A4.2). Overall, TA concentrations at the sediment surface were lower ( $2950.99 \mu\text{mol L}^{-1}$ ) compared to those of the subsurface ( $3330.38 \mu\text{mol L}^{-1}$ ) (Depth;  $F\text{-test}_{(1,31)} = 12.58$ ,  $p\text{-value} = 0.001$ ; Supplementary Table A4.2) and this was mainly due to the high TA concentrations in the subsurface of the nutrient plots ( $3630.58 \mu\text{mol L}^{-1}$ ). The interaction effect was significant due to differences between depths in the nutrient samples (TukeyHSD;  $p\text{.adj.} = 0.001$ ), and the subsurface samples of the control and the nutrient samples (TukeyHSD;  $p\text{.adj.} = 0.02$ ; Figure 4.2E).



**Figure 4.2** Porewater concentrations of A nitrate/nitrite, B ammonium, C phosphate, D dissolved organic carbon, E total alkalinity, from different depths and treatments in mangrove sediments in New Zealand. The boxplots indicate the median with the interquartile range (IQR) between the 25<sup>th</sup> and the 75<sup>th</sup> percentile and the whiskers extend  $1.5 \times \text{IQR}$ . Lower case letters indicate statistical differences between levels. Where no such letters are displayed, no significances were detected.

### 4.3.3 Submerged fluxes

There was a slight efflux of  $\text{NO}_x$  from the sediments to the water column in the light chambers and a similarly slight influx in the dark, which was consistent across treatments. However, the statistical test showed only weak evidence to support this observation (Exposure;  $F_{1,32} = 3.70$ ;  $p$ -value = 0.063; Supplementary Table A4.3). The mean  $\text{NO}_x$  influx in dark chambers was  $-0.925 \mu\text{mol N m}^{-2} \text{hr}^{-1}$  and the mean efflux from the light chambers was  $1.002 \mu\text{mol N m}^{-2} \text{hr}^{-1}$ . It is important to note that the majority of the measured  $\text{NO}_x$  was in the form of nitrate with nitrite levels being close to or below the detection limit. The ammonium flux was not significantly different between the treatments or light exposures, with a median efflux of ( $1.51 \mu\text{mol N m}^{-2} \text{hr}^{-1}$ ). Phosphate fluxes were on average positive ( $0.767 \mu\text{mol P m}^{-2} \text{hr}^{-1}$ ) across all factors but no significant differences between light and dark, and nutrients and control were found (Treatment  $\times$  Exposure;  $F_{1,32} = 1.47$ ;  $p$ -value = 0.23; Supplementary Table A4.3).

Fluxes of DOC were significantly different between treatments (Treatment;  $F_{1,29} = 4.8$ ;  $p$ -value = 0.037; Supplementary Table A4.3). This was due to differences in the dark chambers with a negative flux in the control plots ( $-4.84 \text{ mg C m}^{-2} \text{hr}^{-1}$ ) compared to the nutrient plots ( $8.94 \text{ mg C m}^{-2} \text{hr}^{-1}$ ) (TukeyHSD;  $p$ .adj. = 0.06). In the light incubations the DOC fluxes averaged around  $-1.66 \text{ mg C m}^{-2} \text{hr}^{-1}$ . The oxygen flux from the sediment to the overlying water was overall positive in the light chambers (net primary productivity, NPP) and negative in the dark chambers (respiration) (Exposure;  $F_{1,31} = 7.34$ ;  $p$ -value = 0.011; Supplementary Table A4.3). However, it did not vary between treatments ( $F_{1,31} = 0.18$ ;  $p$ -value = 0.67). Sediment respiration was low, with a mean of  $-125 \mu\text{mol O}_2 \text{ m}^{-2} \text{hr}^{-1}$  for control plots and  $-360 \mu\text{mol O}_2 \text{ m}^{-2} \text{hr}^{-1}$  for nutrient plots. Mean NPP of the sediments was  $511 \mu\text{mol O}_2 \text{ m}^{-2} \text{hr}^{-1}$  and  $560 \mu\text{mol O}_2 \text{ m}^{-2} \text{hr}^{-1}$  for control and nutrient plots, respectively. The sediment was a source of total alkalinity to the overlying water as there was a small but net positive flux recorded in the chambers ( $23 \mu\text{mol m}^{-2} \text{hr}^{-1}$ ).

### 4.3.4 Emerged fluxes

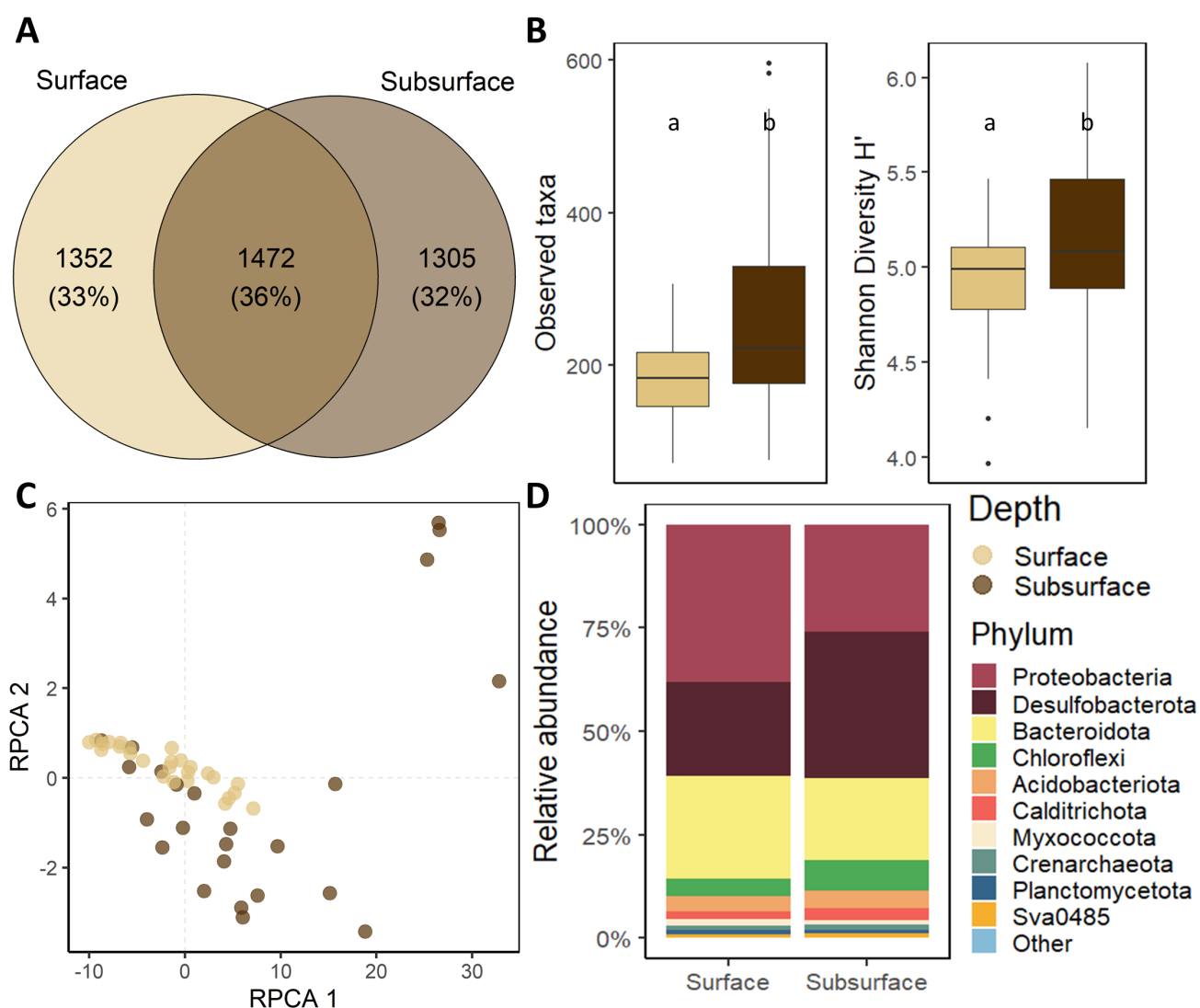
Gas fluxes of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  between the sediment and the atmosphere were all positive but did not detect effects from the enrichment treatment. The  $\text{CO}_2$  efflux was about one order of magnitude higher than the  $\text{CH}_4$  efflux, which again was about one order of magnitude higher than the  $\text{N}_2\text{O}$  emissions. The average  $\text{CO}_2$  efflux from control plots was slightly higher than in the nutrient plots, however, this difference was not significant (Treatment;  $F_{1,32} = 1.802$ ;  $p$ -value = 0.19; Supplementary Table A4.4) and possibly due to a few high measurements.



Across all treatments, the average  $\text{CO}_2$  efflux from the emerged sediment was  $39.86 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$ . Similarly, the  $\text{CH}_4$  efflux was not significantly higher from the nutrient plots as compared to the control plots (Treatment:  $F_{1,23} = 2.61$ ; p-value = 0.12; Supplementary Table A4.4). These results are affected by the high variability of the  $\text{CH}_4$  measurements which range from  $-0.28$  to  $14.37 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$  in the nutrient plots and  $0.36$  to  $6.33 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ . The efflux of  $\text{N}_2\text{O}$  was also highly variable but similar across all treatments (Treatment;  $F_{1,24} = 1.14$ ; p-value = 0.3; Supplementary Table A4.4). The average  $\text{N}_2\text{O}$  efflux from the nutrient plots in the light was the highest with rates of  $0.44 \mu\text{mol N}_2\text{O m}^{-2} \text{ hr}^{-1}$  compared to the overall mean of  $0.19 \mu\text{mol N}_2\text{O m}^{-2} \text{ hr}^{-1}$ .

#### 4.3.5 New Zealand mangrove sediment microbiome

The bacterial communities of mangrove sediments in Aotearoa New Zealand differed between surface and subsurface samples (2824, and 2777 ASVs, respectively) and were dominated by the presence of a few abundant, and a long tail of rare taxa (Supplementary Figure A4.3). Out of 4129 ASVs present, roughly one third was unique to the surface and another third unique to the subsurface layers, while the remaining ASVs (also about one third) were shared between the two compartments (Figure 4.3A). In the surface, most ASVs belonged to the phylum of *Proteobacteria* (relative abundance, 36.5%), followed by *Bacteroidota* (24.1%), and *Desulfobacterota* (22.1%). All other phyla made up the remaining 17.3% with no single contribution above 5% (Figure 4.3D). At class level, *Gammaproteobacteria* (31.4%), *Bacteroidia* (23.2%), and *Desulfobacteria* (11.1%) were dominant across samples. The same phyla and classes dominated the communities in the subsurface, however, the order changed to *Desulfobacterota* (33.8%; *Desulfobacteria* 23.6%), *Proteobacteria* (24.3%; *Gammaproteobacteria* 21.9%), and *Bacteroidota* (18.6%; *Bacteroidia* 17.8%). The remaining 23.3% of phyla in the subsurface were led by *Chloroflexi* (6.9%), and *Acidobacteriota* (4.1%) (Figure 4.3D). The subsurface layers contained more taxa per sample (Observed taxa; p-value = 0.001) as well as a higher diversity score (Shannon diversity; p-value = 0.007) than the surface layers (Figure 4.3B; Supplementary Table A4.5). This difference between depth layers in the control samples was visualised by robust aitchinson principal component analysis (RPCA) (Figure 4.3C). Multivariate analysis of the bacterial community composition confirmed a significant effect of depth (mvabund; LRT = 8666.032, p-value < 0.001, Supplementary Table A4.6) and specifically between depths in the control samples (pairwise test; LRT = 7189.7, p.adj. < 0.001).

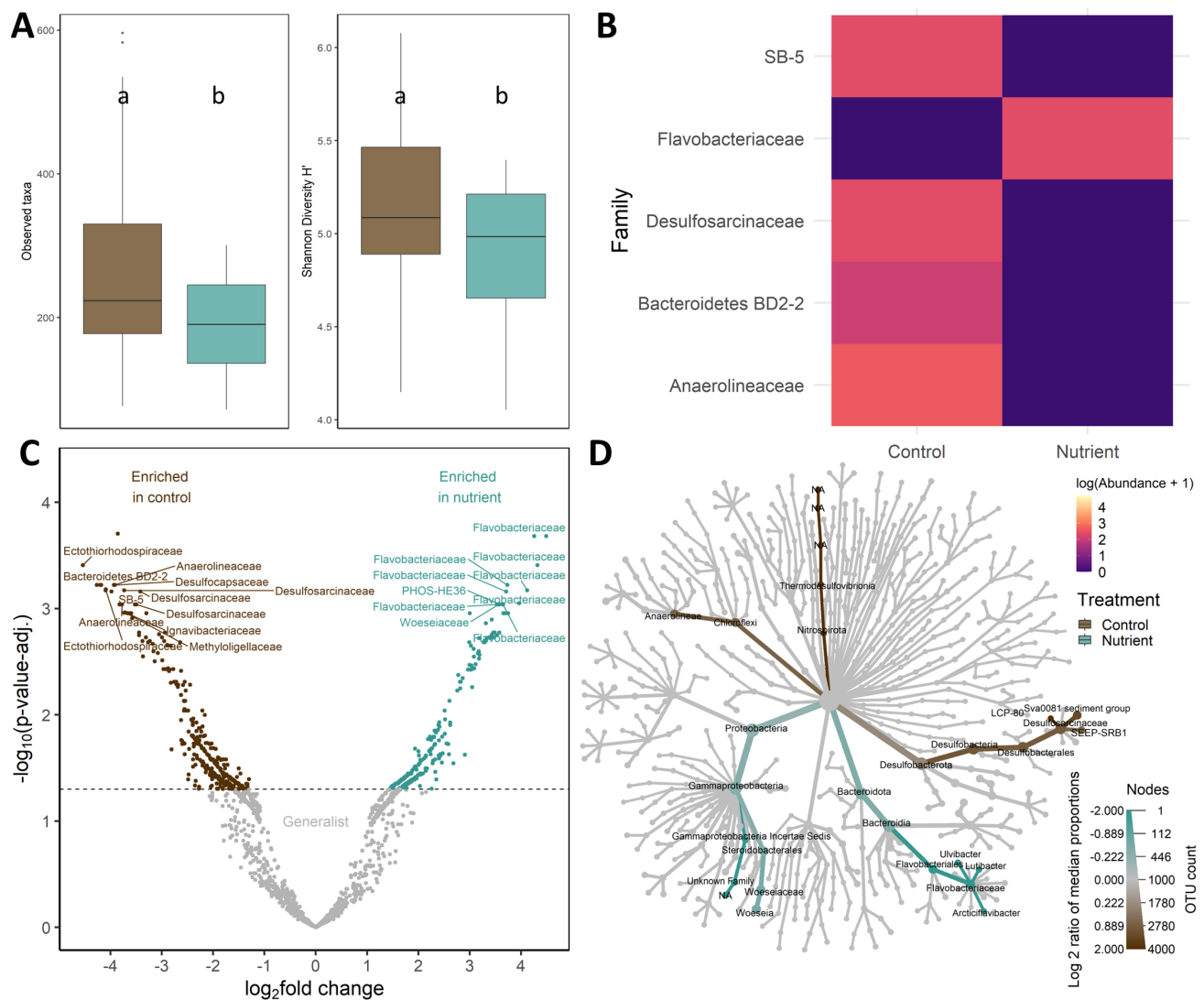


**Figure 4.3** Characterisation of differences in the bacterial communities between the surface and the subsurface samples in temperate mangrove sediments in Aotearoa New Zealand. **A** The Venn-diagram showing the total number of unique and shared ASVs between the two depth layers. **B** Alpha diversity, expressed by the number of observed species and Shannon diversity index. The boxplots indicate the median with the interquartile range (IQR) between the 25<sup>th</sup> and the 75<sup>th</sup> percentile and the whiskers extend 1.5\*IQR. Lower case letters indicate statistical differences between levels. **C** RPCA plot showing the differentiation of the two communities (beta diversity). **D** Relative abundance of taxa at phylum level showing the top 10 most abundant phyla (remaining grouped as "Others").

#### 4.3.6 Effects of nutrient enrichment on subsurface microbiome

The multivariate test identified a significant interaction effect (mvabund; LRT = 4803, p-value < 0.001). However, the bacterial community composition varied much less between treatments in the surface layers (pairwise test; LRT = 5066, p.adj. = 0.032) than in the subsurface layers (pairwise test; LRT = 6571, p.adj. = 0.002). Moreover, the number of observed species in the

surface layers did not vary significantly between treatments (Observed taxa;  $p$ -value = 0.17, Supplementary Figure A4.4) but were significantly lower in the nutrient compared to the control treatments in the subsurface ( $p$ -value = 0.02). The Shannon diversity index showed the same relationship of no significance between treatments in surface samples (Shannon diversity;  $p$ -value = 0.21) but significant differences in subsurface samples ( $p$ -value = 0.04). Therefore, we focus our attention on the comparison of the bacterial communities between treatments in the subsurface layer. The families identified to be most important drivers of the differentiation between treatments in the subsurface layer are generally more abundant in the control samples apart from the family *Flavobacteriaceae*, which is more abundant in the nutrient treatments (Figure 4.4B). Differential abundance analysis of the subsurface communities revealed a total of 636 differential abundant taxa of which 259 were significantly enriched in the nutrient samples and 377 were enriched in the control samples (Figure 4.4C). Taxa that were most significantly enriched in the control samples were from the phyla *Proteobacteria* (family *Ectothiorhodospiraceae*), *Bacteroidota* (family *Bacteroidetes* BD2-2, and SB-5), *Chloroflexi* (family *Anaerolineae*), and *Desulfobacterota* (family *Desulfocapsaceae*), among others. The most significantly enriched taxa in the nutrient samples included *Proteobacteria* (order BD7-8; and family *Woeseiaceae*), *Bacteroidota* (family *Flavobacteriaceae*), and *Acidobacteriota* (family *Thermoanaerobaculaceae*), among others. Overall, the nutrient treatment led to an increased abundance of *Proteobacteria* (*Gammaproteobacteria*) and *Bacteroidota*, while the control treatments supported higher abundances of *Desulphobacterota*, *Chloroflexi*, and *Nitrospirota* (Figure 4.4D).



**Figure 4.4** Diversity measures and distribution of differentially abundant taxa between the treatments in the subsurface samples. **A** Species richness and Shannon diversity index between both treatments. The box-plots indicate the median with the interquartile range (IQR) between the 25<sup>th</sup> and the 75<sup>th</sup> percentile and the whiskers extend 1.5\*IQR. Lower case letters indicate statistical differences between levels. **B** Taxa at family level, that drive the differentiation between treatments in the subsurface layer as identified by the univariate adjustment of the mvabund algorithm. **C** Volcano plot showing the ASVs that are differentially abundant (twofold changes,  $p_{\text{adj}} < 0.05$ ) in control and nutrient treatments. The 30 most significant ASVs are presented at family level. **D** The significant ( $p_{\text{adj}} < 0.05$ ) differential abundance plotted on the taxonomic tree of the underlying community. Branches with teal colours are enriched in the nutrient treatments whereas brown branches are enriched in controls. The intensity of the colour represents the  $\log_2$  fold change and the size of the node represents its abundance within the dataset.

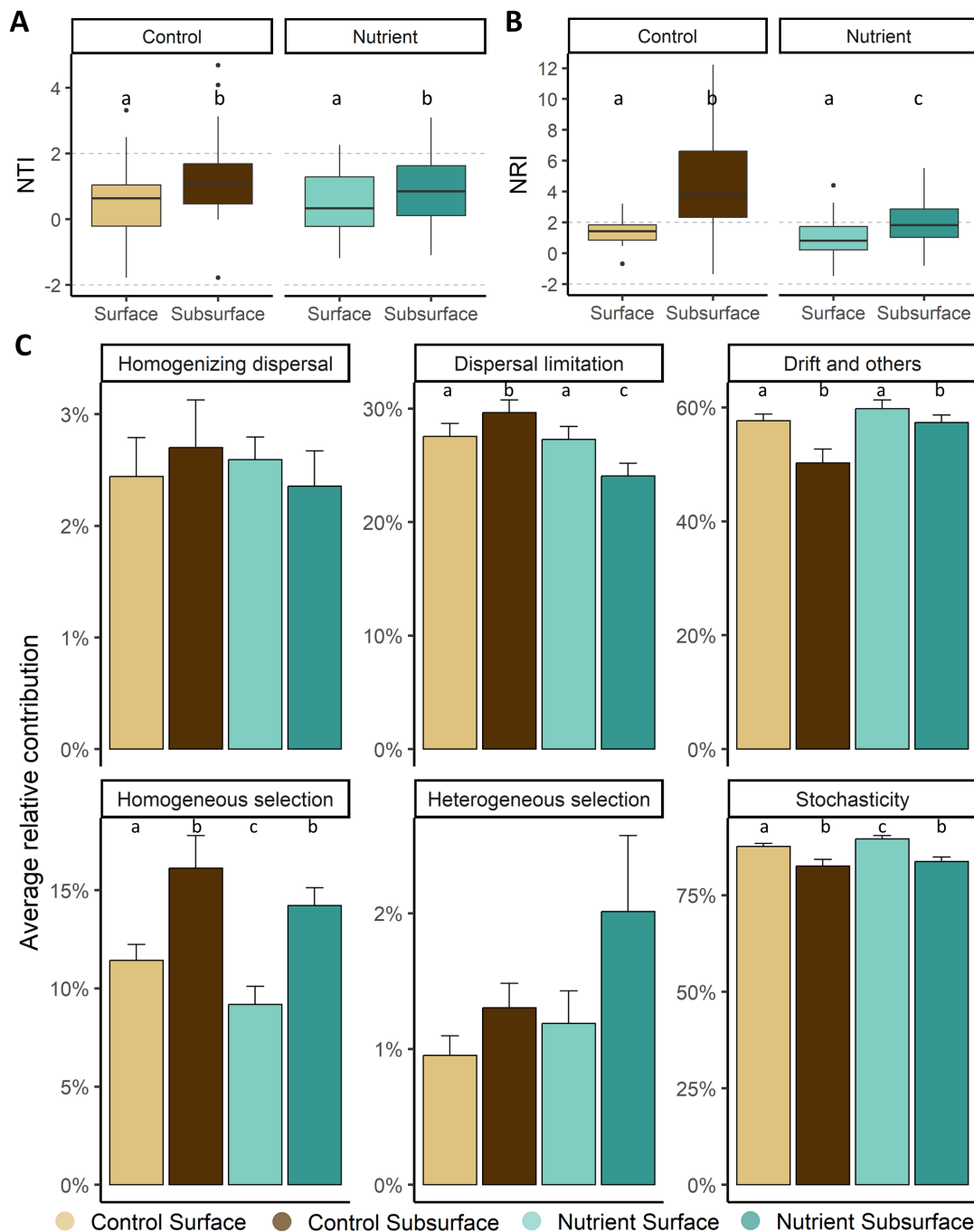
#### 4.3.7 Assembly mechanisms highlight differences between depth layers

Differences in phylogenetic patterns between samples were assessed using the NTI and NRI scores (Stegen et al., 2012). The NTI scores  $< 1.96$  indicated a dominance of stochastic processes across all samples with only marginally higher values in the subsurface samples

compared to the subsurface samples ( $F\text{-test}_{(1,99)} = 6.64$ ;  $p\text{-value} = 0.01$ , Figure 4.5A; Supplementary Table A4.7). The factor treatment showed no significant effect ( $F\text{-test}_{(1,99)} = 1.39$ ;  $p\text{-value} = 0.24$ ). The NRI score showed a significant interaction effect between treatment and depth ( $F\text{-test}_{(1,99)} = 4.58$ ;  $p\text{-value} = 0.03$ , Figure 4.5B; Supplementary Table A4.7) which was driven by differences between depths of the control samples (TukeyHSD;  $p.\text{adj.} < 0.001$ ) and differences between treatments in the subsurface layer (TukeyHSD;  $p.\text{adj.} < 0.001$ ).

The stochastic processes of drift and dispersal limitation were the most dominant assembly mechanisms identified by the iCAMP methodology with a relative contribution ranging between 41.7% - 64.5% and 23.7% - 32.9%, respectively, across all treatments and layers (Figure 4.5C). Of the deterministic mechanisms, homogeneous selection was the more dominant than heterogeneous selection (relative contribution, 6.7% - 22.9% and 0.5% - 4.4%, respectively). The surface samples differed significantly from the subsurface samples (Figure 4.5C; Supplementary Table A4.8). In the surface samples, effects of the nutrient treatment were only detectable in the homogeneous selection which showed a lower relative contribution in the nutrient samples (Cohen's  $d = 2.6$ ,  $p\text{-value} = 0.03$ , Supplementary Table A4.8). In the nutrient treatment, communities showed a significantly higher ratio of drift (Cohen's  $d = -3.6$ ;  $p\text{-value} = 0.005$ ), but a lower ratio of dispersal limitation (Cohen's  $d = 5.0$ ;  $p\text{-value} < 0.001$ ). The stochasticity (all stochastic processes combined: homogenising dispersal, dispersal limitation, and drift and others) explains between 75.8% and 92.0% of community assembly mechanisms from all samples. The stochastic processes are more important in the surface communities than in the subsurface, which is consistent across the two treatments (control, Cohen's  $d = -3.8$ ;  $p\text{-value} = 0.001$ ; nutrient, Cohen's  $d = -6.0$ ;  $p\text{-value} < 0.001$ ; Supplementary Table A4.8). However, the effect of the nutrient treatment in the subsurface is masked when assessing the combined stochasticity (Cohen's  $d = -0.8$ ,  $p\text{-value} = 0.3$ ).

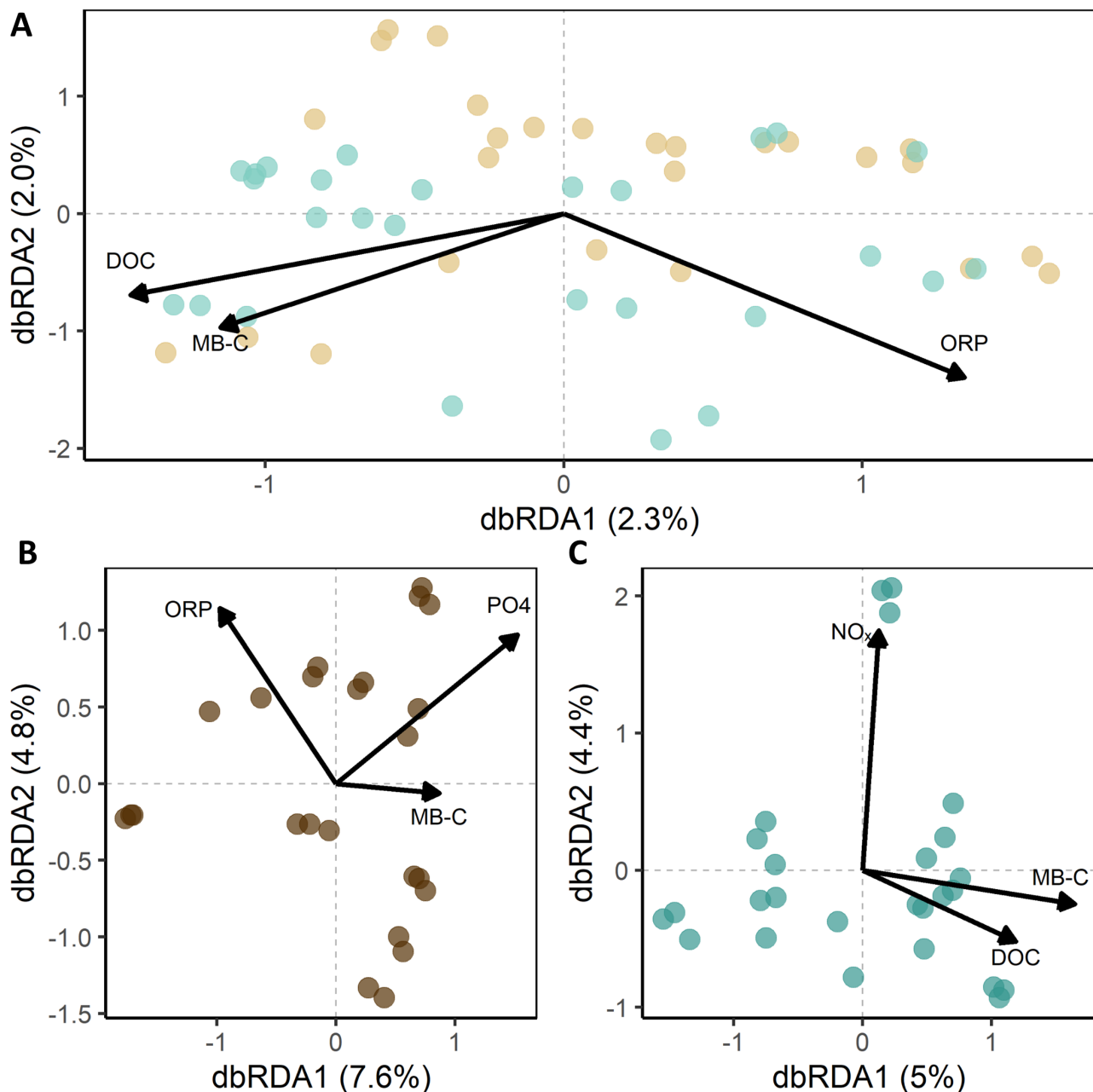




**Figure 4.5** Patterns of phylogenetic diversity structure of the bacterial community from different depths and treatments. **A** Nearest taxon index (NTI) is calculated from the mean distance to the nearest neighbour on the phylogenetic tree. **B** Net relatedness index (NRI) computes the mean pairwise distances between each taxon on the phylogenetic tree. Both indexes are fitted against null models to give a standardised measure of effect size of phylogenetic clustering or overdispersion. The area between the two dashed lines indicates that stochastic processes dominate. The boxplots indicate the median with the interquartile range (IQR) between the 25<sup>th</sup> and the 75<sup>th</sup> percentile and the whiskers extend 1.5\*IQR. **C** Average contributions of bacterial community assembly processes. These processes were calculated from distance based phylogenetic structure analysis following the iCamp methodology. Stochastic assembly processes (top row) are summed to calculate stochasticity (bottom right). Homogeneous and heterogeneous selection are deterministic processes. Bars and errors represent means and standard deviations of the permutational analysis. Lower case letters indicate statistical differences between levels. Where no such letters are displayed, no significances were detected.

#### **4.3.8 Environmental association between sediment variables and the microbiome**

Environmental factors influencing the microbiome in each treatment and depth layer were assessed by comparing the amount compositional variance that each variable was able to explain. In a model that included all samples across treatments and depths (permutational ANOVA of Bray-Curtis dbRDA; F-test<sub>(7,95)</sub> = 3.16, p-value = 0.001; Supplementary Table A4.9), water content (p-value = 0.01),  $\text{NH}_4^+$  (p-value = 0.01), ORP (p-value = 0.01), MB-C (p-value = 0.01) were returned as significant variables shaping the bacterial communities (Supplementary Figure A4.5). However, the two first dimensions were able to explain only 3% of variation within the community. The explanatory power of the ordination increased when looking at the treatment and depth combinations individually (Figure 4.6). In the surface layer (of the combined control and nutrient samples) the selected model was significant (F-test<sub>(3,49)</sub> = 1.05, p-value = 0.009; Supplementary Table A4.9; Figure 4.6A), but only DOC and ORP were returned as significant environmental variables (p-value = 0.05, and 0.05, respectively). The full model constraining the variance in the subsurface of the control samples was significant (F-test<sub>(3,20)</sub> = 1.37, p-value = 0.001; Supplementary Table A4.9; Figure 4.6B). The permutational analysis of the model terms returned  $\text{PO}_4^{3-}$  (p-value = 0.01), and MB-C (p-value = 0.01) as significant variables, whereas ORP (p-value = 0.07) indicated a weak level of association. In the subsurface of the nutrient samples, the ordination was significant (F-test<sub>(3,22)</sub>, p-value = 0.001; Supplementary Table A4.9; Figure 4.6C). Here, the significant environmental variables were MB-C (p-value = 0.01), DOC (p-value = 0.04), and  $\text{NO}_x$  (p-value = 0.04).



**Figure 4.6** Constrained ordination dbRDA, to represent the association of environmental variables with the bacterial community composition for **A** Surface samples (Control and nutrient combined), **B** Control Subsurface, and **C** Nutrient Subsurface. Variables for parsimonious models were selected independently for each ordination.

## 4.4 Discussion

In this study we assessed the sediment bacterial community response to a manipulative *in situ* fertilisation experiment in a temperate *A. marina* subsp. *australasica* mangrove forest in Aotearoa New Zealand. This study represents the first characterisation of the bacterial

community of New Zealand mangroves and to our knowledge only the second (after Palacios et al., 2021), to characterise the bacterial community composition in temperate mangrove sediments worldwide (Japan, South Africa, South Australia, New Zealand). To the best of the authors' knowledge, no other studies have described the 16S rRNA genetic diversity in temperate mangrove sediments of other mangrove species. Our results indicate a distinction between the communities inhabiting the surface (0-2 cm) and the subsurface (5-7 cm) layers, that is partly determined by the physicochemical environment and partly due to the homogenising force of hydrodynamic movement. No effect of the nutrient treatment was detectable in the surface communities, but a legacy of the fertilisation effect remained in the subsurface, indicating prolonged disturbance effects in the less dynamic environment. Here we characterise the naturally occurring (control) bacterial community of New Zealand mangrove surface and subsurface sediments, before discussing the effects of nutrient enrichment on the subsurface communities including community assembly mechanisms and environmental associations.

#### **4.4.1 Microbial communities differ by depth**

The sediments of mangrove forests are spatially, and temporally variable environments dominated by strong physicochemical gradients and micro-niches that create a habitat for highly specialised and adapted organisms (Luis et al., 2019). Our results confirm this spatial compartmentalisation along a vertical depth profile and show the defining influence this has on the microbial communities inhabiting each depth layer, most likely due to hydrodynamic forcing and advective porewater flow (Böer et al., 2009). In dynamic shallow coastal environments, the surface layers of sediments are consistently disturbed through tides, wind, and waves. This leads to increased exchanges of solutes and organisms between the sedimentary surface layer and the overlying body of water. Previous work has demonstrated the homogenising effects of increased dispersal through hydrodynamic disturbance on sedimentary microbial communities (Fodelianakis et al., 2019; Chen et al., 2021; Thomson et al., 2022b). While abundance and diversity of the bacterial community generally decreases with depth (von Hoyningen-Huene et al., 2022), this study found an increase of both metrics in the subsurface layers. This is most likely an artifact of the shallow sampling depth of even the subsurface layer (5-7 cm) at which defining environmental factors such as TOM and porewater nutrients are still high and variable. However, the fact that the sedimentary variables did not show significant differences between depths suggests that bacterial variation between surface and subsurface layers are due to unobserved differences, such as light exposure and the

availability of alternative electron acceptors other than oxygen (Sinkko et al., 2019; Chen et al., 2021; Bonthond et al., 2023). Most dominantly, the relative abundance of *Desulphobacterota* (and more specifically the family *Desulfosarcinaceae*) was higher in the subsurface samples. Members of these groups have been associated with a sulphate reducing metabolism and are predominantly strict anaerobes (Kuever, 2014; Galushko and Kuever, 2019). The genera SEEP-SRB1, and Sva0081 sediment group, from the family *Desulfosarcinaceae*, have been observed as important consumers of acetate and H<sub>2</sub> in marine sediments indicating the importance of these metabolic pathways in mangrove sediments (Dyksma et al., 2018a, 2018b). In contrast, the increased occurrence of *Gammaproteobacteria* (family *Woesiaceae*) and *Alphaproteobacteria* (families *Rhodobacteraceae* and *Ectothiorhodospiraceae*) in the surface layers emphasises these environments as hotspots of coupled carbon and sulphur cycling (Pujalte et al., 2013; Dyksma et al., 2016).

#### 4.4.2 Nutrient enrichment effects remain detectable in the subsurface community

The level of enrichment as represented by the porewater concentrations of dissolved nutrients was not as strong as expected based on previous studies using this methodology (Douglas et al., 2016; Gladstone-Gallagher et al., 2020). This may be due to the longer incubation period chosen for this study (4 months versus 10 weeks) or differences in system properties such as sediment grain size and inter-sedimentary hydrodynamic flow. While the period of continuous fertilisation of the fertiliser used was specified as 5-6 months, these times may be greatly reduced for usage in waterlogged, dynamic coastal sediments. Very high outliers measured in the porewaters of some nutrient plots indicate an enrichment legacy in some distinct areas of the plots, whereas higher mud content may have limited the diffusion from the locations where fertiliser was added.

Despite the weak signal of a remaining fertilisation effect, the microbial community differed significantly between treatments. This difference was especially pronounced in the subsurface samples, while the surface samples showed no (or only weak) changes between the treatments. In the nutrient treatment, the alpha diversity metrics decreased relative to the controls, indicating a simplification of the bacterial community. Nutrient enrichment has been reported to decrease microbial diversity in a range of ecosystems (Thrush et al., 2006; Aires et al., 2019; Xu et al., 2022), potentially due to the alleviation of selective pressure, giving a competitive advantage to a few generalist taxa (Zhang et al., 2019b; Ferguson et al., 2021). Differential abundance analysis revealed that organisms from the phyla *Desulfobacterota*,



*Chloroflexi*, and *Nitrospirota* were significantly associated with the control treatment, while *Proteobacteria*, and *Bacteroidota* were associated with the nutrient treatments. Increased abundance of the *Gammaproteobacterial* family *Woeseiaceae* in nutrient enriched sediments is hard to interpret since this widely distributed and prevalent family has been reported as functionally extremely variable and plastic (Hoffmann et al., 2020). In genomes recovered from coastal sediments, genes for hydrogen- and sulphur-based chemolithoautotrophy have been identified, as well as the potential for denitrification (Baker et al., 2015; Musmann et al., 2017). Members of the family *Flavobacteriaceae*, the most abundant family in the phylum *Bacteroidota*, were enriched in the nutrient treatment. These taxa have been associated with the digestion of complex polysaccharides (McBride, 2014). The production of marine macroalgae, as well as mangrove biomass may be enhanced with increased nutrient availability potentially facilitating this shift. In the phylum of *Desulfobacterota*, *Desulfobulbales* were enriched in the nutrient samples, while *Desulfosarcinaceae* were enriched in the control samples. Members from both families are known to be mainly sulphate reducers, however, recent work has proposed them as important  $H_2$  scavengers in marine sediments (Dyksma et al., 2018b). The functional effects of this differentiation of these two families driven by the nutrient enrichment are, however, unknown (Ward et al., 2021). While several other studies reported changes in prominent nitrogen cycling taxa (Craig et al., 2021; Dai et al., 2021; Xie et al., 2022), the legacy effects in the communities observed in this study indicate changes to sulphur cycling taxa and the breakdown of more complex organic matter. This indicates a legacy effect of nutrient enrichment in the tightly coupled sulphur and carbon metabolisms that outlasts the geochemical enrichment signal in the porewater. The functional importance of this legacy however remains speculative and further research is needed to evaluate its effects on carbon dynamics in mangrove forest sediments.

#### 4.4.3 More deterministic bacterial community assembly processes in the subsurface layers

The dominance of stochastic community assembly mechanisms, and in particular phylogenetic drift, in mangrove sediments corroborates the findings of previous studies from Saudi Arabia and China (Thomson et al., 2022a; Liu et al., 2023). While the stochastic processes remain dominant across all samples, they decrease significantly in the subsurface compared to the surface samples which is likely due to the increased specificity of the subsurface community regarding niche space and resource limitation (Luis et al., 2019). The study from Saudi Arabia found that more restricting conditions in the sediments due to reduced tidal influence resulted

in a stark decrease of phylogenetic drift and a concurrent increase in dispersal limitation in shaping the bacterial community (Thomson et al., 2022a). Studies from various terrestrial systems report a much lower influence of stochasticity (range 20-55%) than rates reported from mangrove forests (range: 55-92%) (Gu et al., 2022; Li et al., 2022; Chen et al., 2023b, 2023a). Interestingly, other studies from aquatic systems (lake and subtidal coastal sediments) also report relatively low contributions of stochasticity to bacterial assembly processes (20%, and 30 – 60%, respectively), which suggests that high stochasticity may be a tidally-driven phenomenon (Gong et al., 2022; Pearman et al., 2023). West and Whitman (2022) were able to demonstrate that even infrequent mixing of soil samples resulted in a substantial increase of stochastic assembly processes in microbial communities. This likely explains the observed differences between the 'conserved' subsurface and the 'disturbed' surface layers in mangrove forests, as well as the homogenising influence of tidally driven water movement in intertidal areas. The depth related differences in assembly mechanisms were weakened (or reversed) in the nutrient treatments, leading to assembly patterns in the subsurface of the nutrient samples that were more similar to those of the surface samples. This shows a potential homogenising effect that the excess of a limiting resource may have on the community.

#### **4.4.4 Environmental sensitivity of microbial communities may be reduced by nutrient enrichment**

Environmental variables that were associated with the bacterial community of the natural (control) samples differed partly between the surface and the subsurface layers, but the strength of this relationship was higher in the subsurface samples as indicated by the overall model fit and the variation explained by the first two axes of the constrained ordination (Figure 4.6A, B). Variables representing nutrient enrichment were only important in the subsurface, while MB-C, DOC, and ORP appeared to be important drivers regardless of the depth layer. The fact that nitrate/nitrite was a better predictor of the bacterial community in the enriched subsurface is interesting as ammonium is by far the most abundant form of nitrogen present in the sediments (Figure 4.2A, B). Previous studies have highlighted a sensitivity of microbial communities to nitrate concentrations (Wang et al., 2016), however, others have found ammonium to be the stronger predictor of community composition (Fernandes et al., 2022). A recent study by Wang et al. (2021) found the mangrove forest to be a permanent sink of nitrate, despite high levels of ammonium. The levels of total organic matter (displayed throughout the literature in various associated forms, i.e. total carbon, organic matter, sediment organic carbon, etc.) are well known to exert a strong influence

on the microbial communities in mangrove sediments (Fernandes et al., 2022; Fiard et al., 2022; Hu et al., 2022). In the subsurface,  $\text{PO}_4^{3-}$  and MB-C were both significant drivers of the community composition, with water content and ORP showing only weak associations.

In the fertilised subsurface samples, the strength of the bacterial association with environmental variables decreased, possibly indicating a disassociation of the microbial community from the sedimentary environment with eutrophication. However, as in the control treatments, the environmental drivers explained more variation in the bacterial community in the subsurface layers, corroborating our previously stated hypothesis, that more specialised taxa inhibit the less disturbed subsurface sediments. DOC and MB-C were both important variables in constraining bacterial community variance in the nutrient treatments. Increases in microbial biomass have been reported from enrichment studies and likely reflect the increased energy supply to the system (Craig et al., 2021). The correlation with DOC may well be reversed, in that the communities present in enriched sediments produce more DOC which would also explain the increased fluxes of DOC to the overlying water in the dark incubations (Supplementary Figure A4.2E).

#### **4.4.5 Ecosystem function responses to nutrient enrichment**

A major challenge in microbial ecological studies is to bridge the gap between observed differences in community structure and measurable changes in ecosystem functions at reasonable scales (Bier et al., 2015; Hall et al., 2018). While microbial processes underly all major organic matter conversions, transient changes in responses to disturbances must be evaluated by their effect size and impact relative to the status quo. In this study, we were unable to identify significant changes in the majority of processes which we observed. This is in large parts due to the extremely high variability of the recorded data, combined with inherent spatial and temporal variability in the studied ecosystem. Finer sampling techniques are needed to resolve this variability for more accurate inclusion into predictive models. Furthermore, the influence of bioturbation by macrofauna on the local variability of nutrient concentrations and microbial communities needs to be taken into account (Booth et al., 2023). Generally, the system studied here presents a source of all three measured greenhouse gases to the atmosphere at low tide, while being an overall sink of DOC at high tide. With the exclusion of photosynthesis (dark chamber measurement), however, the fertilised sediments became a source of DOC to the overlying water column, potentially signalling increased plant or microbial activity in the fertilised plots. This hypothesis of increased heterotrophic

activity is supported by increased concentrations of total alkalinity in subsurface porewaters of enriched plots which may be due to an increased metabolism of microbial consumers (Krumins et al., 2013; Middelburg et al., 2020; Saderne et al., 2020). However, the signal of these differences is fairly small, and more detailed work is needed to verify their importance to the carbon cycle in mangrove ecosystems.

## **4.5 Conclusion**

This study presents a first overview of the bacterial community composition in temperate mangrove sediments from Aotearoa New Zealand and adds a valuable datapoint to the global distribution of bacterial communities in these important habitats. Furthermore, we were able to show that the contrasting environments of the surface and the subsurface layers resulted in different signal related to nutrient enrichment of the sediment. While the dynamic and tidally disturbed surface sediments showed no response to nutrient additions after 4.5 months, the community composition of the subsurface layers varied between treatments, even after the chemical signal could not be detected anymore. These findings highlight the importance to better understand microbial responses to anthropogenic disturbances and underscore the need for longer term monitoring of effects after enrichment events. Subtle shifts in the microbial community composition may have far reaching consequences on the cycling of major nutrients in mangrove sediments. Our results indicate a legacy of altered sulphur (and associated carbon) cycling after the enrichment effect has worn off, which likely has implications for the continued loss of carbon from the sediments.

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**The mangroves** that fill pockets of the Purangi estuary on the Coromandel Peninsular are some of the tallest I encountered during this field campaign growing up to 6 m tall. In many places in New Zealand, stunted growth forms predominate in response to the restricting conditions including temperature and wind exposure. However, when the conditions are right, they can grow to formidable size.





# 5 | Synoptic discussion

## 5.1 Significance and key findings

Coastal ecosystems worldwide are under enormous pressure from land-derived stressors due to an ever-increasing human footprint on the planet (Doney, 2010; Steffen et al., 2015). Mangrove forests have been proposed as sentinels of our coasts due to their ability to filter and cycle nutrients, retain heavy metals and plastic pollution, while at the same time sequestering more carbon than most other systems (Reef et al., 2010; Donato et al., 2011; Martin et al., 2020). However, they seem to also emerge as vulnerable when having to compete with coastal developments (i.e. competition for space), or when nutrient enrichment leads to physiological changes that reduce their resilience to environmental change (Duke et al., 2007; Lovelock et al., 2009; Polidoro et al., 2010). This existing dichotomy in our understanding of the effects of anthropogenic pressures on mangrove ecosystems calls for a more nuanced investigation of the ecological processes affected by land-derived stressors.

This thesis contributes to a more comprehensive understanding of the effects of catchment human land use on temperate mangrove forests and its direct and indirect influences on important ecosystem functions. The three data chapters combined, provide a line of sight from human land use intensity in the catchments to regionally generalisable trends of changes in forest structure and physicochemical parameters in the sediments (Chapter 2), and how these environmental factors influence the important process of organic matter degradation (Chapter 3). Furthermore, the effects of nutrient enrichment, a prominent anthropogenic stressor (also identified as such in Chapter 2 and 3) are shown to outlast its biogeochemical signal in the subsurface sediments in mangroves (Chapter 4) (Figure 5.1).

Overall, this work provides a holistic view on the effects of anthropogenic impacts on temperate mangrove ecosystems across multiple scales and identifies areas of future research to better understand and manage human interactions with this important coastal ecosystem.

## 5.2 Summary of results

The three research chapters in this thesis are based on a combination of observational data utilising environmental gradients and *in situ* experiments to assess the effects of land-derived

stressors on mangrove ecosystems and their functioning. Considering the main research question of this thesis, I can conclude that:

*Human land use impacts multiple aspects of mangrove ecosystems related to forest structure and physicochemical variables in the sediments, some of which directly influence bacterial communities in the sediments and the rate at which organic matter degrades. Therefore, increasing human land use can lead to a cryptic ecological degradation in mangrove ecosystems that may limit the carbon storage potential of the system.*

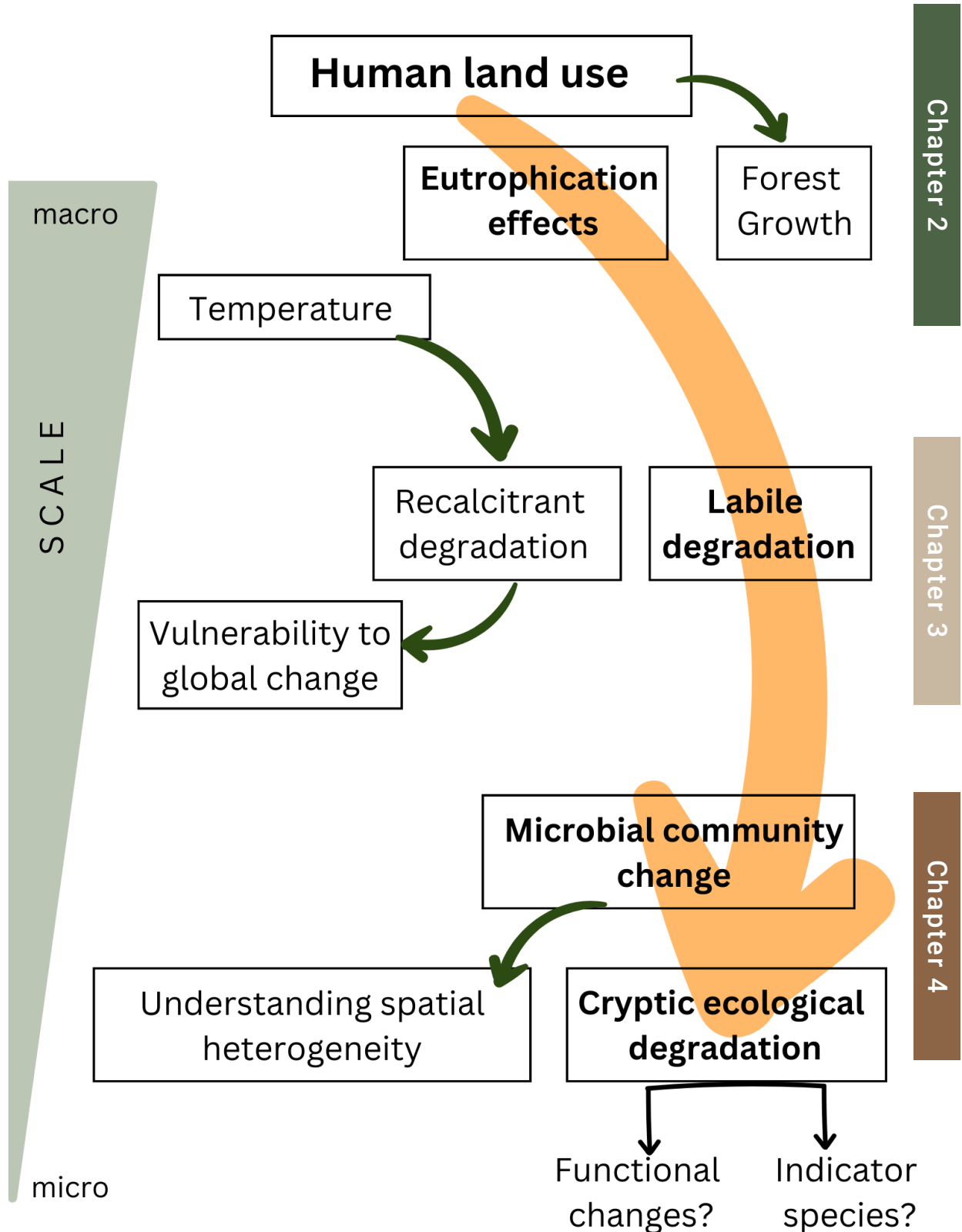
More specifically, I can draw the following conclusions from each chapter:

1. **Mangrove forest structure and several sedimentary variables relating to eutrophication are directly impacted by the amount of human land use in the catchments.** I was able to show across a wide range of sites, that higher amounts of area in the catchments that was utilised for human land use (urban and agricultural areas), related to denser forest growth and increased reproduction. This is a commonly observed effect in the early stages of eutrophication. Moreover, human land use was correlated with changes in porewater chemistry that suggest nutrient enrichment as a strong driver of ecological change. This chapter highlights the need for an integrated approach when evaluating anthropogenic impacts on complex natural systems.
2. **Eutrophication accelerates the rate of labile organic matter loss from mangrove sediments, while degradation of more recalcitrant material is related to sediment temperature.** The comprehensive analysis of environmental variables together with the deployment of standardised organic matter substrates enabled me to identify environmental controls of organic matter degradation in temperate mangrove forests. The strength of the association and the environmental factors controlling degradation varied between substrates. This finding is important to consider to accurately model degradation processes in coastal ecosystems. Furthermore, variables related to human-induced eutrophication (see Chapter 2) were associated with the accelerated loss of labile organic matter, while the climatic variable temperature controlled the breakdown of the more stable substrate. These findings indicate a weakening of the carbon storage potential through accelerated loss of labile organic matter in response to anthropogenic impacts.
3. **Nutrient enrichment remains detectable in subsurface bacterial communities of temperate mangrove sediments, even after the biochemical signal is lost.** This chapter provides the first dataset on bacterial communities in mangrove sediments

in Aotearoa New Zealand and one of the first in temperate mangrove sediments worldwide. Differences between the surface and subsurface layers of mangrove sediments, were most likely due to the homogenising force of tidal movement, resulted in contrasting effects of nutrient enrichment on the bacterial communities. At 4.5 months after the fertilisation an effect was detectable in the subsurface layer, however the surface bacterial community was indistinguishable from control sediments. With this *in situ* manipulative field experiment I was able to show, that changes in the bacterial communities due to nutrient enrichment prevailed for longer than the elevated concentrations of nutrient in the porewater. The differences were mainly in the abundance of carbon and sulphur cycling taxa, indicating that a legacy of enrichment may influence these important nutrient cycles. These findings highlight the spatial resolution and understanding needed to detect impacts on the bacterial communities in heterogeneous mangrove sediments. Moreover, it warns that functional changes in the microbial community may prevail even after the eutrophication effect is no longer detectable.



## The Eutrophication Cascade



**Figure 5.1** Conceptual figure showing the eutrophication effect detected in Chapter 2 and how this human impact drives changes in ecosystem processes (Chapter 3) and microbial community structure (Chapter 4).

## 5.3 Synopsis

Human activities on land influence adjacent coastal areas and more often than not, these interactions compromise the ecosystem's functions, services, and resilience. A vast field of research addresses the issue of anthropogenic impacts, and in particular of eutrophication, on coastal ecosystems and the isolated responses of ecosystem components such as growth and nutrient cycling in trees, associated biota, or ecosystem processes (Supplementary table A1.1). Throughout this thesis I was able to identify eutrophication as an important anthropogenic stressor at landscape scale (Chapter 2), trace some of its relevant effects on ecosystem processes (Chapter 3), and provide evidence of ecological homogenisation at the micro scale (Chapter 4). I clearly illustrate the need for a holistic view of disturbance effects on ecosystems to avoid the premature evaluation of processes taken out of context or viewed in isolation. Ecosystems are complex kaleidoscopes of interacting parts which change on temporal and on spatial scales and influence each other in a multitude of ways. Capturing this multitude is one of the greatest challenges in ecological science but also one of its most important objectives.

### 5.3.1 Cryptic effects of nutrient enrichment

In the specific example of mangrove forests, the effects of elevated nutrient levels on mangrove trees have been extensively documented. Most studies report an increase in growth through the alleviation of limiting nutrients which can be evaluated as an elevated ecosystem service through biomass production and carbon fixation (Feller et al., 2010). However, emerging literature (including this thesis) warns of cryptic ecological degradation in response to eutrophication that may become apparent through a reduced resilience of the trees to extreme weather events (Lovelock et al., 2009), reduction in other ecosystem services such as the increased loss of labile organic matter (Chapter 3; Ouyang et al., 2023), or subtle but lingering effects of a changed microbial community metabolism (Chapter 4). Capturing this 'unseen' ecological deterioration is extremely important for an accurate assessment of the effects of external stressors on the health and sustained functioning of ecosystems. I show here that microbial indicators may be a sensitive tool for tracking environmental impacts in mangrove forest sediments due to their collective 'memory' that can outlasts the specific disturbance effect (Chapter 4). While this is by no means an original thought, further evidence from my work suggests that it is of critical importance to understand the spatial heterogeneity present in the studied system to maximise the likelihood of detecting an effect.

### **5.3.2 Human land use drives changes at landscape scale**

When evaluating the results from Chapters 2 and 3, it is important to consider the scale at which the assessments were conducted. I utilised an existing gradient of human land use, within a relatively narrow latitudinal range to reduce confounding factors of climatic variation, while site-specific variation of environmental factors remained substantial. Nonetheless, I observed clear patterns of human influence on several components of mangrove forests, that transcended site specific differences. Furthermore, the predictor used in this study was solely based on the relative amount of area in the catchment that was utilised for either agricultural or urban practices and neglected differences in more specific land use intensities, or the connectivity between the catchment and estuary due to freshwater inputs. I therefore consider the effects detected in Chapters 2 and 3 as fairly conservative indicators of anthropogenic impacts on mangrove forests in New Zealand, that hold across a wide regional scale (Thrush et al., 2000). In line with the existing literature, I found some tree-specific variables to be positively influenced by human land use (Lovelock et al., 2010), while porewater variables showed signs of eutrophication and an altered biochemical state (Barroso et al., 2022; Conrad et al., 2023). The fact that increased human land use promotes mangrove forest expansion in estuaries of New Zealand is of particular local interest, as management efforts in many places aim to reduce their spread (Le Heron et al., 2022). This thesis presents regional-scale evidence, that management strategies should focus on mitigating eutrophication and sedimentation at the catchment level, rather than the clearance and destruction of established ecosystems which comes with significant legacy effects to the cleared areas (Lundquist et al., 2012; Bulmer et al., 2017; Stokes et al., 2023).

A noteworthy observation from Chapter 2 was the fact that forestry related land use (exotic forest plantations and cleared area from harvest) was indistinguishable from native forest, in its effects on mangrove systems. Most likely, the multi-decadal rotations of the forestry sector in New Zealand mean that during growth seasons, the effects in the estuaries are similar to those of native forests, while directly after harvest, a signal of increased sedimentation in the receiving ecosystems may be expected (Fahey et al., 2003; Phillips et al., 2005). This pattern is likely exacerbated by severe weather events which act as a vector to transport the vulnerable surface sediments to the coasts, as evidenced by recent severe weather events (Page et al., 1999; Marden et al., 2023).

### 5.3.3 Decomposition emerges as vulnerable to human land use and temperature

The human land use gradient presented in Chapter 2 further enabled me to assess the drivers of degradation processes in mangrove forests, by providing contrasting environmental settings across the different forests. This study confirmed previous work from terrestrial soil systems by indicating that organic matter which differs in its structural composition (for example leaves compared to wood) is controlled by a different set of environmental variables (Fierer et al., 2005; Qin et al., 2019; Jia et al., 2020). In mangrove sediments, the more labile components are broken down fairly quickly which is generally controlled by the chemical signature in the sediments and foliar nutrient composition. Importantly, I show, that the rate at which the labile organic matter loss occurs is strongly influenced by the level of eutrophication in the system. This relationship was represented by the  $\delta^{15}\text{N}$  values of the leaves, which is a proxy commonly used as longer-term integrative measure of nutrient enrichment, and nitrate concentrations in the porewater, both being important predictors of degradation of labile material. These relationships between nutrient enrichment and increased litter degradation were corroborated in a recent review on litter decomposition in blue carbon habitats (Ouyang et al., 2023). While these results suggest losses of organic matter in eutrophied systems, they also provide a mechanism for the informed management and optimisation of carbon stocks in mangrove ecosystems. Furthermore, nitrate concentrations above a threshold of about  $1 \mu\text{mol N L}^{-1}$  seemed to increase the temperature sensitivity of labile organic material. This finding is consistent with work by Wang et al. (2016), who show that the temperature dependence of aquatic microbes is greatest at extreme nutrient levels. This suggested an interactive relationship between nutrient concentrations and temperature, that has major implications for carbon storage in mangrove forests amid the challenges posed by climate change.

In contrast to the results highlighted above, the breakdown of more recalcitrant material was mainly controlled by temperature with sedimentary environmental variables being of lesser importance. This means that an increase in sediment temperature due to climate change will disproportionately affect the vulnerability of the more recalcitrant fraction of degrading organic material, further reducing the carbon storage efficiency of the system (Fierer et al., 2005; Davidson and Janssens, 2006; Craine et al., 2010). Specifically, the lower end of daily temperature variation was more associated with the degradation of organic material, suggesting that the daily temperature minima, instead of the maxima, are limiting to the microbial metabolism in temperate regions. These findings hold implications for understanding shifts in microbial process rates in a changing and warming world. Moreover, this finding

links into the discussion from Chapter 4 where the shift of the community metabolism after enrichment potentially enables the breakdown of more complex carbon sources and an alleviation of temperature inhibition could provide additional energy for this process. Lastly, the finding of a temperature threshold at lower daily temperatures will be important to consider when comparing degradation processes in temperate and in tropical systems. While this threshold may be present and limiting in a temperate setting, it may be surpassed in tropical sediments with other factors becoming more limiting to the microbial activity. Based on this line of thinking, the consequences of nutrient enrichment in tropical sediments may have a stronger impact on the losses of recalcitrant plant material, given that the limiting temperature threshold has already been exceeded. While these results advocate for caution of positive feedback loops induced by anthropogenic nutrient enrichment in interplay with global change in mangrove forests, it is important to note, that the data supporting these hypotheses is scarce and the connection in many places is theoretical.

#### **5.3.4 Ecosystem variability changes microbial response to fertilisation**

The degradation processes observed in Chapter 3 are majorly controlled by the microbial activity in the sediments. Therefore, a common approach is to understand and quantify the relationship between microbial communities and processes to predict how external influences may affect ecosystem functions (Fierer et al., 2007; Hall et al., 2018; Morrissey et al., 2023). This is currently a very active field of research and success in answering such questions has been variable (Bier et al., 2015; Fierer et al., 2021). This thesis, alongside existing literature, underpins the importance of understanding the temporal and spatial variability of the study system to ask hypothesis-driven research questions. The results I present in Chapter 4 illustrate this spatial heterogeneity and how it impacts the outcomes from the conducted enrichment experiment. By separating the surface and the subsurface layers I was able to show that the bacterial communities of the surface sediments seem to be influenced more strongly by homogenising forces, which was facilitated by stochastic community assembly processes. While I am unable to definitively identify the driving homogenising forces, I suspect tidal, and wind-driven hydrodynamic movement to be a major contributor (Chen et al., 2021). Furthermore, light and oxygen availability may play a role by alleviating energy deficiencies and creating conditions favourable for more generalist taxa. Bioturbation of the sediments by macrofauna may also play impact this process (Booth et al., 2023). This observed homogenising effect in the sediment surface eliminated the influence of nutrient enrichment on the microbial community composition, while it was sustained in the subsurface layers.



Highlighting this three-dimensional aspect of the spatial response adds to our understanding of the system and underpins the importance of more nuanced approaches appropriate to the scale of our subject.

Similarly, it is important to consider the temporal dimension of microbial community changes in response to external stressors. My results, in combination with the literature, suggest a natural succession of communities after a nutrient enrichment event. Nitrogen cycling taxa (and respective functional genes) dominate immediately after the enrichment has taken place (Craig et al., 2021; Dai et al., 2021; Erazo and Bowman, 2021; Palacios et al., 2021; Xie et al., 2022). However, after the excess nitrogen has been removed (through microbial pathways, tree uptake, and/or tidal flushing) I observed mainly changes in community composition that were related to the sulphur and carbon cycles, suggesting, that nutrient enrichment events leave a legacy of altered carbon cycling with unknown implications for the fate of carbon in mangrove sediments.

Lastly, I was unable to identify a clear response of emerged or submerged fluxes between the sediment and the overlying medium to nutrient enrichment. My results suggested an increased concentration of total alkalinity in the subsurface porewaters, which may be an effect of increased metabolic activity in that layer with increased nutrient availability (Saderne et al., 2020). Furthermore, dissolved organic carbon fluxes were higher from the fertilised sediments in the dark incubations. These results, together with the gas fluxes, which showed no responses to nutrient enrichment, suggest that effects of an accelerated microbial metabolism are more likely to be found in the dissolved phase (Appendix I: 6.5). This hypothesis has previously been postulated and it seems logical, that in generally waterlogged systems, measurable responses will more easily be detected in solution rather than gas efflux (Maher et al., 2013). And while the dissolved carbon may still eventually end up in the atmosphere, the multitude of intermediate processes and flows complicate the tracking of this process substantially (Sippo et al., 2017; Santos et al., 2021). This illustrates that the investigation of metabolic processes in waterlogged and tidally influenced sediments requires careful consideration of response variables and scale at a resolution relevant to the research question.

## 5.4 Future research directions

**Understanding system variability at scales relevant to research question.** Mangrove forests are highly dynamic and spatially variable ecosystems (Hu et al., 2021). My research underpins the need to better understand this variability in order to maximise the 'signal to environmental

noise' ratio in field studies. This task is made more difficult by the high inter-site variability at local and global scales. Therefore, I suggest that research is needed to understand levels of local ecosystem heterogeneity (spatially and temporally) at varying scales and identify the environmental factors driving it. Being able to predict ecosystem variability will help to design more efficient field studies and improve model estimates of spatially sensitive processes (Bernhardt et al., 2020; Raffeld et al., 2024). And while this is intrinsically important from an ecological perspective, it is also critical to maintain the scalability of estimates produced from ecological data (Schenone and Thrush, 2022).

However, the scale at which this variability needs to be considered will vary depending on the question that is being investigated. While this may be differences in geomorphic settings and mass balances at landscape scales (~km), the resolution relevant to microbial processes is much smaller (~ $\mu\text{m}$ ) and may have to do with particle sizes or local resource availability. I will use two examples from my thesis to illustrate this: At landscape scale, the integrated measures of environmental factors (in this case temperature, and foliar  $\delta^{15}\text{N}$ ) were the best predictors of the inherently temporal process of degradation (Chapter 3). And at micro scale, it was important to consider the spatial differences between the surface and the subsurface sediments to detect an enrichment effect in the microbial community (Chapter 4). At the very least, I suggest a good reporting standard of the spatial sampling efforts as well as the temporal conditions in respect to the scale of the research question, to be the minimum requirement to allow for comparisons between studies. A better understanding of the ecosystem variability will be greatly beneficent to the relatively understudied discipline of microbial ecology in dynamic coastal sediments, enabling cross study comparisons, and the quantification and scaling of important ecosystem processes (Green and Bohannan, 2006).

**Confining succession of enrichment events.** As I showed in Chapter 4, and discussed with the literature, nutrient enrichment events seem to follow through successional stages from an initial response to upregulated nitrogen cycling capacity and once the nutrient signal is gone, a legacy of carbon and sulphur cycling taxa that may or may not remain. When aiming to track nutrient flows after enrichment events and measuring the impacts of eutrophication a better understanding of the temporal dimension of these successional stages is needed. Results from lab-based incubation studies suggest a fast initial response that may be detectable in sediment effluxes or porewater concentrations. However, this effect may only last for a few hours. Observing such a response *in situ* and tracking its evolution and duration relative to the strength of the enrichment will be highly informative and a critical component to understand related nutrient processing in the sediments.

**Determining the definitive role of tree responses to nutrient enrichment on decomposition.**

Through the discussion of the existing literature, I have shown that tree responses to nutrients are fairly well documented. However, the role of trees in mediating nutrient availability for microbial primary producers in mangrove sediments remains unknown. Understanding the fate of enriched tree growth and its influences on microbially-driven processes in the sediments would enable us to quantitatively evaluate enrichment effects at the ecosystem level. While we have good evidence, that more labile growth occurs with enrichment (higher leaf to wood ratio), and that the nutrient retention efficiency of trees decreases (leading to higher quality litter), we have not been able to translate this into process rates of carbon accumulation and degradation. Closing this gap is essential for capturing the extent of eutrophication induced changes to the ecosystem.

**Interactive effects of nutrients and temperature on decomposition.** My results from Chapter 3 suggest an interaction effect between temperature and nutrient concentrations on decomposition processes. However, the temperature data is limited to a relatively narrow range and only a few high measurements of nitrate/nitrite were recorded. A conclusive confirmation of this interaction would have major implications for decomposition in a warming world and the vulnerability induced by anthropogenic pressures. Evidence already exists in the literature, that suggests that temperate sediments will experience higher rates of carbon remineralisation when exposed to elevated temperatures (Grisi et al., 1998). How this relationship will be altered with increased external nutrient availability is an important fragment of knowledge to improve our knowledge of risks of future change scenarios in mangrove ecosystems.

**Differences and similarities of temperate and tropical systems and the effects of global change on ecosystem processes.** The studies underlying this thesis were performed in temperate mangrove forests, which differ in many ways from their tropical counterparts. And while some of the findings presented here may be transferrable between the climate regions, it is likely that factors controlling ecosystem processes such as growth and decomposition, vary in their importance (Feher et al., 2017; Walden et al., 2023). Degradation in tropical systems has been shown to be nutrient limited, while in temperate systems it was controlled by temperature or carbon accessibility (Grisi et al., 1998; Lovelock et al., 2007; Ameray et al., 2021). Furthermore, responses to disturbance events in tropical systems may be stronger but shorter due to a faster metabolic rate of the ecosystem components. Identifying drivers that differentially effect processes in the respective climates will give an indication of how temperate systems may respond to topicalisation through global warming (Feher et al.,

2017). For example, understanding latitudinal differences between plant productivity and decomposition is important to predict the rate of organic matter accumulation into the future. If temperature effects degradation differently than it effects productivity, this mismatch may lead to increased vulnerability and losses of stored carbon from temperate mangrove forests.

**Terrestrial vs marine ecosystems.** Lastly, throughout this thesis I have increasingly become interested in the differences and similarities between coastal marine and terrestrial systems. The research on terrestrial carbon and nutrient cycling as well as microbial processes is decades ahead of marine research and using that vast pool of knowledge can only be beneficial for informed hypothesis making in the coastal space. However, in order to utilise the existing knowledge effectively, it is important to consider what separates these biomes at a basic level. While soil water content (synonyms: aridity, moisture content, mean annual rainfall) is a main limiting factor of soil processes, it is generally overabundant in coastal sediments. Similarly, microbial primary producers play an important role in coastal sediments, providing a labile source of organic matter that fuels a fast lived energy flow. Together with the dynamic nature of temporal changes to the system introduced by hydrodynamic forces, these factors seem to lead to a high level of resilience to disturbances often exceeding that of terrestrial ecosystems (Bowen et al., 2011). Studying these differences may provide important insights into ecological processes and help shape future research that is informed through transdisciplinary knowledge.

## 5.5 Concluding remarks

Anthropogenic activities are impacting coastal zones worldwide, however the full extent of their impacts on mangrove systems is difficult to assess. Due to their complex nature, multiple ecosystem components react differently to environmental conditions, which highlights the need for ecosystem-based approaches when trying to understand and manage human impacts on natural systems. Furthermore, the non-linearity of ecosystem responses to change can lead to contrasting results from different studies, further complicating the evaluation and the interpretation of 'real' effects. This thesis contributes to the understanding of anthropogenic impacts on mangrove forests at several scales, while attempting to retain a generalisable approach at understanding the intricate interplay of ecological processes and human-induced stressors on these vital coastal ecosystems.

At its core, this research signals a call for a nuanced understanding of the impacts of anthropogenic stressors on mangrove ecosystems. The findings underscore the necessity

to consider not only direct effects such as heightened productivity or reproductive success, but also the secondary effects on ecosystem processes like increased carbon degradation or the disruption of microbial communities. Thereby, this thesis draws attention to the potential ecological degradation taking place after enrichment events which is difficult to assess, but critical to track. The implications of this thesis extend beyond the temperate mangroves of Aotearoa New Zealand. The work contributes valuable insights into the global discourse on coastal resilience, providing a blueprint for addressing the challenges faced by similar ecosystems worldwide. It emphasises the importance of considering variability at multiple scales, urging researchers to understand and confine local ecosystem heterogeneity to enhance the robustness of field studies. Ultimately, it underscores the imperative of adopting a holistic perspective to safeguard the invaluable services provided by mangrove ecosystems and, by extension, coastal ecosystems globally.



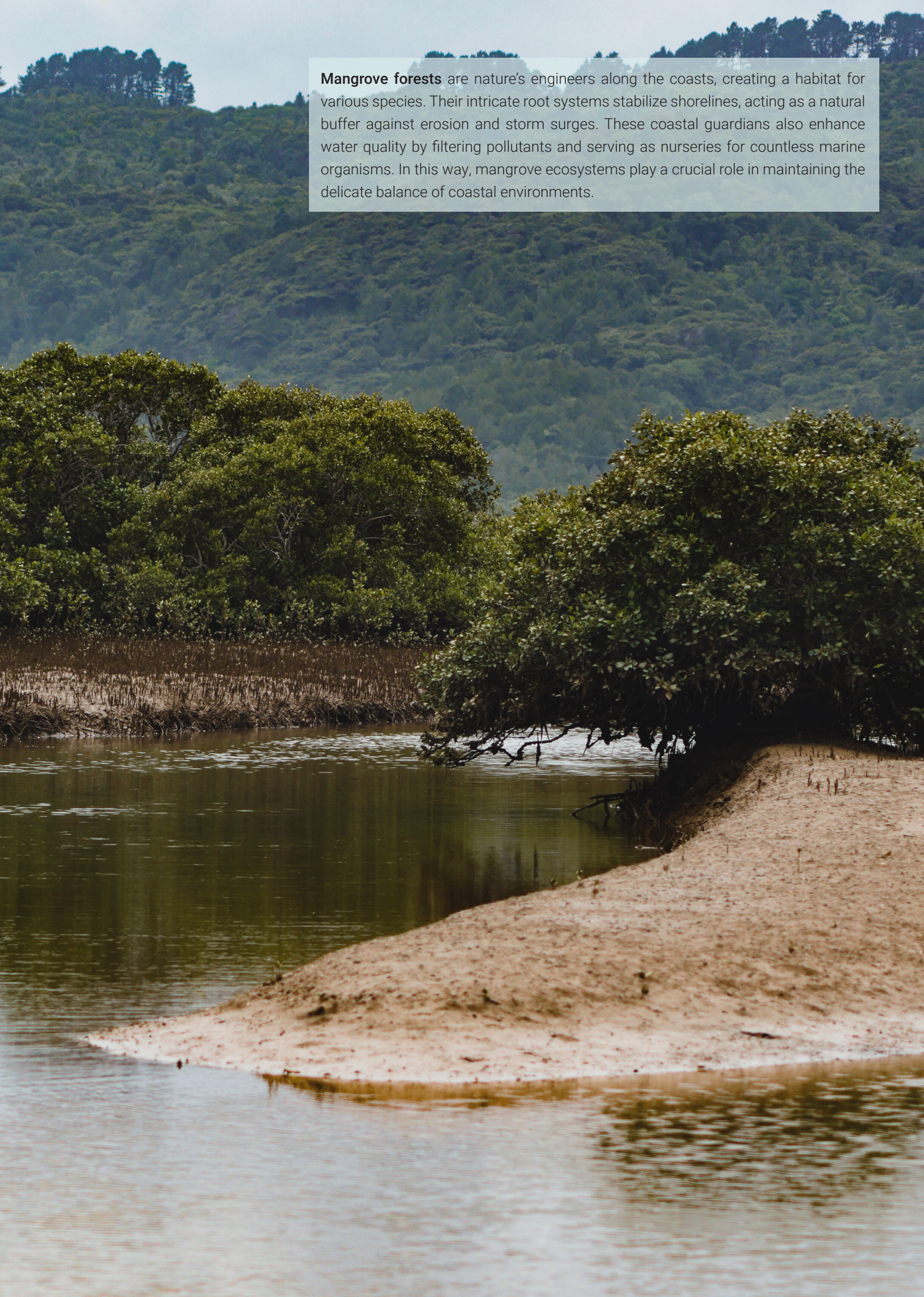
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A photograph of a mangrove forest. In the foreground, there is a sandy beach that curves along the edge of a body of water. The water is calm and reflects the surrounding greenery. On the right side, a large, leafy mangrove tree stands prominently, its roots partially submerged in the water. To the left, a dense line of mangrove trees forms a thick wall. In the background, a large, rolling hill covered in a dense forest of green trees rises up. The sky is a pale, clear blue. A semi-transparent text box is overlaid on the upper right portion of the image.

**Mangrove forests** are nature's engineers along the coasts, creating a habitat for various species. Their intricate root systems stabilize shorelines, acting as a natural buffer against erosion and storm surges. These coastal guardians also enhance water quality by filtering pollutants and serving as nurseries for countless marine organisms. In this way, mangrove ecosystems play a crucial role in maintaining the delicate balance of coastal environments.



## 6 Appendix I Supplementary Materials

### 6.1 Supplementary Materials to Chapter 1

**Supplementary Table A1.1** Results of literature search (n=60) via Google Scholar and SCOPUS (key words: nutrient AND enrichment AND mangrove, key words:16s AND rna AND mangrove AND soil OR sediment AND nutrient) for nutrient enrichment studies in mangrove ecosystems.

| Focus of main results | Microbial processes discussed | Use of NGS | Year published | Geographic region                                                                                                  | Climate                                | Reference                |
|-----------------------|-------------------------------|------------|----------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------|--------------------------|
| Tree responses        |                               |            | 1983           | Queensland, Australia                                                                                              | Subtropical                            | Boto & Wellington (1983) |
| Tree responses        | Yes                           |            | 1985           | Queensland, Australia                                                                                              | Subtropical                            | Boto et al. (1985)       |
| Tree responses        |                               |            | 1995           | Belize                                                                                                             | Tropical                               | Feller (1995)            |
| Tree responses        |                               |            | 1996           | Belize                                                                                                             | Tropical                               | Feller (1996)            |
| Tree responses        |                               |            | 2004           | Panama                                                                                                             | Tropical                               | Lovelock et al. (2004)   |
| Tree responses        |                               |            | 2008           | Lafayette, LA & Louisiana, USA                                                                                     | Tropical, temperate                    | McKee & Rooth (2008)     |
| Tree responses        |                               |            | 2009           | Florida, USA, Panama, Belize, Western Australia, Queensland, New South Wales, Australia & North Island New Zealand | Tropical, subtropical, arid, temperate | Lovelock et al. (2009)   |
| Tree responses        |                               |            | 2009           | Durban, South Africa                                                                                               | Subtropical                            | Naidoo (2009)            |
| Tree responses        |                               |            | 2009           | Florida to Panama                                                                                                  | Tropical, subtropical                  | Feller et al. (2009)     |
| Tree responses        |                               |            | 2010           | South-eastern Australia                                                                                            | Temperate                              | Martin et al. (2010)     |
| Tree responses        |                               |            | 2013           | Florida, USA                                                                                                       | Subtropical                            | Simpson et al. (2013)    |
| Tree responses        |                               |            | 2014           | Shenzhen, China                                                                                                    | Subtropical                            | Chen & Ye (2014)         |
| Tree responses        |                               |            | 2015           | Florida, USA                                                                                                       | Subtropical                            | Feller et al. (2015)     |
| Tree responses        |                               |            | 2016           | Ruvu River Estuary & Zanzibar Tanzania                                                                             | Tropical                               | Mangora (2016)           |
| Tree responses        |                               |            | 2016           | Panama                                                                                                             | Tropical                               | Reef et al. (2016)       |
| Tree responses        |                               |            | 2018           | Texas, USA                                                                                                         | Subtropical                            | Weaver & Armitage (2018) |
| Tree responses        |                               |            | 2020           | Texas, USA                                                                                                         | Subtropical                            | Weaver & Armitage (2020) |
| Tree responses        |                               |            | 2020           | Florida, USA                                                                                                       | Subtropical                            | Dangremond et al. (2020) |



|                           |     |      |                                                                            |                                        |                                  |
|---------------------------|-----|------|----------------------------------------------------------------------------|----------------------------------------|----------------------------------|
| Tree responses            |     | 2022 | Xiamen, China                                                              | Subtropical                            | Wei et al. (2022)                |
| Tree responses            |     | 2023 | Florida, USA                                                               | Subtropical                            | Feller et al. (2023)             |
| Tree responses            |     | 2023 | Florida, USA                                                               | Subtropical                            | Sturchio et al. (2023)           |
| Tree responses            | Yes | 2023 | Southern China                                                             | Subtropical                            | Peng et al. (2023)               |
| Sediment processes        |     | 1996 | Taiwan                                                                     | Subtropical                            | Chiu et al. (1996)               |
| Sediment processes        |     | 2001 | Bangrong, Thailand & Wadden Sea, Denmark                                   | Tropical, temperate                    | Holmboe et al. (2001)            |
| Sediment processes        |     | 2007 | North Island, New Zealand                                                  | Temperate                              | Lovelock et al. (2007)           |
| Sediment processes        |     | 2007 | Belize                                                                     | Tropical                               | Feller et al. (2007)             |
| Sediment processes        | Yes | 2009 | Florida, USA                                                               | Subtropical                            | Whigham et al. (2009)            |
| Sediment processes        | Yes | 2011 | Goa, India                                                                 | Tropical                               | Fernandes & Loka Bharathi (2011) |
| Sediment processes        | Yes | 2013 | Saudi Arabia                                                               | Arid                                   | Keuskamp et al. (2013)           |
| Sediment processes        | Yes | 2014 | Caribbean, Australia & North Island, New Zealand                           | Tropical, subtropical, arid, temperate | Lovelock et al. (2014)           |
| Sediment processes        | Yes | 2015 | Belize                                                                     | Tropical                               | Lovelock et al. (2015)           |
| Sediment processes        | Yes | 2017 | Western Australia                                                          | Arid                                   | Davies et al. (2017)             |
| Sediment processes        | Yes | 2019 | Southeastern Brazil                                                        | Subtropical                            | Reis et al. (2019)               |
| Biota                     |     | 1995 | Belize                                                                     | Tropical                               | Feller (1995)                    |
| Biota                     |     | 2007 | Belize                                                                     | Tropical                               | Feller & Chamberlain (2007)      |
| Biota                     |     | 2008 | New South Wales, Australia                                                 | Temperate                              | Minchinton & McKenzie (2008)     |
| Biota                     |     | 2013 | Florida, Belize & Panama                                                   | Tropical, subtropical                  | Feller et al. (2013)             |
| Biota                     |     | 2015 | Global                                                                     | Tropical, subtropical,                 | He & Silliman (2015)             |
| Biota                     |     | 2017 | North Island, New Zealand & New South Wales, Queensland, Western Australia | Temperate, arid, subtropical, tropical | Feller et al. (2017)             |
| Leaf litter decomposition | Yes | 1999 | Belize                                                                     | Tropical                               | Feller et al. (1999)             |
| Leaf litter decomposition | Yes | 2015 | Belize & Florida, USA                                                      | Tropical, subtropical                  | Keuskamp et al. (2015)           |
| Leaf litter decomposition | Yes | 2017 | Queensland, Australia                                                      | Subtropical                            | Hayes et al. (2017)              |
| Leaf litter decomposition | Yes | 2020 | Florida, USA                                                               | Subtropical                            | Simpson et al. (2020)            |

|                           |     |     |      |                            |                                            |                        |
|---------------------------|-----|-----|------|----------------------------|--------------------------------------------|------------------------|
| Leaf litter decomposition | Yes |     | 2021 | Puerto Rico                | Tropical                                   | Jessen et al. (2021)   |
| Microbial                 | Yes |     | 2015 | Belize                     | Tropical                                   | Keuskamp et al. (2015) |
| Microbial                 | Yes | Yes | 2021 | Florida, USA               | Subtropical                                | Craig et al. (2021)    |
| Microbial                 | Yes | Yes | 2021 | New South Wales, Australia | Temperate                                  | Palacios et al. (2021) |
| Microbial                 | Yes | Yes | 2021 | southeastern China         | Subtropical                                | Dai et al. (2021)      |
| Microbial                 | Yes | Yes | 2022 | Southern China             | Subtropical                                | Xie et al. (2022)      |
| Dissertation              |     |     | 1993 | Belize                     | Tropical                                   | Feller (1993)          |
| Dissertation              | Yes |     | 2016 | Puerto Rico                | Tropical                                   | Jessen (2016)          |
| Dissertation              |     |     | 2016 | Texas, USA                 | Subtropical                                | Weaver (2016)          |
| Dissertation              | Yes |     | 2018 | Western Australia          | Arid, semi-arid, Mediterranean, Temperate, | Davies (2018)          |
| Dissertation              |     |     | 2022 | Texas, USA                 | Subtropical                                | Flores (2022)          |
| Review                    | Yes |     | 1992 | Global                     | Tropical, subtropical,                     | Boto (1992)            |
| Review                    | Yes |     | 2001 | Global                     | Tropical, subtropical,                     | Holguin et al. (2001)  |
| Review                    | Yes |     | 2010 | Global                     | Tropical, subtropical                      | Reef et al. (2010)     |
| Review                    | Yes |     | 2010 | Global                     | Tropical, subtropical,                     | Feller et al. (2010)   |
| Review                    | Yes |     | 2017 | Global                     | Tropical, subtropical,                     | Reis et al. (2017)     |
| Review                    | Yes |     | 2023 | Global                     | Tropical, subtropical,                     | Simpson et al. (2023)  |

## Supplementary Table A1.1 References

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## 6.2 Supplementary Materials to Chapter 2

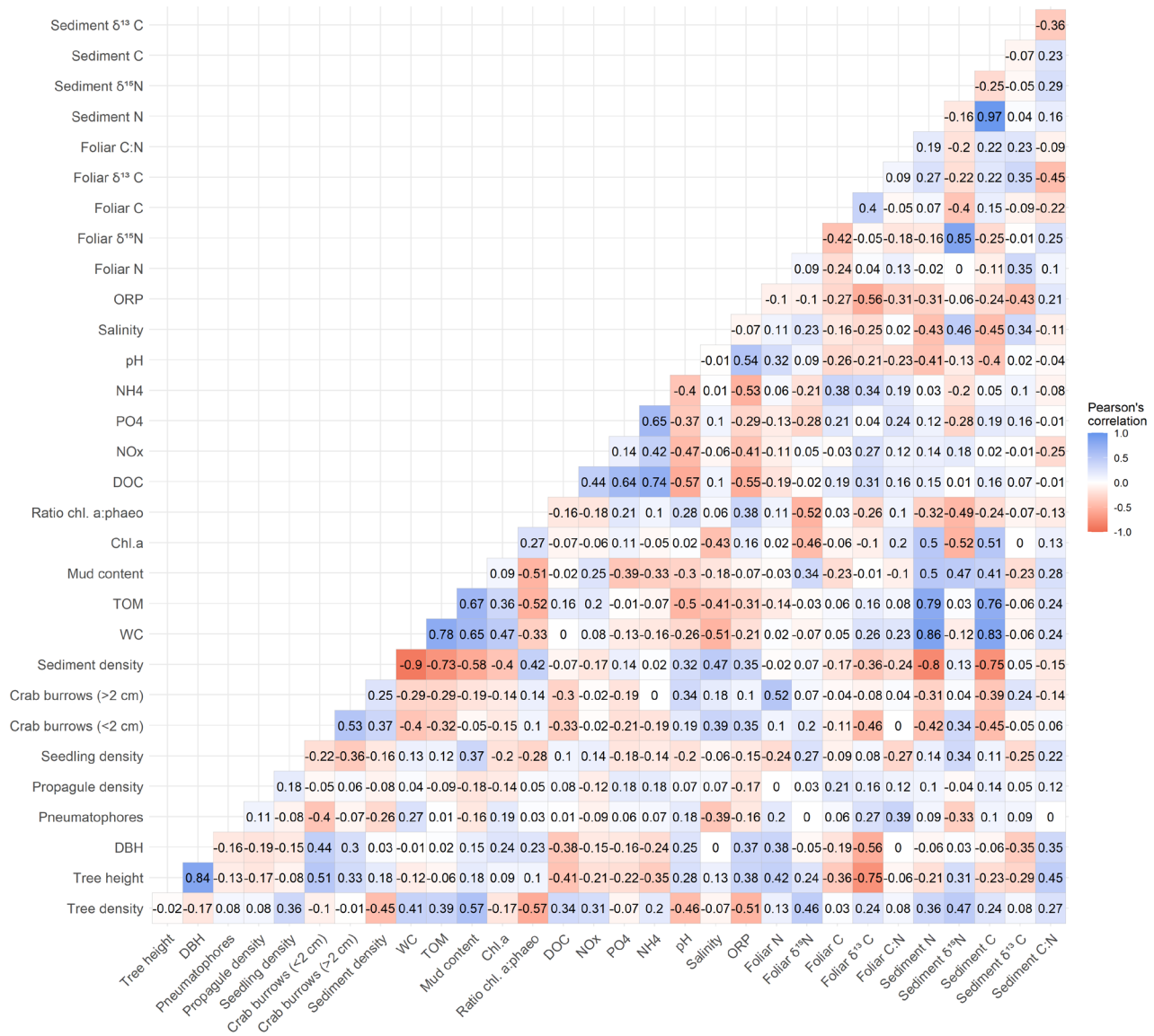
**Supplementary Table A2.1** Summary of average forest variables at each site. Means  $\pm$  standard deviations are calculated from three replicates at each site and variables without error were single measurements or were pooled for analysis. (DBH = diameter at breast height; N = nitrogen; C = carbon).

| Site | Tree density % | DBH cm | Pneumatophores m <sup>2</sup> | Propagules m <sup>2</sup> | Seedlings m <sup>2</sup> | Crab burrows <2cm m <sup>2</sup> | Crab burrows >2cm m <sup>2</sup> | Root depth cm | Foliar N % | Foliar $\delta^{15}\text{N}$ ‰ | Foliar C % | Foliar $\delta^{13}\text{C}$ ‰ | Foliar C:N |
|------|----------------|--------|-------------------------------|---------------------------|--------------------------|----------------------------------|----------------------------------|---------------|------------|--------------------------------|------------|--------------------------------|------------|
| ATH  | 70             | 12.4   | 576.00 $\pm$ 57.69            | 46.67 $\pm$ 10.07         | 4.00 $\pm$ 4.00          | 62.67 $\pm$ 23.09                | 5.3 $\pm$ 4.6                    | 1.0           | 2.09       | 3.87                           | 43.90      | -25.03                         | 21.02      |
| BOW  | 85             | 11.6   | 730.67 $\pm$ 178.41           | 53.33 $\pm$ 12.86         | 18.67 $\pm$ 10.07        | 57.33 $\pm$ 24.11                | 9.3 $\pm$ 2.3                    | 6.0           | 2.07       | 5.24                           | 44.49      | -24.56                         | 12.58      |
| COR  | 90             | 14.6   | 533.33 $\pm$ 148.67           | 1.33 $\pm$ 2.31           | 10.67 $\pm$ 10.07        | 1514.67 $\pm$ 413.01             | 10.7 $\pm$ 9.2                   | 5.0           | 1.92       | 6.37                           | 43.14      | -26.31                         | 21.45      |
| HEI  | 45             | 11.4   | 474.67 $\pm$ 40.27            | 22.67 $\pm$ 16.17         | 18.67 $\pm$ 10.07        | 170.67 $\pm$ 56.76               | 5.3 $\pm$ 2.3                    | 5.0           | 1.78       | 5.47                           | 42.75      | -26.66                         | 10.74      |
| KAT  | 95             | 21.0   | 800.00 $\pm$ 27.71            | 30.67 $\pm$ 6.11          | 21.33 $\pm$ 12.86        | 98.67 $\pm$ 55.18                | 12.0 $\pm$ 0.0                   | 3.0           | 2.10       | 4.73                           | 44.54      | -24.96                         | 22.44      |
| MAN  | 75             | 30.0   | 325.33 $\pm$ 113.51           | 1.33 $\pm$ 2.31           | 12.00 $\pm$ 10.58        | 372.00 $\pm$ 59.19               | 6.7 $\pm$ 2.3                    | 5.0           | 2.12       | 4.66                           | 43.71      | -26.36                         | 9.81       |
| MAT  | 100            | 13.4   | 1082.67 $\pm$ 117.94          | 30.67 $\pm$ 4.62          | 8.00 $\pm$ 0.00          | 29.33 $\pm$ 32.33                | 13.3 $\pm$ 23.1                  | 7.0           | 2.00       | 6.08                           | 42.91      | -26.03                         | 24.06      |
| OMO  | 90             | 9.5    | 757.33 $\pm$ 92.38            | 92.00 $\pm$ 8.00          | 29.33 $\pm$ 8.33         | 2.67 $\pm$ 4.62                  | 0.0 $\pm$ 0.0                    | 1.5           | 2.00       | 3.51                           | 43.85      | -24.81                         | 21.50      |
| OPU  | 50             | 14.0   | 698.67 $\pm$ 51.43            | 12.00 $\pm$ 4.00          | 12.00 $\pm$ 8.00         | 105.33 $\pm$ 16.17               | 14.7 $\pm$ 8.3                   | 4.0           | 1.85       | 3.41                           | 43.93      | -26.11                         | 8.21       |
| PAU1 | 75             | 24.8   | 816.00 $\pm$ 238.93           | 17.33 $\pm$ 6.11          | 10.67 $\pm$ 8.33         | 277.33 $\pm$ 76.04               | 21.3 $\pm$ 6.1                   | 4.0           | 2.15       | 4.51                           | 43.74      | -24.41                         | 23.75      |
| PAU2 | 50             | 25.6   | 821.33 $\pm$ 130.31           | 30.67 $\pm$ 15.14         | 10.67 $\pm$ 9.24         | 277.33 $\pm$ 56.05               | 16.0 $\pm$ 8.0                   | 5.0           | 2.11       | 4.11                           | 43.47      | -26.05                         | 23.74      |
| POU  | 60             | 14.7   | 821.33 $\pm$ 208.21           | 5.33 $\pm$ 2.31           | 1.33 $\pm$ 2.31          | 264.00 $\pm$ 16.00               | 17.3 $\pm$ 2.3                   | 5.0           | 2.06       | 4.10                           | 43.80      | -26.87                         | 23.94      |
| PUR  | 60             | 35.5   | 501.33 $\pm$ 33.31            | 18.67 $\pm$ 8.33          | 6.67 $\pm$ 4.62          | 293.33 $\pm$ 92.38               | 16.0 $\pm$ 4.0                   | 4.0           | 2.32       | 4.13                           | 42.81      | -28.30                         | 19.92      |
| RAK  | 100            | 16.4   | 448.00 $\pm$ 55.43            | 29.33 $\pm$ 12.22         | 1.33 $\pm$ 2.31          | 784.00 $\pm$ 193.99              | 64.0 $\pm$ 16.0                  | 1.5           | 1.99       | 5.04                           | 44.02      | -25.26                         | 21.98      |
| RAN  | 75             | 26.9   | 848.00 $\pm$ 142.21           | 10.67 $\pm$ 10.07         | 0.00 $\pm$ 0.00          | 750.67 $\pm$ 611.10              | 21.3 $\pm$ 10.1                  | 6.0           | 2.26       | 4.78                           | 44.00      | -26.46                         | 13.20      |
| REA  | 100            | 14.0   | 805.33 $\pm$ 208.82           | 2.67 $\pm$ 2.31           | 20.00 $\pm$ 8.00         | 45.33 $\pm$ 24.44                | 2.7 $\pm$ 4.6                    | 0.5           | 1.82       | 6.82                           | 43.23      | -24.94                         | 21.24      |
| TAH  | 70             | 11.7   | 1306.67 $\pm$ 339.54          | 9.33 $\pm$ 10.07          | 10.67 $\pm$ 12.22        | 1.33 $\pm$ 2.31                  | 4.0 $\pm$ 4.0                    | 12.0          | 2.24       | 6.87                           | 42.75      | -24.67                         | 20.37      |
| TAI1 | 60             | 18.3   | 560.00 $\pm$ 42.33            | 9.33 $\pm$ 9.24           | 6.67 $\pm$ 4.62          | 13.33 $\pm$ 9.24                 | 0.0 $\pm$ 0.0                    | 1.0           | 1.94       | 3.76                           | 43.74      | -25.73                         | 11.03      |
| TAI2 | 45             | 21.8   | 752.00 $\pm$ 48.00            | 21.33 $\pm$ 12.22         | 4.00 $\pm$ 4.00          | 97.33 $\pm$ 34.02                | 5.3 $\pm$ 6.1                    | 1.0           | 1.97       | 2.84                           | 44.28      | -26.64                         | 20.58      |
| TBB  | 85             | 21.3   | 602.67 $\pm$ 51.43            | 137.33 $\pm$ 60.04        | 100.00 $\pm$ 24.33       | 874.67 $\pm$ 351.03              | 5.3 $\pm$ 9.2                    | 4.0           | 1.72       | 8.55                           | 43.29      | -27.31                         | 13.13      |
| TEK  | 45             | 23.7   | 458.67 $\pm$ 136.08           | 5.33 $\pm$ 2.31           | 5.33 $\pm$ 6.11          | 466.67 $\pm$ 34.02               | 6.7 $\pm$ 8.3                    | 3.0           | 1.96       | 5.25                           | 42.19      | -26.63                         | 21.22      |
| TRB  | 100            | 23.0   | 384.00 $\pm$ 120.80           | 30.67 $\pm$ 4.62          | 25.33 $\pm$ 12.86        | 1002.67 $\pm$ 101.61             | 42.7 $\pm$ 24.4                  | 15.0          | 2.43       | 10.06                          | 42.78      | -26.83                         | 9.63       |
| TUA  | 75             | 9.9    | 1002.67 $\pm$ 124.28          | 57.33 $\pm$ 14.05         | 6.67 $\pm$ 8.33          | 65.33 $\pm$ 2.31                 | 4.0 $\pm$ 0.0                    | 1.5           | 1.98       | 6.67                           | 44.45      | -23.99                         | 18.43      |
| TYE  | 80             | 19.3   | 474.67 $\pm$ 152.07           | 0.00 $\pm$ 0.00           | 34.67 $\pm$ 12.86        | 29.33 $\pm$ 44.06                | 0.0 $\pm$ 0.0                    | 1.0           | 1.89       | 5.56                           | 43.80      | -25.32                         | 11.90      |
| WAI  | 55             | 20.9   | 533.33 $\pm$ 122.20           | 14.67 $\pm$ 2.31          | 4.00 $\pm$ 0.00          | 226.67 $\pm$ 34.02               | 0.0 $\pm$ 0.0                    | 1.0           | 1.41       | 5.11                           | 44.36      | -27.16                         | 22.08      |
| WEB  | 95             | 15.6   | 352.00 $\pm$ 112.00           | 21.33 $\pm$ 16.17         | 64.00 $\pm$ 32.00        | 106.67 $\pm$ 94.35               | 6.7 $\pm$ 8.3                    | 1.0           | 1.82       | 5.48                           | 43.51      | -25.33                         | 9.93       |
| WHA1 | 65             | 30.8   | 874.67 $\pm$ 90.98            | 46.67 $\pm$ 26.03         | 6.67 $\pm$ 2.31          | 84.00 $\pm$ 10.58                | 13.3 $\pm$ 6.1                   | 2.5           | 2.22       | 5.41                           | 43.44      | -25.28                         | 19.45      |
| WHA2 | 85             | 17.8   | 768.00 $\pm$ 16.00            | 21.33 $\pm$ 8.33          | 40.00 $\pm$ 17.44        | 10.67 $\pm$ 8.33                 | 0.0 $\pm$ 0.0                    | 4.0           | 1.67       | 4.53                           | 44.03      | -26.11                         | 10.54      |
| WHA3 | 45             | 23.4   | 640.00 $\pm$ 112.00           | 5.33 $\pm$ 4.62           | 0.00 $\pm$ 0.00          | 234.67 $\pm$ 12.86               | 25.3 $\pm$ 6.1                   | 6.0           | 1.97       | 5.60                           | 43.71      | -25.99                         | 9.32       |
| WHI  | 100            | 30.7   | 997.33 $\pm$ 113.51           | 4.00 $\pm$ 4.00           | 5.33 $\pm$ 2.31          | 186.67 $\pm$ 60.58               | 9.3 $\pm$ 4.6                    | 8.0           | 2.16       | 5.66                           | 43.10      | -27.78                         | 20.64      |

**Supplementary Table A2.2** Average values  $\pm$  standard deviation of sedimentary variables at each site.

Means are calculated from three replicates at each site and variables without error were single measurements or were pooled for analysis. (TOM = total organic matter; Chl  $\alpha$  = chlorophyll  $\alpha$ ; Phaeo = chlorophyll  $\alpha$  : phaeophytin ratio; DOC = dissolved organic carbon; NO<sub>x</sub> = nitrate and nitrite; PO<sub>4</sub> = phosphate; NH<sub>4</sub> = ammonium; ORP = oxygen reduction potential; Sed = sediment; N = nitrogen; C = carbon)

| Site | Sediment density<br>g m <sup>-3</sup> | TOM<br>%       | Mud content<br>% | Chl $\alpha$<br>$\mu\text{g g}^{-1}$ DW | Chl $\alpha$ : Phaeo | DOC<br>ppm        | NO <sub>x</sub><br>$\mu\text{mol N L}^{-1}$ | PO <sub>4</sub><br>$\mu\text{mol P L}^{-1}$ | NH <sub>4</sub><br>$\mu\text{mol N L}^{-1}$ | pH  | Salinity | ORP<br>mV | Sed N<br>% | Sed $\delta^{15}\text{N}$<br>‰ | Sed C<br>% | Sed $\delta^{13}\text{C}$<br>‰ | Sed C:N |
|------|---------------------------------------|----------------|------------------|-----------------------------------------|----------------------|-------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|-----|----------|-----------|------------|--------------------------------|------------|--------------------------------|---------|
| ATH  | 0.65 $\pm$ 0.26                       | 14.3 $\pm$ 1.1 | 62.0 $\pm$ 3.2   | 83.9 $\pm$ 6.5                          | 3.0 $\pm$ 0.9        | 68.1 $\pm$ 1.6    | 0.43 $\pm$ 0.08                             | 7.8 $\pm$ 2.7                               | 189.1 $\pm$ 24.8                            | 6.8 | 34.94    | -3.7      | 0.56       | 3.68                           | 7.00       | -22.12                         | 11.04   |
| BOW  | 0.84 $\pm$ 0.05                       | 15.8 $\pm$ 0.4 | 78.4 $\pm$ 2.1   | 52.3 $\pm$ 2.9                          | 2.0 $\pm$ 0.3        | 48.1 $\pm$ 4.6    | 2.10 $\pm$ 1.54                             | 3.0 $\pm$ 0.8                               | 174.0 $\pm$ 10.0                            | 7.0 | 23.91    | 37.5      | 0.50       | 5.05                           | 5.39       | -23.20                         | 10.96   |
| COR  | 1.34 $\pm$ 0.10                       | 12.5 $\pm$ 0.7 | 93.7 $\pm$ 2.8   | 29.1 $\pm$ 0.8                          | 1.5 $\pm$ 0.1        | 35.6 $\pm$ 6.4    | 1.93 $\pm$ 1.02                             | 1.6 $\pm$ 0.8                               | 30.5 $\pm$ 8.6                              | 6.5 | 32.16    | 184.6     | 0.28       | 7.17                           | 2.73       | -23.25                         | 10.86   |
| HEI  | 2.77 $\pm$ 0.26                       | 4.2 $\pm$ 0.3  | 31.5 $\pm$ 2.1   | 19.7 $\pm$ 4.1                          | 17.9 $\pm$ 21.8      | 49.5 $\pm$ 3.6    | 0.30 $\pm$ 0.10                             | 6.0 $\pm$ 1.0                               | 72.9 $\pm$ 4.0                              | 7.3 | 33.87    | 237.6     | 0.05       | 4.79                           | 0.40       | -23.02                         | 8.65    |
| KAT  | 0.50 $\pm$ 0.11                       | 17.5 $\pm$ 0.7 | 84.0 $\pm$ 2.0   | 48.7 $\pm$ 3.2                          | 1.2 $\pm$ 0.3        | 94.3 $\pm$ 27.3   | 4.95 $\pm$ 4.87                             | 3.3 $\pm$ 0.8                               | 189.2 $\pm$ 10.2                            | 6.8 | 29.69    | -172.5    | 0.41       | 4.68                           | 4.08       | -22.54                         | 11.23   |
| MAN  | 1.20 $\pm$ 0.16                       | 15.1 $\pm$ 0.6 | 92.8 $\pm$ 0.5   | 62.6 $\pm$ 13.7                         | 4.2 $\pm$ 1.6        | 54.1 $\pm$ 20.0   | 0.79 $\pm$ 0.38                             | 4.0 $\pm$ 0.3                               | 74.4 $\pm$ 6.0                              | 6.7 | 33.28    | 199.8     | 0.40       | 4.93                           | 3.74       | -23.38                         | 11.42   |
| MAT  | 0.37 $\pm$ 0.06                       | 26.5 $\pm$ 1.8 | 73.5 $\pm$ 3.9   | 115.3 $\pm$ 41.7                        | 1.2 $\pm$ 0.2        | 62.6 $\pm$ 7.3    | 0.12 $\pm$ 0.05                             | 17.2 $\pm$ 7.6                              | 104.6 $\pm$ 5.4                             | 6.6 | 21.17    | 11.3      | 0.73       | 4.23                           | 8.08       | -21.07                         | 21.50   |
| OMO  | 0.97 $\pm$ 0.19                       | 19.4 $\pm$ 1.4 | 82.7 $\pm$ 0.7   | 55.7 $\pm$ 3.7                          | 3.2 $\pm$ 0.3        | 358.7 $\pm$ 78.3  | 0.46 $\pm$ 0.05                             | 25.2 $\pm$ 1.3                              | 216.9 $\pm$ 10.7                            | 6.5 | 34.10    | 43.3      | 0.48       | 4.33                           | 6.36       | -22.97                         | 19.05   |
| OPU  | 1.22 $\pm$ 0.16                       | 6.5 $\pm$ 0.7  | 42.6 $\pm$ 5.9   | 65.6 $\pm$ 6.6                          | 7.2 $\pm$ 0.4        | 32.5 $\pm$ 10.5   | 0.49 $\pm$ 0.18                             | 2.9 $\pm$ 0.9                               | 91.3 $\pm$ 16.9                             | 7.2 | 26.45    | 341.3     | 0.22       | 3.23                           | 2.43       | -24.79                         | 9.63    |
| PAU1 | 1.19 $\pm$ 0.23                       | 12.4 $\pm$ 2.2 | 69.9 $\pm$ 9.4   | 62.4 $\pm$ 19.8                         | 3.8 $\pm$ 2.6        | 23.9 $\pm$ 1.1    | 0.92 $\pm$ 0.29                             | 2.1 $\pm$ 0.3                               | 53.1 $\pm$ 12.3                             | 6.9 | 26.13    | 170.2     | 0.34       | 4.17                           | 3.79       | -23.08                         | 9.78    |
| PAU2 | 0.86 $\pm$ 0.06                       | 13.6 $\pm$ 0.9 | 67.5 $\pm$ 4.0   | 78.2 $\pm$ 6.2                          | 6.0 $\pm$ 0.6        | 23.8 $\pm$ 2.2    | 0.26 $\pm$ 0.10                             | 1.6 $\pm$ 0.4                               | 62.6 $\pm$ 20.0                             | 7.0 | 23.30    | 228.6     | 0.33       | 3.38                           | 4.33       | -24.95                         | 22.54   |
| POU  | 1.92 $\pm$ 0.22                       | 5.5 $\pm$ 1.1  | 21.2 $\pm$ 4.2   | 52.3 $\pm$ 8.8                          | 10.1 $\pm$ 1.0       | 55.3 $\pm$ 16.2   | 0.38 $\pm$ 0.23                             | 5.8 $\pm$ 0.2                               | 141.8 $\pm$ 22.6                            | 7.0 | 35.11    | 285.9     | 0.14       | 4.29                           | 1.35       | -22.35                         | 12.72   |
| PUR  | 1.89 $\pm$ 0.13                       | 6.8 $\pm$ 0.3  | 30.4 $\pm$ 2.1   | 53.1 $\pm$ 4.5                          | 35.4 $\pm$ 47.5      | 60.9 $\pm$ 8.2    | 1.42 $\pm$ 1.27                             | 11.5 $\pm$ 0.3                              | 253.4 $\pm$ 76.0                            | 6.9 | 33.20    | 156.4     | 0.20       | 5.14                           | 2.38       | -24.65                         | 22.50   |
| RAK  | 0.85 $\pm$ 0.16                       | 17.0 $\pm$ 1.0 | 83.3 $\pm$ 1.3   | 47.6 $\pm$ 4.2                          | 2.4 $\pm$ 0.2        | 83.2 $\pm$ 16.5   | 5.73 $\pm$ 2.94                             | 6.0 $\pm$ 1.4                               | 246.6 $\pm$ 28.2                            | 6.4 | 33.99    | 53.1      | 0.46       | 5.65                           | 4.79       | -22.74                         | 12.56   |
| RAN  | 1.43 $\pm$ 0.63                       | 10.2 $\pm$ 3.9 | 49.8 $\pm$ 23.0  | 83.2 $\pm$ 52.8                         | 4.1 $\pm$ 2.2        | 87.8 $\pm$ 19.2   | 0.47 $\pm$ 0.36                             | 12.0 $\pm$ 4.0                              | 179.7 $\pm$ 52.4                            | 7.0 | 30.24    | 203.6     | 0.30       | 4.48                           | 3.21       | -23.13                         | 25.09   |
| REA  | 0.87 $\pm$ 0.10                       | 18.6 $\pm$ 0.9 | 91.3 $\pm$ 2.0   | 50.1 $\pm$ 4.4                          | 0.8 $\pm$ 0.2        | 405.5 $\pm$ 100.1 | 21.06 $\pm$ 6.34                            | 13.3 $\pm$ 2.7                              | 292.8 $\pm$ 10.0                            | 6.4 | 20.74    | 21.0      | 0.41       | 5.66                           | 4.49       | -25.30                         | 17.61   |
| TAH  | 0.81 $\pm$ 0.17                       | 13.9 $\pm$ 1.7 | 77.8 $\pm$ 8.5   | 45.9 $\pm$ 2.2                          | 1.5 $\pm$ 0.4        | 55.6 $\pm$ 18.9   | 5.03 $\pm$ 8.39                             | 2.2 $\pm$ 2.1                               | 76.0 $\pm$ 26.5                             | 7.0 | 30.20    | 26.2      | 0.41       | 5.41                           | 4.02       | -22.91                         | 7.32    |
| TAI1 | 0.76 $\pm$ 0.03                       | 25.7 $\pm$ 0.7 | 87.9 $\pm$ 1.7   | 113.8 $\pm$ 11.7                        | 4.1 $\pm$ 2.3        | 66.6 $\pm$ 22.8   | 0.57 $\pm$ 0.31                             | 4.7 $\pm$ 2.9                               | 152.8 $\pm$ 43.6                            | 6.8 | 18.90    | 286.0     | 0.67       | 3.83                           | 8.58       | -25.08                         | 23.16   |
| TAI2 | 1.51 $\pm$ 0.44                       | 11.8 $\pm$ 0.9 | 51.5 $\pm$ 2.4   | 91.9 $\pm$ 10.2                         | 22.2 $\pm$ 13.3      | 67.7 $\pm$ 29.5   | 1.63 $\pm$ 1.05                             | 22.5 $\pm$ 19.3                             | 154.7 $\pm$ 79.9                            | 6.5 | 29.59    | 123.6     | 0.30       | 3.33                           | 3.80       | -24.06                         | 12.00   |
| TBB  | 1.29 $\pm$ 0.19                       | 13.4 $\pm$ 0.6 | 87.6 $\pm$ 2.5   | 23.3 $\pm$ 2.8                          | 1.1 $\pm$ 0.2        | 46.1 $\pm$ 7.8    | 0.39 $\pm$ 0.32                             | 2.3 $\pm$ 0.7                               | 31.8 $\pm$ 8.4                              | 6.8 | 37.09    | 228.6     | 0.33       | 8.21                           | 3.80       | -25.29                         | 31.42   |
| TEK  | 1.18 $\pm$ 0.16                       | 12.6 $\pm$ 0.5 | 87.1 $\pm$ 3.7   | 120.5 $\pm$ 35.0                        | 5.7 $\pm$ 1.0        | 45.7 $\pm$ 4.7    | 2.93 $\pm$ 2.44                             | 3.1 $\pm$ 1.6                               | 45.8 $\pm$ 30.9                             | 7.2 | 35.34    | 259.8     | 0.32       | 5.56                           | 3.10       | -22.05                         | 14.34   |
| TRB  | 1.51 $\pm$ 0.07                       | 9.9 $\pm$ 0.4  | 94.2 $\pm$ 2.7   | 12.0 $\pm$ 0.7                          | 0.6 $\pm$ 0.1        | 34.2 $\pm$ 4.7    | 0.16 $\pm$ 0.02                             | 1.8 $\pm$ 0.9                               | 52.5 $\pm$ 12.6                             | 7.1 | 36.65    | 178.8     | 0.21       | 8.49                           | 1.82       | -23.54                         | 24.12   |
| TUA  | 1.80 $\pm$ 0.41                       | 3.9 $\pm$ 1.4  | 14.1 $\pm$ 5.1   | 30.6 $\pm$ 4.6                          | 6.7 $\pm$ 0.8        | 122.3 $\pm$ 8.7   | 0.27 $\pm$ 0.02                             | 10.5 $\pm$ 5.4                              | 291.5 $\pm$ 59.3                            | 6.9 | 31.29    | 44.6      | 0.02       | 4.56                           | 0.13       | -23.02                         | 10.45   |
| TYE  | 0.89 $\pm$ 0.13                       | 17.7 $\pm$ 0.7 | 85.5 $\pm$ 1.0   | 51.3 $\pm$ 4.3                          | 1.5 $\pm$ 0.4        | 78.1 $\pm$ 20.2   | 0.99 $\pm$ 0.85                             | 6.5 $\pm$ 5.0                               | 127.8 $\pm$ 38.2                            | 6.5 | 31.05    | 40.0      | 0.42       | 5.60                           | 5.07       | -23.68                         | 19.59   |
| WAI  | 1.16 $\pm$ 0.17                       | 16.3 $\pm$ 1.7 | 69.0 $\pm$ 6.9   | 65.2 $\pm$ 8.1                          | 2.1 $\pm$ 0.2        | 85.2 $\pm$ 18.0   | 0.46 $\pm$ 0.32                             | 13.9 $\pm$ 2.2                              | 108.6 $\pm$ 22.9                            | 6.8 | 31.15    | 186.7     | 0.42       | 4.99                           | 6.09       | -25.89                         | 11.5    |
| WEB  | 1.09 $\pm$ 0.13                       | 15.1 $\pm$ 0.6 | 86.8 $\pm$ 1.9   | 70.6 $\pm$ 10.6                         | 1.9 $\pm$ 0.3        | 135.7 $\pm$ 19.6  | 10.28 $\pm$ 4.12                            | 4.4 $\pm$ 0.6                               | 173.9 $\pm$ 18.7                            | 6.5 | 30.08    | 58.6      | 0.42       | 5.71                           | 3.67       | -23.90                         | 8.50    |
| WHA1 | 0.67 $\pm$ 0.20                       | 10.2 $\pm$ 1.4 | 79.9 $\pm$ 2.3   | 83.1 $\pm$ 3.0                          | 9.1 $\pm$ 1.9        | 31.4 $\pm$ 5.9    | 0.44 $\pm$ 0.14                             | 4.7 $\pm$ 1.0                               | 78.7 $\pm$ 12.5                             | 7.3 | 23.94    | 278.7     | 0.28       | 4.26                           | 2.88       | -24.50                         | 12.11   |
| WHA2 | 0.65 $\pm$ 0.13                       | 16.5 $\pm$ 0.6 | 92.8 $\pm$ 0.7   | 71.2 $\pm$ 6.3                          | 2.8 $\pm$ 0.2        | 27.2 $\pm$ 4.2    | 0.26 $\pm$ 0.14                             | 1.2 $\pm$ 0.1                               | 40.7 $\pm$ 6.9                              | 7.0 | 19.15    | 253.5     | 0.55       | 4.49                           | 6.28       | -25.30                         | 22.20   |
| WHA3 | 1.80 $\pm$ 0.21                       | 18.1 $\pm$ 0.4 | 49.4 $\pm$ 6.1   | 53.4 $\pm$ 4.9                          | 1.7 $\pm$ 0.1        | 37.7 $\pm$ 8.5    | 0.37 $\pm$ 0.26                             | 5.6 $\pm$ 4.6                               | 94.3 $\pm$ 35.5                             | 7.1 | 28.05    | 208.4     | 0.36       | 4.74                           | 4.39       | -23.11                         | 9.94    |
| WHI  | 0.84 $\pm$ 0.06                       | 16.5 $\pm$ 0.3 | 95.2 $\pm$ 1.0   | 109.7 $\pm$ 11.5                        | 4.1 $\pm$ 0.2        | 42.9 $\pm$ 7.7    | 0.28 $\pm$ 0.24                             | 1.5 $\pm$ 0.5                               | 68.5 $\pm$ 3.4                              | 7.1 | 25.49    | 244.8     | 0.16       | 4.78                           | 1.63       | -25.49                         | 22.45   |



**Supplementary Figure A2.1** Pearson's correlation matrix of all environmental variables (forest and sedimentary variables). Numbers inside each box represent the correlation (r) of each variable combination. TOM = total organic matter; Chl a = chlorophyll a; ratio chl a : phaeo = chlorophyll a : phaeophytin ratio; DOC = dissolved organic carbon; NO<sub>x</sub> = nitrate and nitrite; PO<sub>4</sub> = phosphate; NH<sub>4</sub> = ammonium; ORP = oxygen reduction potential; N = nitrogen; C = carbon.

**Supplementary Table A2.3** The variables returned from the univariate comparisons in relation to %Human land use. Variables are sorted by the F-statistic (F.test) as a measure of strength of association between the predictor and the response. 'coef' denotes the standardised coefficient indicating the strength and direction of the effect and 'p.uni' gives the adjusted (resampled) p-value of the univariate regression model. (TOM = total organic matter; Chl a = chlorophyll a; Chl a : Phaeo = chlorophyll a : phaeophytin ratio; DOC = dissolved organic carbon; NO<sub>x</sub> = nitrate and nitrite; PO<sub>4</sub> = phosphate; NH<sub>4</sub> = ammonium; OPR = oxygen reduction potential; Sed = sediment; N = nitrogen; C = carbon).

| Variable                   | Variable type | F-statistic | p-value | Standardized coefficient | Relationship with %Human |
|----------------------------|---------------|-------------|---------|--------------------------|--------------------------|
| ORP                        | Sediment      | 76.13       | 0.002   | -0.48                    | Negative                 |
| Tree density               | Forest        | 46.72       | 0.002   | 0.41                     | Positive                 |
| DOC                        | Sediment      | 23.33       | 0.002   | 0.32                     | Positive                 |
| pH                         | Sediment      | 21.55       | 0.002   | -0.31                    | Negative                 |
| Seedling density           | Forest        | 18.79       | 0.002   | 0.29                     | Positive                 |
| Propagule density          | Forest        | 17.81       | 0.002   | 0.29                     | Positive                 |
| Chl a : Phaeo ratio        | Sediment      | 15.69       | 0.003   | -0.27                    | Negative                 |
| NH <sub>4</sub>            | Sediment      | 14.37       | 0.004   | 0.26                     | Positive                 |
| Foliar δ <sup>15</sup> N   | Forest        | 11.58       | 0.017   | 0.24                     | Positive                 |
| NO <sub>x</sub>            | Sediment      | 10.60       | 0.022   | 0.23                     | Positive                 |
| TOM                        | Sediment      | 7.72        | 0.076   | 0.20                     | Positive                 |
| Bulk density               | Sediment      | 7.56        | 0.081   | -0.20                    | Negative                 |
| Foliar δ <sup>13</sup> C   | Forest        | 6.89        | 0.126   | 0.19                     | Positive                 |
| DBH                        | Forest        | 6.83        | 0.126   | -0.19                    | Negative                 |
| Crab burrows (<2 cm)       | Forest        | 6.43        | 0.139   | -0.18                    | Negative                 |
| Mud content                | Sediment      | 5.41        | 0.217   | 0.17                     | Positive                 |
| PO <sub>4</sub>            | Sediment      | 4.79        | 0.265   | 0.16                     | Positive                 |
| Sediment C:N ratio         | Sediment      | 3.06        | 0.517   | 0.13                     | Positive                 |
| Chlorophyll a              | Sediment      | 2.89        | 0.517   | -0.12                    | Negative                 |
| Sediment δ <sup>13</sup> C | Sediment      | 2.62        | 0.517   | 0.12                     | Positive                 |
| Foliar C                   | Forest        | 1.63        | 0.705   | -0.09                    | Negative                 |
| Crab burrows (>2 cm)       | Forest        | 1.19        | 0.787   | -0.08                    | Negative                 |
| Salinity                   | Sediment      | 0.69        | 0.875   | 0.06                     | Positive                 |
| Foliar N                   | Forest        | 0.41        | 0.894   | 0.05                     | Positive                 |
| Pneumatophores             | Forest        | 0.35        | 0.894   | -0.04                    | Negative                 |
| Foliar C:N ratio           | Forest        | 0.23        | 0.894   | 0.04                     | Positive                 |

**Supplementary Table A2.4** The variables returned from the univariate comparisons in relation to %Urban and %Agricultural land use. Variables are sorted by the F-statistic (F.test) as a measure of strength of association between the predictor and the response. 'coef' denotes the standardised coefficient indicating the strength and direction of the effect and 'p.uni' gives the adjusted (resampled) p-value of the univariate regression model. (TOM = total organic matter; Chl a = chlorophyll a; Chl a : Phaeo = chlorophyll a : phaeophytin ratio; DOC = dissolved organic carbon; NO<sub>x</sub> = nitrate and nitrite; PO<sub>4</sub> = phosphate; NH<sub>4</sub> = ammonium; OPR = oxygen reduction potential; Sed = sediment; N = nitrogen; C = carbon).

| Variable                    | Variable type | Urban  |       |       | Agricultural |       |       | Urban × Agricultural |       |       | Interaction effect |
|-----------------------------|---------------|--------|-------|-------|--------------|-------|-------|----------------------|-------|-------|--------------------|
|                             |               | F.test | coef  | p.uni | F.test       | coef  | p.uni | F.test               | coef  | p.uni |                    |
| ORP                         | Sediment      | 12.21  | -0.33 | 0.011 | 53.72        | -0.96 | 0.002 | 3.95                 | -0.13 | 0.582 | Antagonistic       |
| Tree density                | Forest        | 41.84  | 0.47  | 0.002 | 23.34        | 0.48  | 0.002 | 0.48                 | 0.05  | 0.996 | Antagonistic       |
| DOC                         | Sediment      | 7.99   | 0.51  | 0.084 | 18.07        | 1.67  | 0.004 | 23.14                | 0.35  | 0.002 | Additive           |
| Salinity                    | Sediment      | 23.17  | -0.30 | 0.002 | 16.33        | 0.92  | 0.006 | 4.85                 | 0.16  | 0.433 | Antagonistic       |
| pH                          | Sediment      | 19.04  | -0.45 | 0.003 | 9.98         | -0.79 | 0.028 | 3.32                 | -0.14 | 0.706 | Antagonistic       |
| NO <sub>x</sub>             | Sediment      | 7.42   | 0.48  | 0.107 | 8.64         | 1.43  | 0.058 | 15.03                | 0.31  | 0.013 | Additive           |
| TOM                         | Sediment      | 30.17  | 0.42  | 0.002 | 0.31         | 0.01  | 0.990 | 0.01                 | -0.01 | 1.000 | Individual         |
| Bulk density                | Sediment      | 26.16  | -0.31 | 0.002 | 0.54         | 0.32  | 0.965 | 1.38                 | 0.10  | 0.968 | Individual         |
| Seedling density            | Forest        | 11.96  | 0.37  | 0.012 | 12.94        | 0.82  | 0.006 | 3.09                 | 0.14  | 0.744 | Antagonistic       |
| Chl a : Phaeo ratio         | Sediment      | 17.30  | -0.37 | 0.004 | 6.70         | -0.46 | 0.138 | 0.69                 | -0.07 | 0.991 | Antagonistic       |
| NH <sub>4</sub>             | Sediment      | 0.14   | 0.10  | 0.904 | 17.94        | 0.87  | 0.004 | 2.81                 | 0.14  | 0.777 | Individual         |
| Propagule density           | Forest        | 0.09   | -0.11 | 0.904 | 18.48        | 0.05  | 0.004 | 0.66                 | -0.07 | 0.996 | Individual         |
| Mud content                 | Sediment      | 17.56  | 0.32  | 0.005 | 1.65         | 0.05  | 0.875 | 0.02                 | -0.01 | 1.000 | Individual         |
| Chlorophyll a               | Sediment      | 3.44   | 0.13  | 0.457 | 13.73        | -0.68 | 0.006 | 1.48                 | -0.10 | 0.964 | Individual         |
| Crab burrows (<2cm)         | Forest        | 14.54  | -0.34 | 0.008 | 0.98         | -0.22 | 0.938 | 0.20                 | -0.04 | 0.996 | Individual         |
| Foliar δ <sup>15</sup> N    | Forest        | 4.36   | 0.08  | 0.335 | 7.94         | -0.05 | 0.072 | 0.62                 | -0.07 | 0.996 | No effect          |
| Foliar δ <sup>13</sup> C    | Forest        | 4.14   | 0.26  | 0.353 | 5.09         | 0.64  | 0.295 | 1.83                 | 0.12  | 0.935 | No effect          |
| DBH                         | Forest        | 7.15   | -0.32 | 0.109 | 2.21         | -0.56 | 0.797 | 1.62                 | -0.11 | 0.958 | No effect          |
| Sediment C:N ratio          | Sediment      | 2.92   | -0.07 | 0.495 | 0.99         | -0.79 | 0.938 | 6.31                 | -0.22 | 0.228 | No effect          |
| Crab burrows (>2 cm)        | Forest        | 8.70   | -0.26 | 0.060 | 0.00         | 0.01  | 0.997 | 0.00                 | 0.00  | 1.000 | No effect          |
| Pneumatophore density       | Forest        | 3.07   | 0.11  | 0.495 | 3.88         | -0.48 | 0.487 | 0.86                 | -0.08 | 0.991 | No effect          |
| Foliar C                    | Forest        | 3.11   | -0.01 | 0.495 | 0.05         | 0.57  | 0.997 | 2.71                 | 0.15  | 0.785 | No effect          |
| Foliar N                    | Forest        | 1.73   | -0.23 | 0.717 | 1.83         | -0.26 | 0.861 | 1.06                 | -0.09 | 0.981 | No effect          |
| PO <sub>4</sub>             | Sediment      | 1.61   | 0.16  | 0.717 | 1.49         | 0.36  | 0.876 | 0.54                 | 0.07  | 0.996 | No effect          |
| Sediments δ <sup>13</sup> C | Sediment      | 1.64   | 0.11  | 0.717 | 0.11         | 0.02  | 0.997 | 0.00                 | -0.00 | 1.000 | No effect          |
| Foliar C:N ratio            | Forest        | 0.74   | 0.03  | 0.867 | 0.10         | -0.26 | 0.997 | 0.41                 | -0.06 | 0.996 | No effect          |



**Supplementary Table A2.5** New Zealand Land Cover Database (LCDB) classes at version 5; LCDB5, Landcare Research, <http://www.Iris.scinfo.org.nz>

| Class Code | Class Name                 | Class Description                                                                                                                                                                                                                                                                  |
|------------|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 0          | Not land                   | Used where the shoreline has moved between timesteps and no other appropriate class is available to represent an area outside the coastline.                                                                                                                                       |
| 1          | Built-up Area (Settlement) | Commercial, industrial or residential buildings, including associated infrastructure and amenities, not resolvable as other classes. Low density 'lifestyle' residential areas are included where hard surfaces, landscaping and gardens dominate other land covers.               |
| 2          | Urban Parkland/Open Space  | Open, mainly grassed or sparsely-treed, amenity, utility and recreation areas. The class includes parks and playing fields, public gardens, cemeteries, golf courses, berms and other vegetated areas usually within or associated with built-up areas.                            |
| 5          | Transport Infrastructure   | Artificial surfaces associated with transport such as arterial roads, rail-yards and airport runways. Skid sites and landings associated with forest logging are sometimes also included.                                                                                          |
| 6          | Surface Mine or Dump       | Bare surfaces arising from open-cast and other surface mining activities, quarries, gravel-pits and areas of solid waste disposal such as refuse dumps, clean-fill dumps and active reclamation sites.                                                                             |
| 10         | Sand or Gravel             | Bare surfaces dominated by unconsolidated materials generally finer than coarse gravel (60 mm). Typically mapped along sandy seashores and the margins of lagoons and estuaries, lakes and rivers and some areas subject to surficial erosion, soil toxicity and extreme exposure. |
| 12         | Landslide                  | Bare surfaces arising from mass-movement erosion generally in mountain- lands and steep hill-country.                                                                                                                                                                              |
| 14         | Permanent Snow and Ice     | Areas where ice and snow persists through late summer. Typically occurring above 1800 m but also at lower elevations as glaciers.                                                                                                                                                  |
| 16         | Gravel or Rock             | Bare surfaces dominated by unconsolidated or consolidated materials generally coarser than coarse gravel (60 mm). Typically mapped along rocky seashores and rivers, sub-alpine and alpine areas, scree slopes and erosion pavements.                                              |
| 15         | Alpine Grass/Herbfield     | Typically sparse communities above the actual or theoretical treeline dominated by herbaceous cushion, mat, turf, and rosette plants and lichens. Grasses are a minor or infrequent component, whereas stones, boulders and bare rock are usually conspicuous.                     |
| 20         | Lake or Pond               | Essentially-permanent, open, fresh-water without emerging vegetation including artificial features such as oxidation ponds, amenity, farm and fire ponds and reservoirs as well as natural lakes, ponds and tarns.                                                                 |
| 21         | River                      | Flowing open fresh-water generally more than 30 m wide and without emerging vegetation. It includes artificial features such as canals and channels as well as natural rivers and streams.                                                                                         |
| 22         | Estuarine Open Water       | Standing or flowing saline water without emerging vegetation including estuaries, lagoons, and occasionally lakes occurring in saline situations such as inter-dune hollows and coastal depressions.                                                                               |

|    |                                              |                                                                                                                                                                                                                                                                                                                                                                                                 |
|----|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 30 | Short-rotation Cropland                      | Land regularly cultivated for the production of cereal, root, and seed crops, hops, vegetables, strawberries and field nurseries, often including intervening grassland, fallow land, and other covers not delineated separately.                                                                                                                                                               |
| 33 | Orchards, Vineyards or Other Perennial Crops | Land managed for the production of grapes, pip, citrus and stone fruit, nuts, olives, berries, kiwifruit, and other perennial crops. Cultivation for crop renewal is infrequent and irregular but is sometimes practiced for weed control.                                                                                                                                                      |
| 40 | High Producing Exotic Grassland              | Exotic sward grassland of good pastoral quality and vigour reflecting relatively high soil fertility and intensive grazing management. Clover species, ryegrass and cocksfoot dominate with lucerne and plantain locally important, but also including lower-producing grasses exhibiting vigour in areas of good soil moisture and fertility.                                                  |
| 41 | Low Producing Grassland                      | Exotic sward grassland and indigenous short tussock grassland of poor pastoral quality reflecting lower soil fertility and extensive grazing management or non-agricultural use. Browntop, sweet vernal, danthonia, fescue and Yorkshire fog dominate, with indigenous short tussocks (hard tussock, blue tussock and silver tussock) common in the eastern South Island and locally elsewhere. |

|    |                                  |                                                                                                                                                                                                                                                                                                                                                   |
|----|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 43 | Tall Tussock Grassland           | Indigenous snow tussocks in mainly alpine mountain-lands and red tussock in the central North Island and locally in poorly-drained valley floors, terraces and basins of both islands.                                                                                                                                                            |
| 44 | Depleted Grassland               | Areas, of mainly former short tussock grassland in the drier eastern South Island high country, degraded by over-grazing, fire, rabbits and weed invasion among which <i>Hieracium</i> species are conspicuous. Short tussocks usually occur, as do exotic grasses, but bare ground is more prominent.                                            |
| 45 | Herbaceous Freshwater Vegetation | Herbaceous wetland communities occurring in freshwater habitats where the water table is above or just below the substrate surface for most of the year. The class includes rush, sedge, restiad, and sphagnum communities and other wetland species, but not flax nor willows which are mapped as Flaxland and Deciduous Hardwoods respectively. |
| 46 | Herbaceous Saline Vegetation     | Herbaceous wetland communities occurring in saline habitats subject to tidal inundation or saltwater intrusion. Commonly includes club rush, wire rush and glasswort, but not mangrove which is mapped separately.                                                                                                                                |
| 47 | Flaxland                         | Areas dominated by New Zealand flax usually swamp flax (harakeke) in damp sites but occasionally mountain flax (wharariki) on cliffs and mountain slopes.                                                                                                                                                                                         |

|    |                                  |                                                                                                                                                                                                                                                                                                                                                       |
|----|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 50 | Fernland                         | Bracken fern, umbrella fern, or ring fern, commonly on sites with low fertility and a history of burning. Manuka, gorse, and/or other shrubs are often a component of these communities and will succeed Fernland if left undisturbed.                                                                                                                |
| 51 | Gorse and/or Broom               | Scrub communities dominated by gorse or Scotch broom generally occurring on sites of low fertility, often with a history of fire, and insufficient grazing pressure to control spread. Left undisturbed, this class can be transitional to                                                                                                            |
| 52 | Mānuka and/or Kānuka             | Scrub dominated by mānuka and/or kānuka, typically as a successional community in a reversion toward forest. Mānuka has a wider ecological tolerance and distribution than kānuka with the latter somewhat concentrated in the north with particular prominence on the volcanic soils of the central                                                  |
| 54 | Broadleaved Indigenous Hardwoods | Lowland scrub communities dominated by indigenous mixed broadleaved shrubs such as wineberry, mahoe, five-finger, <i>Pittosporum</i> spp, fuchsia, tutu, titoki and tree ferns. This class is usually indicative of advanced succession                                                                                                               |
| 55 | Sub Alpine Shrubland             | Highland scrub dominated by indigenous low-growing shrubs including species of <i>Hebe</i> , <i>Dracophyllum</i> , <i>Olearia</i> , and <i>Cassinia</i> . Predominantly occurring above the actual or theoretical treeline, this class is also recorded where temperature inversions have created cooler micro-climates at lower elevations e.g., the |
| 56 | Mixed Exotic Shrubland           | Communities of introduced shrubs and climbers such as boxthorn, hawthorn, elderberry, blackberry, sweet brier, buddleja, and old man's beard.                                                                                                                                                                                                         |
| 58 | Matagouri or Grey Scrub          | Scrub and shrubland comprising small-leaved, often divaricating shrubs such as matagouri, <i>Coprosma</i> spp, <i>Muehlenbeckia</i> spp., <i>Cassinia</i> spp., and <i>Parsonsia</i> spp. These, from a distance, often have a grey appearance.                                                                                                       |
| 80 | Peat Shrubland (Chatham Islands) | Low-growing shrubland communities usually dominated by <i>Dracophyllum</i> spp. in association with <i>Cyathodes</i> spp. and ground ferns. Mapped only on the Chatham Islands.                                                                                                                                                                       |
| 81 | Dune Shrubland (Chatham Islands) | Low-growing shrubland communities dominated by <i>Leucopogon</i> spp., <i>Pimelia arenaria</i> and <i>Coprosma</i> spp., in association with sedges and scattered herbs and grasses. Mapped only on the Chatham Islands.                                                                                                                              |

|    |                     |                                                                                                                                                                                                                                                                                                              |
|----|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 70 | Mangrove            | Shrubs or small trees of the New Zealand mangrove ( <i>Avicennia marina</i> subspecies <i>australascia</i> ) growing in harbours, estuaries, tidal creeks and rivers north of Kawhia on the west coast and Ohiwa on the east coast.                                                                          |
| 64 | Forest - Harvested  | Predominantly bare ground arising from the harvesting of exotic forest or, less commonly, the clearing of indigenous forest. Replanting of exotic forest (or conversion to a new land use) is not evident and nor is the future use of land                                                                  |
| 68 | Deciduous Hardwoods | Exotic deciduous woodlands, predominantly of willows or poplars but also of oak, elm, ash or other species. Commonly alongside inland water (or as part of wetlands), or as erosion-control, shelter and amenity plantations                                                                                 |
| 69 | Indigenous Forest   | Tall forest dominated by indigenous conifer, broadleaved or beech species.                                                                                                                                                                                                                                   |
| 71 | Exotic Forest       | Planted or naturalised forest predominantly of radiata pine but including other pine species, Douglas fir, cypress, larch, acacia and eucalypts. Production forestry is the main land use in this class with minor areas devoted to mass- movement erosion-control and other areas of naturalised (wildling) |



## 6.3 Supplementary Materials to Chapter 3

### Supplementary Protocol 1: ROMA plates

Rapid Organic Matter Assay (ROMA) plates were prepared as described in O'Meara et al. (2017). Briefly, plates (180 × 90 mm) were made from 15 mm thick acrylic sheets and holding 18 wells of 0.9 mL volume each. The wells are arranged in three vertical rows which are 3 cm apart and are separated along the vertical profile at 1, 3, 5, 7, 10, and 15 cm from the top of the plate. The wells were filled with a mixture of food grade agar, microcrystalline cellulose, and powdered bran flakes to achieve a concentration of 0.029 g C mL<sup>-1</sup>. The medium was prepared using 10 g food grade agar, 21.66 g cellulose (CAS 9004-34-6; Thermofisher) and 13.32 g of powdered bran flakes (Edmonds brand) in 1 L of deionized water and heated to a boil until the mixture began to thicken. The wells were gradually filled with the hand-warm medium avoiding air bubbles and sinking of the bran particles until the wells were flush with the surface of the plate. Excess medium from overfilling was removed using a razor blade. A 25 µm nylon mesh screen was used to cover the wells to exclude macro- and meiofauna from accessing the wells. Once prepared, the plates were stored in a cool, dark, and highly humid environment to avoid desiccation and shrinkage of agar. The plates were prepared the night before deployment. Three ROMA plates were used at each site. A flat-bladed spade was used to open a gap in the sediment to a depth of approximately 20 cm into which the ROMA plate was pushed, the open wells facing towards the fringe of the mangrove stand. The plates were placed so that the upper edge was just below the sediment surface and the gap was closed by gently pressing the sediment back into place. After 12 days the plates were retrieved by gently pulling them vertically out of the sediment using the attached string with tag and stored wrapped in wet towels on ice until analysis the same day. The plates were gently rinsed with deionized water, tapped dry with paper towels, and analysed by measuring the volume of agar lost from the wells. For each well, a 1 mL syringe (BD syringe Luer-lock) filled with 0.9 mL water was used to fill the well until the water was flush with the surface of the plate. The volume replaced by water was equivalent to the volume of substrate lost to degradation over time. A carbon decomposition rate could then be calculated by:

$$k_{ROMA} = \frac{(V_{initial} - V_{final}) * 0.029}{t} \quad \text{Eq. (A1)}$$

Where  $k_{ROMA}$  is the carbon degradation rate in g of Carbon per day,  $V_{initial}$  is the initial agar volume (0.9 mL),  $V_{final}$  is the volume of agar after the incubation, and  $t$  is the time (in days) of the incubation. The value of 0.029 (g C mL<sup>-1</sup>) is the concentration of carbon of the agar substrate. To avoid misinterpretation of the degradation coefficient by ill-fitting regressions, a simple measure to describe the degradation profiles was chosen. The average C consumption of the wells from rows 3, 5, and 7 (corresponding to cm depth) was used to achieve a value for C consumption from a depth that matched the burial depth of the tea bags.



## Supplementary Protocol 2: Tea bag index

Following the method outlined by Keuskamp et al. (2013), we buried two types of tea (Lipton green tea (EAN:87 22700 05552 5) and Lipton rooibos tea (EAN:87 22700 18843 8)) in mangrove sediments to assess the rate at which they decompose. Green tea represents a more labile material source, (representative of leaves and fine roots) and is expected to decompose faster, whereas the rooibos tea (from here on called 'red tea') is made up of more recalcitrant material with expected slower rates of decomposition, such as woody plant components (Keuskamp et al., 2013). The tea was contained in the original tea bags to allow for water flow and microbial access but restrict consumption by meio- and macrofauna. Before burial, each tea bag (tea, bag, string, and tag) was dried to a constant weight at 60 °C and then weighed. An average weight of each empty bag with string and tag was established empirically by weighing ten green tea bag and ten red tea bags. Differences between empty bags from green and red tea were tested using ANOVA (p-value = 0.82) and used to calculate a single average value seeing that there was no statistical difference between the weights of the bags. The variation around the mean from all bags was about 1.7%. The difference between the total weight of each bag and the average weight of an empty bag provided the initial weight of the tea in each bag. Once retrieved, the tea bags were washed in clean water and dried to constant weight at 60 °C. The dried tea was extracted from the bag and weighed to obtain the remaining weight after incubation. The difference between the initial weight of each tea bag and the remaining weight after incubation was the amount of tea lost during incubation. As a modification to the original TBI, we incubated additional bags for 12 months, as some studies have found long-term incubation data to be better related to environmental factors (Marley et al., 2019). We used the time series data of each tea type to calculate decomposition rates by fitting a single-term exponential decay model to the data at each site, using a nonlinear least squares regression with the Levenberg-Marquardt algorithm as the measure of estimation (Levenberg 1944). The model was fitted using the `nlsLM()` command from the `minpack.lm` package v1.2-3 (Elzhov et al., 2023) and defined as:

$$y = y_0 e^{-kt} \quad \text{Eq. (A2)}$$

where  $y$  is the percent mass remaining at a time point,  $y_0$  is the initial mass at  $t_0$ , and  $t$  is the time elapsed since the beginning of the incubation (in days). The decomposition rate was calculated from the red tea only, as there was not much change between the remaining mass of the green tea at 3 months compared to that after 12 months.

Calculations for decomposition rate constant ( $k$ ) and stabilization factor ( $S$ ) were performed following the TBI-protocol from Keuskamp et al. (2013):

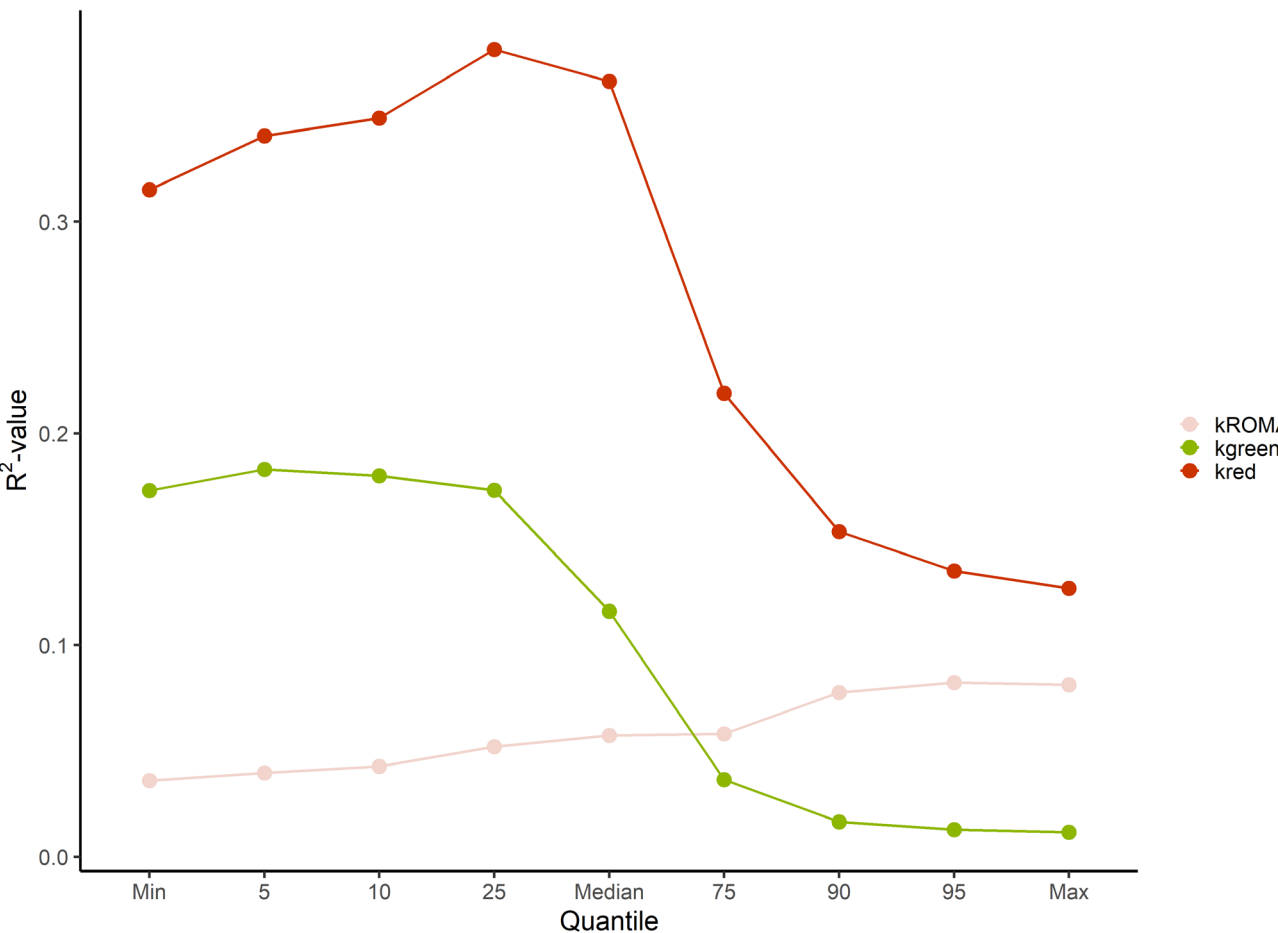
$$W_r(t) = a_r * e^{-kt} + (1 - a_r) \quad \text{Eq. (A3)}$$

$$S = 1 - \frac{a_g}{H_g}, \quad \text{Eq. (A4)}$$

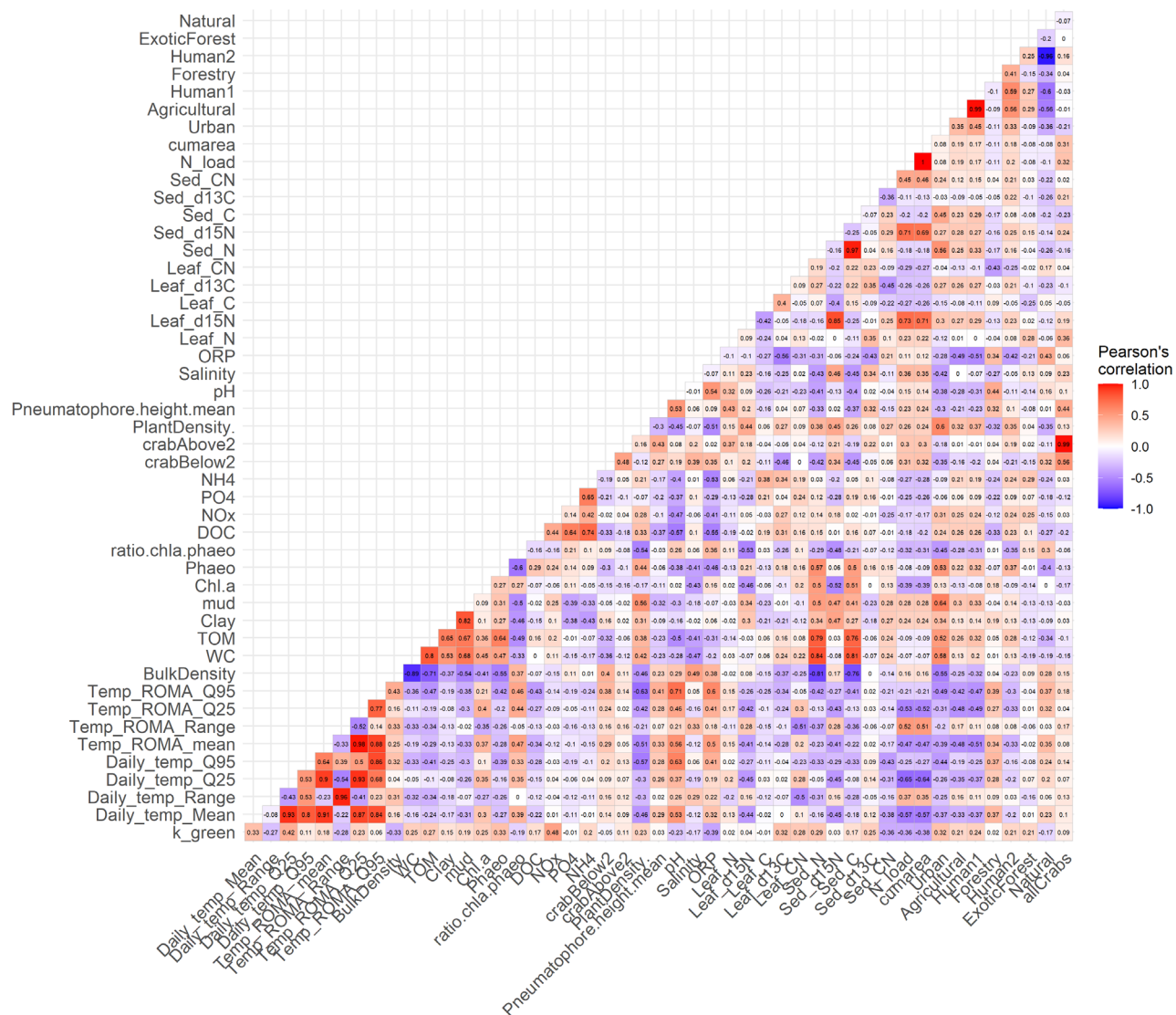
$$a_r = H_r(1 - S). \quad \text{Eq. (A5)}$$

Where  $W_r(t)$  describes the substrate weight of red tea after incubation time ( $t$  in days),  $a_r$  is the labile and  $1 - a_r$  is the recalcitrant fraction of the substrate, and  $k$  is the decomposition rate constant.  $S$  describes the stabilization factor,  $a_g$  is the decomposable fraction of green tea (based on the mass loss during incubation), and  $H_g$  is the hydrolyzable fraction of green tea. The decomposable fraction of red tea was calculated using Eq. (A4), based on its hydrolyzable fraction ( $H_r$ ) and stabilization factor ( $S$ ). With  $W_r(t)$  and  $a_r$  known,  $k$  is calculated using Eq. (A2). The decomposition rate calculated from the tea bag index was tested but yielded no relationship with any of the predictor variables, so it is not reported on in the manuscript. This is consistent with several studies that found the decomposition rate calculated from the tea bag index to be a very poor indicator of environmental variability (MacDonald et al. 2018; Mueller et al. 2018).

To enable the results from this study to be compared with other TBI studies, the decomposition rate and stabilisation factor are presented in supplementary table A3.1.



**Supplementary Figure A3.1** Correlation values of different temperature quantiles with the degradation rate of different substrates. Individual linear models were fitted to each quantile to establish the strength ( $R^2$ ) of the relationship.



**Supplementary Figure A3.2** Correlation matrix of all environmental variables.

**Supplementary Table A3.1** Summary table of the degradation metrics. A subset of these was used as response variables for the model selection. Red values were imputed by the random forest algorithm. Decomposition rate ( $k_{\text{TBI}}$ ) and stabilisation factor ( $S_{\text{TBI}}$ ) were calculated using the tea bag index (TBI) methodology.

| Site | Decomposition rate ( $k_{\text{TBI}}$ ) | Stabilization factor ( $S_{\text{TBI}}$ ) | Green tea (% loss after 3 months) | Green tea (% loss after 12 months) | Red tea (% loss after 3 months) | Red tea (% loss after 12 months) | Decomposition rate ( $k_{\text{ROMA}}$ ) | Decomposition rate ( $k_{\text{green}}$ ) | Decomposition rate ( $k_{\text{red}}$ ) |
|------|-----------------------------------------|-------------------------------------------|-----------------------------------|------------------------------------|---------------------------------|----------------------------------|------------------------------------------|-------------------------------------------|-----------------------------------------|
| ATH  | 0.0083 ± 0.0015                         | 0.16 ± 0.03                               | 70.28 ± 0.59                      | 78.58 ± 1.66                       | 24.05 ± 2.16                    | 33.41 ± 1.33                     | 0.20 ± 0.040                             | 0.012                                     | 0.0010                                  |
| BOW  | 0.0087 ± 0.0006                         | 0.07 ± 0.02                               | 78.10 ± 1.40                      | 82.71 ± 0.24                       | 26.66 ± 0.90                    | 36.98 ± 0.88                     | 0.28 ± 0.084                             | 0.017                                     | 0.0011                                  |
| COR  | 0.0100 ± 0.0010                         | 0.08 ± 0.02                               | 77.39 ± 1.54                      | 81.02 ± 1.48                       | 28.99 ± 2.14                    | 35.48 ± 0.72                     | 0.36 ± 0.005                             | 0.016                                     | 0.0012                                  |
| HEI  | 0.0057 ± 0.0015                         | 0.20 ± 0.01                               | 67.62 ± 1.21                      | 76.52 ± 1.88                       | 16.35 ± 3.92                    | 37.20 ± 2.80                     | 0.42 ± 0.012                             | 0.011                                     | 0.0013                                  |
| KAT  | 0.0100 ± 0.0036                         | 0.11 ± 0.04                               | 74.56 ± 3.03                      | 84.06 ± 1.05                       | 28.55 ± 5.42                    | 37.42 ± 3.00                     | 0.33 ± 0.207                             | 0.015                                     | 0.0013                                  |
| MAN  | 0.0077 ± 0.0021                         | 0.18 ± 0.03                               | 69.12 ± 2.51                      | 27.31 ± 1.74                       | 23.49 ± 3.64                    | 57.47 ± 14.94                    | 0.52 ± 0.068                             | 0.012                                     | 0.0011                                  |
| MAT  | 0.0073 ± 0.0015                         | 0.08 ± 0.05                               | 77.19 ± 4.15                      | 81.24 ± 0.71                       | 25.62 ± 2.38                    | 35.51 ± 1.62                     | 0.36 ± 0.067                             | 0.015                                     | 0.0012                                  |
| OMO  | 0.0060 ± 0.0000                         | 0.16 ± 0.01                               | 70.55 ± 1.00                      | 78.08 ± 1.62                       | 20.11 ± 0.63                    | 32.83 ± 3.89                     | 0.38 ± 0.021                             | 0.012                                     | 0.0010                                  |
| OPU  | 0.0067 ± 0.0012                         | 0.17 ± 0.03                               | 69.92 ± 2.35                      | 81.36 ± 2.67                       | 20.89 ± 2.96                    | 33.07 ± 2.00                     | 0.42 ± 0.145                             | 0.012                                     | 0.0010                                  |
| PAU1 | 0.0100 ± 0.0010                         | 0.15 ± 0.01                               | 71.57 ± 0.75                      | 83.83 ± 1.45                       | 27.35 ± 0.89                    | 37.79 ± 0.75                     | 0.52 ± 0.138                             | 0.013                                     | 0.0012                                  |
| PAU2 | 0.0073 ± 0.0012                         | 0.14 ± 0.02                               | 72.75 ± 1.48                      | 82.71 ± 1.75                       | 21.76 ± 1.03                    | 38.86 ± 2.66                     | 0.40 ± 0.081                             | 0.014                                     | 0.0013                                  |
| POU  | 0.0067 ± 0.0006                         | 0.17 ± 0.02                               | 69.50 ± 2.02                      | 80.83 ± 3.33                       | 20.58 ± 0.85                    | 37.09 ± 3.63                     | 0.41 ± 0.060                             | 0.012                                     | 0.0012                                  |
| PUR  | 0.0080 ± 0.0020                         | 0.16 ± 0.02                               | 70.89 ± 1.88                      | 81.55 ± 1.36                       | 25.18 ± 4.44                    | 34.31 ± 1.32                     | 0.44 ± 0.030                             | 0.013                                     | 0.0012                                  |
| RAK  | 0.0097 ± 0.0006                         | 0.06 ± 0.01                               | 78.93 ± 1.20                      | 85.95 ± 1.42                       | 30.75 ± 0.21                    | 45.59 ± 2.71                     | 0.60 ± 0.098                             | 0.016                                     | 0.0017                                  |
| RAN  | 0.0083 ± 0.0006                         | 0.12 ± 0.02                               | 73.84 ± 1.58                      | 83.49 ± 1.83                       | 26.02 ± 1.70                    | 38.18 ± 4.41                     | 0.39 ± 0.036                             | 0.014                                     | 0.0012                                  |
| REA  | 0.0073 ± 0.0006                         | 0.10 ± 0.03                               | 75.68 ± 2.48                      | 81.41 ± 1.93                       | 24.22 ± 1.06                    | 36.00 ± 1.14                     | 0.41 ± 0.053                             | 0.014                                     | 0.0011                                  |
| TAH  | 0.0077 ± 0.0006                         | 0.09 ± 0.02                               | 76.25 ± 1.53                      | 80.90 ± 0.76                       | 25.72 ± 1.44                    | 33.37 ± 2.26                     | 0.54 ± 0.070                             | 0.015                                     | 0.0010                                  |
| TAI1 | 0.0093 ± 0.0012                         | 0.18 ± 0.01                               | 69.71 ± 1.41                      | 83.31 ± 0.57                       | 27.04 ± 2.66                    | 30.42 ± 3.33                     | 0.35 ± 0.033                             | 0.012                                     | 0.0010                                  |
| TAI2 | 0.0090 ± 0.0017                         | 0.15 ± 0.01                               | 70.34 ± 2.68                      | 80.38 ± 1.63                       | 23.18 ± 2.25                    | 34.75 ± 0.59                     | 0.39 ± 0.032                             | 0.013                                     | 0.0011                                  |
| TBB  | 0.0143 ± 0.0045                         | 0.22 ± 0.02                               | 65.94 ± 1.36                      | 71.54 ± 2.50                       | 30.37 ± 3.08                    | 35.42 ± 0.79                     | 0.44 ± 0.017                             | 0.009                                     | 0.0011                                  |
| TEK  | 0.0103 ± 0.0006                         | 0.05 ± 0.01                               | 80.04 ± 1.21                      | 82.73 ± 2.73                       | 31.57 ± 1.39                    | 45.18 ± 1.50                     | 0.51 ± 0.093                             | 0.017                                     | 0.0017                                  |
| TRB  | 0.0077 ± 0.0006                         | 0.17 ± 0.01                               | 69.99 ± 1.19                      | 75.35 ± 3.17                       | 24.49 ± 1.98                    | 31.09 ± 8.57                     | 0.45 ± 0.047                             | 0.012                                     | 0.0010                                  |
| TUA  | 0.0083 ± 0.0012                         | 0.12 ± 0.03                               | 73.79 ± 2.22                      | 81.56 ± 0.17                       | 25.52 ± 2.32                    | 35.86 ± 1.15                     | 0.48 ± 0.228                             | 0.014                                     | 0.0012                                  |
| TYE  | 0.0080 ± 0.0010                         | 0.10 ± 0.02                               | 75.59 ± 1.88                      | 80.60 ± 1.59                       | 25.69 ± 1.53                    | 33.24 ± 1.22                     | 0.30 ± 0.028                             | 0.015                                     | 0.0009                                  |
| WEB  | 0.0097 ± 0.0015                         | 0.04 ± 0.00                               | 80.75 ± 1.85                      | 80.81 ± 1.76                       | 31.67 ± 1.87                    | 34.13 ± 3.55                     | 0.36 ± 0.037                             | 0.015                                     | 0.0011                                  |
| WHA1 | 0.0083 ± 0.0012                         | 0.11 ± 0.01                               | 74.57 ± 0.85                      | 85.66 ± 0.40                       | 26.07 ± 1.32                    | 45.54 ± 1.66                     | 0.39 ± 0.093                             | 0.018                                     | 0.0012                                  |
| WHA2 | 0.0080 ± 0.0020                         | 0.18 ± 0.02                               | 68.78 ± 2.01                      | 81.98 ± 1.21                       | 20.59 ± 1.55                    | 32.50 ± 3.39                     | 0.40 ± 0.104                             | 0.014                                     | 0.0017                                  |
| WHA3 | 0.0083 ± 0.0012                         | 0.15 ± 0.03                               | 71.86 ± 2.92                      | 84.67 ± 3.85                       | 25.15 ± 1.77                    | 41.16 ± 2.54                     | 0.35 ± 0.021                             | 0.012                                     | 0.0010                                  |
| WHI  | 0.0080 ± 0.0026                         | 0.10 ± 0.03                               | 74.56 ± 1.96                      | 82.47 ± 1.45                       | 24.65 ± 3.57                    | 37.29 ± 0.79                     | 0.42 ± 0.058                             | 0.013                                     | 0.0015                                  |

**Supplementary Table A3.2** Variable importance controlling ROMA decomposition ( $k_{ROMA}$ ) as calculated from the random forest model. Variables are ranked by importance within the model. Mean and SD values were calculated using 150 bootstraps.

| Rank | Variable                              | variable importance<br>Mean | variable importance<br>SD |
|------|---------------------------------------|-----------------------------|---------------------------|
| 1    | Sediment C                            | 8.70                        | 3.27                      |
| 2    | Foliar N                              | 6.71                        | 2.05                      |
| 3    | Foliar C                              | 6.60                        | 2.15                      |
| 4    | Crab burrow (<2 cm)                   | 6.41                        | 1.58                      |
| 5    | ORP                                   | 6.37                        | 2.20                      |
| 6    | NOx                                   | 6.00                        | 1.63                      |
| 7    | NH4                                   | 5.91                        | 1.47                      |
| 8    | Crab burrow (>2 cm)                   | 5.91                        | 1.40                      |
| 9    | Foliar d15C                           | 5.82                        | 1.71                      |
| 10   | Sediment C:N                          | 5.78                        | 1.33                      |
| 11   | Sediment d13C                         | 5.46                        | 1.26                      |
| 12   | Salinity                              | 5.44                        | 0.94                      |
| 13   | Pneumatophore height                  | 5.14                        | 1.16                      |
| 14   | %Human land use                       | 5.14                        | 1.31                      |
| 15   | Foliar d15N                           | 5.05                        | 1.15                      |
| 16   | DOC                                   | 4.87                        | 1.24                      |
| 17   | Chlorophyll<br>a:Phaeophytin          | 4.81                        | 0.76                      |
| 18   | Mud content                           | 4.61                        | 1.28                      |
| 19   | pH                                    | 4.53                        | 0.92                      |
| 20   | Foliar C:N                            | 4.43                        | 1.12                      |
| 21   | Chlorophyll a                         | 4.34                        | 0.79                      |
| 22   | Daily temperature (25 <sup>th</sup> ) | 4.02                        | 0.95                      |
| 23   | Tree density                          | 3.70                        | 0.91                      |



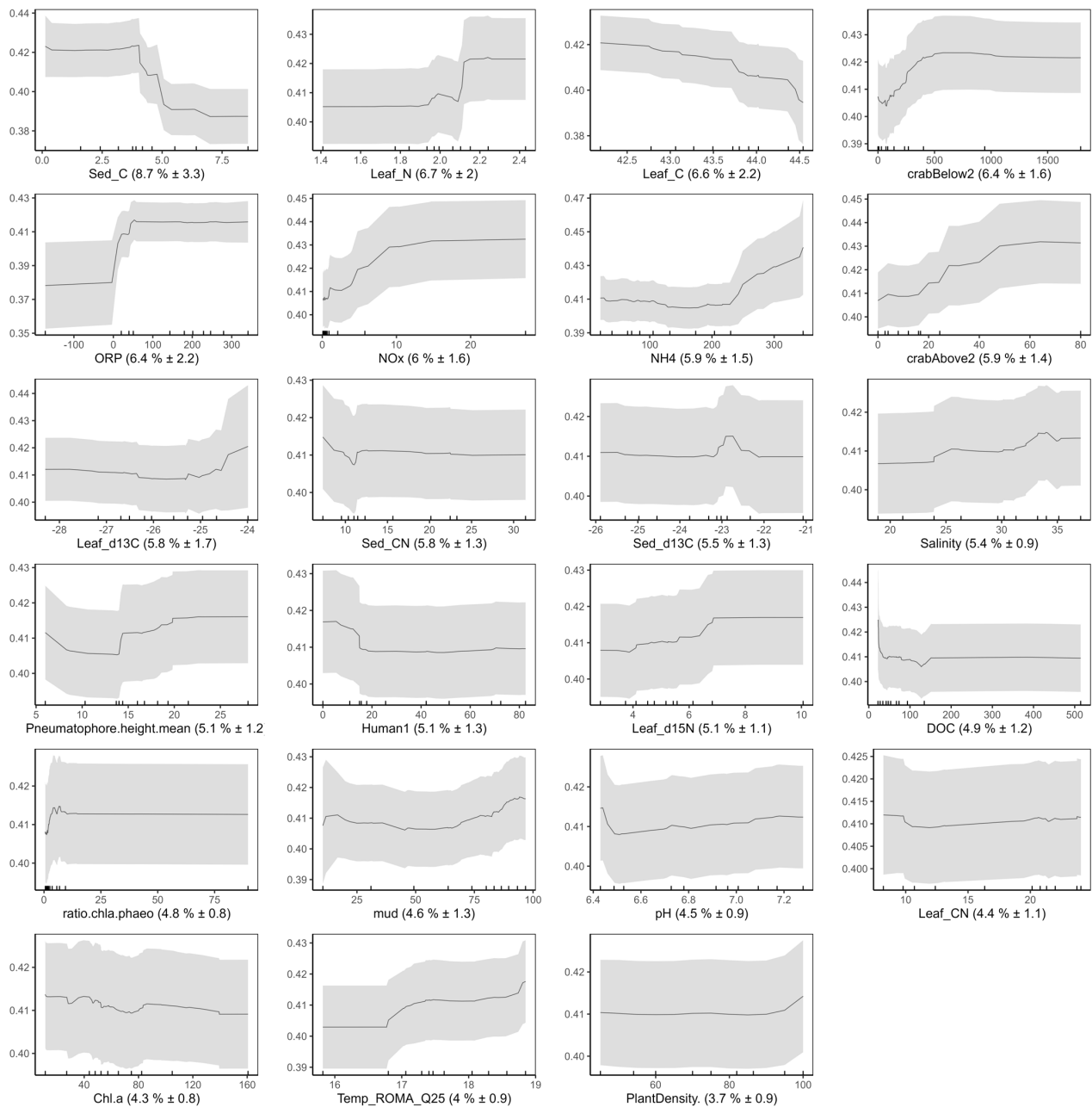
**Supplementary Table A3.3** Variable importance controlling green tea decomposition ( $k_{\text{green}}$ ) as calculated from the random forest model. Variables are ranked by importance within the model. Mean and SD values were calculated using 150 bootstraps.

| Rank | Variable                              | variable importance<br>Mean | variable importance<br>SD |
|------|---------------------------------------|-----------------------------|---------------------------|
| 1    | Foliar d15N                           | 13.33                       | 3.68                      |
| 2    | Salinity                              | 7.75                        | 1.39                      |
| 3    | Foliar C:N                            | 7.68                        | 1.26                      |
| 4    | NOx                                   | 6.67                        | 1.71                      |
| 5    | %Human land use                       | 6.58                        | 1.06                      |
| 6    | Sediment C:N                          | 6.27                        | 1.13                      |
| 7    | Sediment C                            | 6.08                        | 1.06                      |
| 8    | Sediment d13C                         | 5.67                        | 0.98                      |
| 9    | Chlorophyll a                         | 5.52                        | 1.10                      |
| 10   | Pneumatophore height                  | 5.51                        | 0.78                      |
| 11   | Foliar N                              | 5.46                        | 1.16                      |
| 12   | Foliar C                              | 5.44                        | 0.81                      |
| 13   | ORP                                   | 5.42                        | 1.10                      |
| 14   | Daily temperature (25 <sup>th</sup> ) | 5.35                        | 0.92                      |
| 15   | Foliar d13C                           | 5.25                        | 0.93                      |
| 16   | pH                                    | 4.73                        | 0.84                      |
| 17   | Chlorophyll<br>a:Phaeophytin          | 4.27                        | 0.78                      |
| 18   | Tree density                          | 4.14                        | 0.83                      |
| 19   | Mud content                           | 4.11                        | 0.65                      |
| 20   | Crab burrows (<2 cm)                  | 3.62                        | 0.88                      |
| 21   | DOC                                   | 3.12                        | 0.64                      |
| 22   | NH <sub>4</sub>                       | 3.01                        | 0.76                      |
| 23   | Crab burrows (>2 cm)                  | 2.64                        | 0.77                      |

Supplementary Table A3.4 Variable importance controlling red tea decomposition ( $k_{red}$ ) as calculated from the random forest model. Variables are ranked by importance within the model. Mean and SD values were calculated using 150 bootstraps.

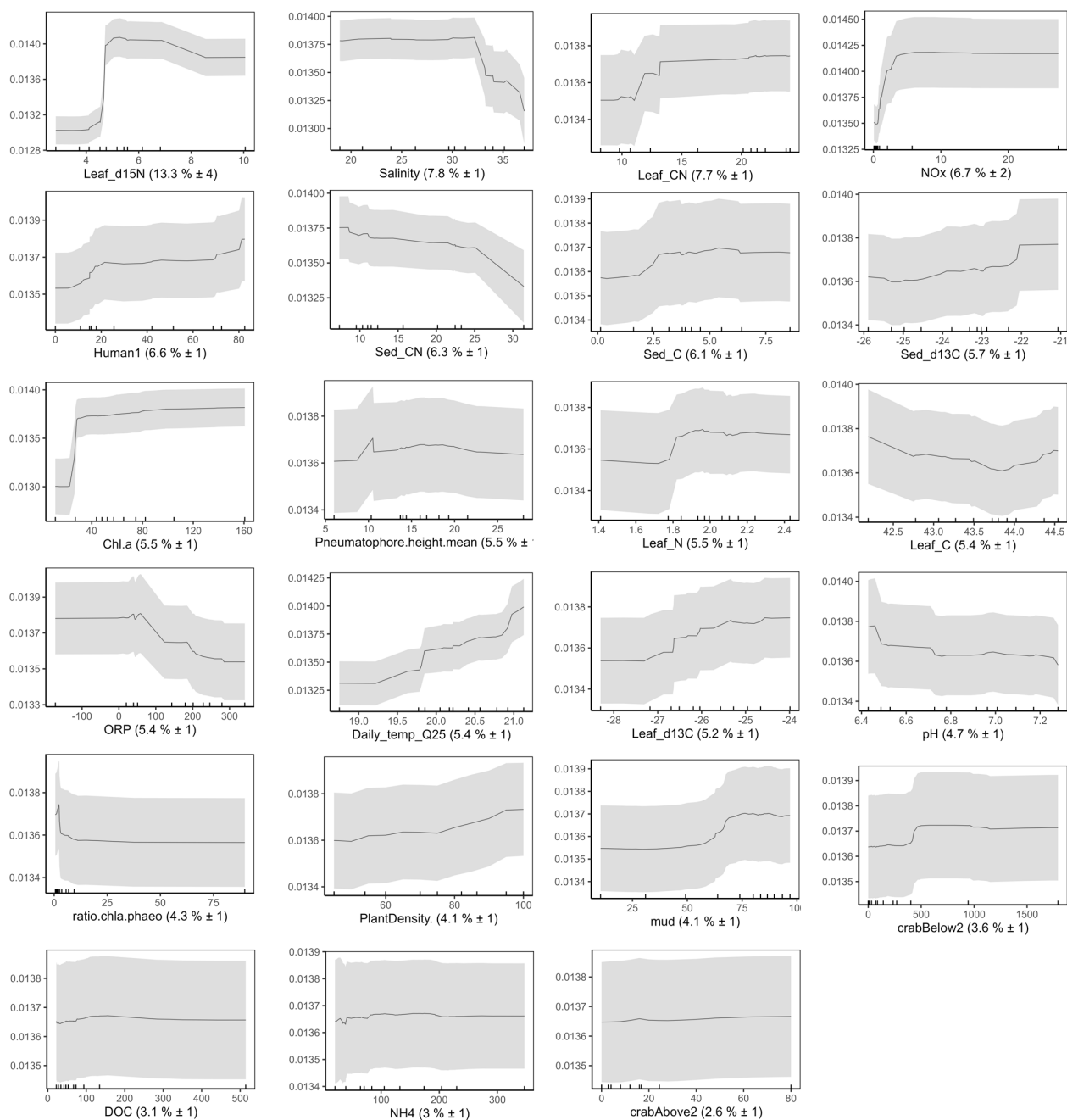
| Rank | Variable                              | variable importance<br>Mean | variable importance<br>SD |
|------|---------------------------------------|-----------------------------|---------------------------|
| 1    | Daily temperature (25 <sup>th</sup> ) | 15.06                       | 5.15                      |
| 2    | pH                                    | 9.66                        | 3.01                      |
| 3    | Sediment C                            | 8.39                        | 1.75                      |
| 4    | Foliar N                              | 8.21                        | 3.21                      |
| 5    | Foliar C:N                            | 7.99                        | 2.15                      |
| 6    | Foliar d15N                           | 7.41                        | 2.34                      |
| 7    | %Human land use                       | 6.93                        | 1.87                      |
| 8    | Foliar C                              | 5.88                        | 1.96                      |
| 9    | Sediment C:N                          | 5.80                        | 1.87                      |
| 10   | Sediment d13C                         | 5.50                        | 1.57                      |
| 11   | Foliar d13C                           | 5.06                        | 1.15                      |
| 12   | Pneumatophore height                  | 4.73                        | 1.20                      |
| 13   | ORP                                   | 4.49                        | 1.20                      |
| 14   | Tree density                          | 4.18                        | 1.17                      |
| 15   | Mud content                           | 4.10                        | 1.09                      |
| 16   | Crab burrows (<2 cm)                  | 3.95                        | 1.38                      |
| 17   | Crab burrows (>2 cm)                  | 3.73                        | 1.45                      |
| 18   | Salinity                              | 3.71                        | 1.25                      |
| 19   | NOx                                   | 3.41                        | 1.17                      |
| 20   | NH4                                   | 3.01                        | 0.98                      |
| 21   | Chlorophyll a                         | 2.72                        | 0.74                      |
| 22   | DOC                                   | 2.71                        | 1.07                      |
| 23   | Chlorophyll:Phaeophytin               | 2.57                        | 1.01                      |

## Partial dependence: ROMA



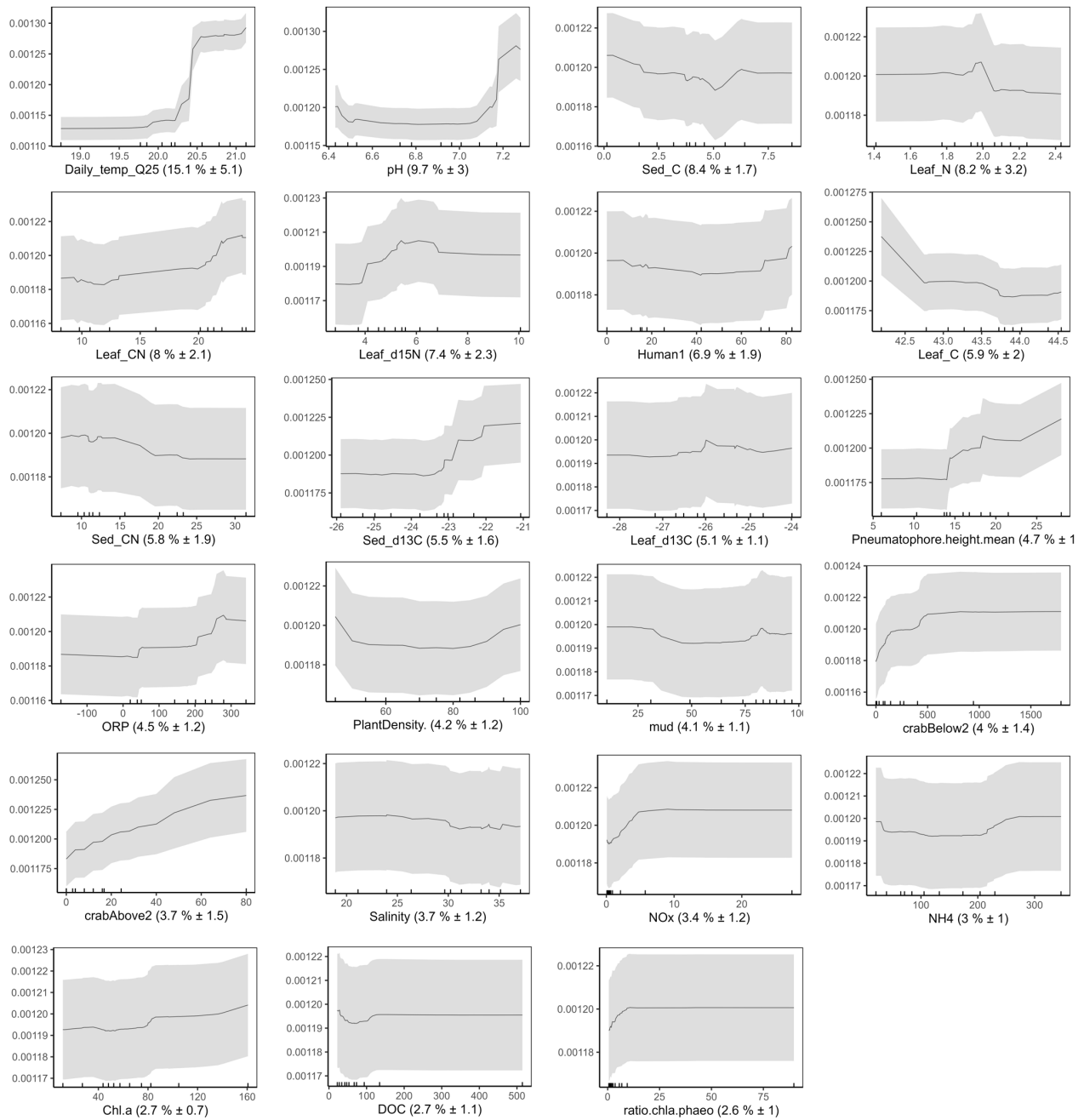
**Supplementary Figure A3.3** Partial dependence plots of all variables' relationship with degradation rate of ROMA ( $k_{ROMA}$ ) ordered by relative importance to the model performance. Black lines are means and ribbons represent the standard deviation from 150 bootstrap iterations.

## Partial dependence: Green tea



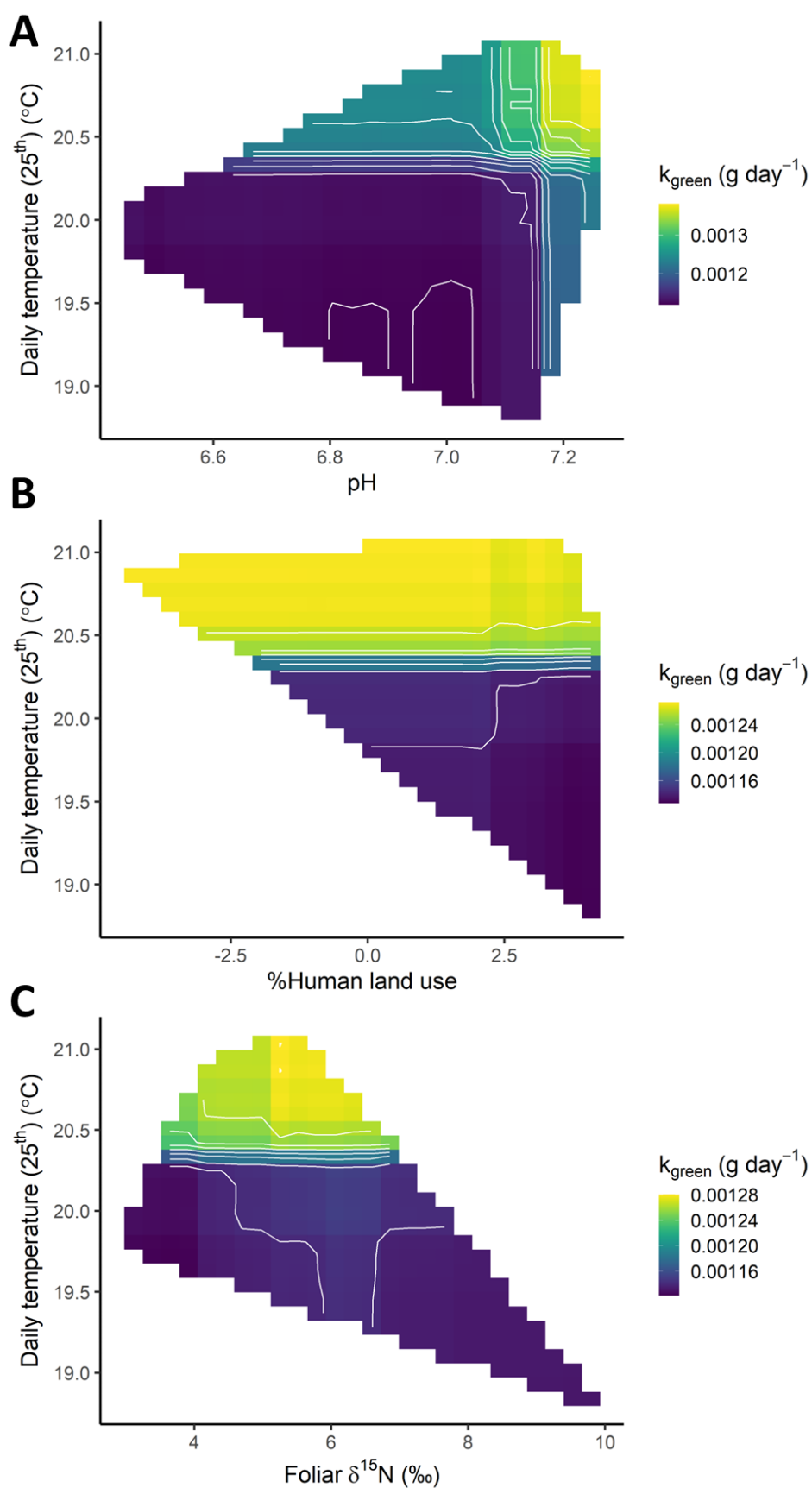
**Supplementary Figure A3.4** Partial dependence plots of all variables' relationship with degradation rate of green tea ( $k_{\text{green}}$ ) ordered by relative importance to the model performance. Black lines are means and ribbons represent the standard deviation from 150 bootstrap iterations.

## Partial dependence: Red tea



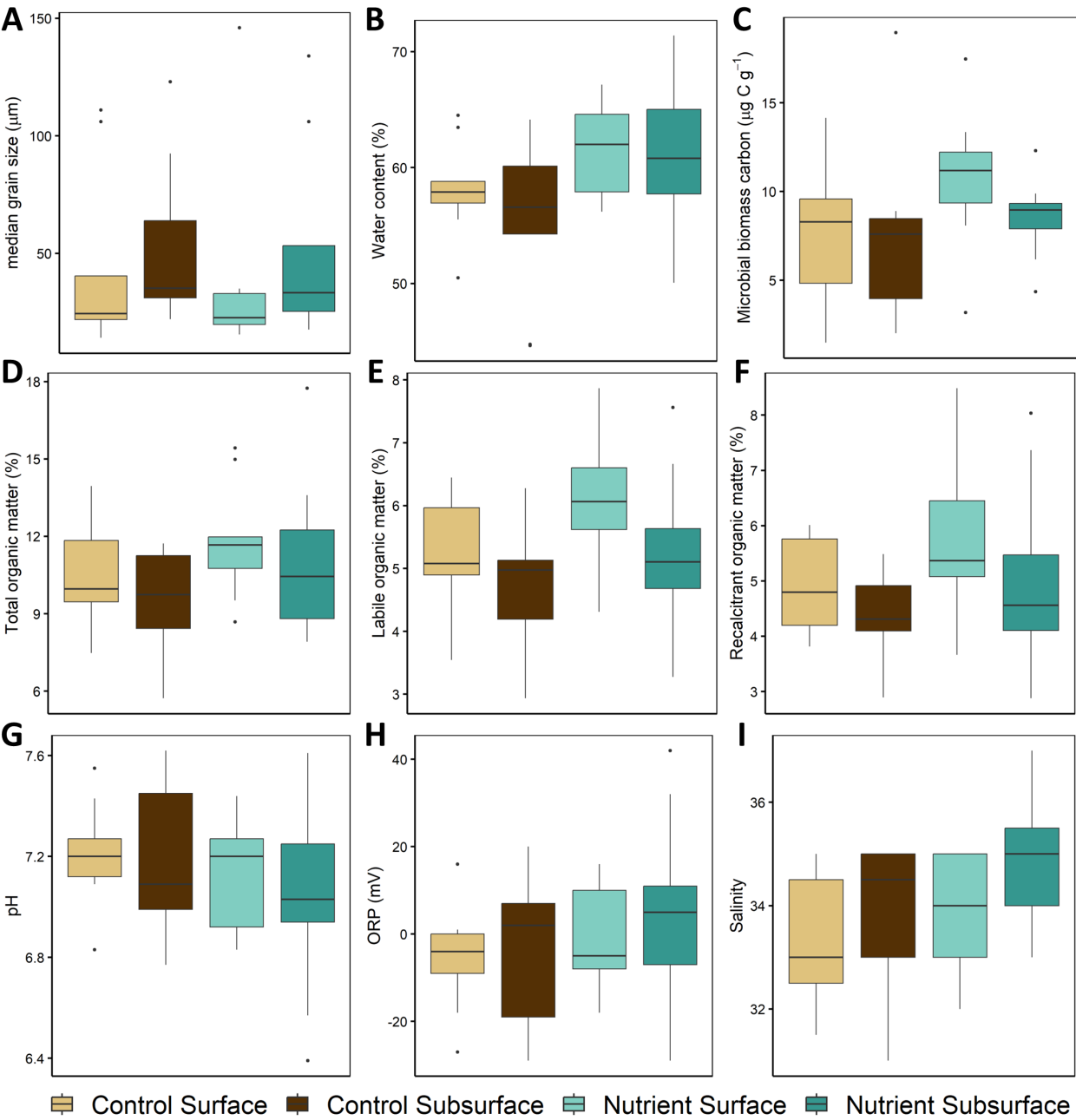
**Supplementary Figure A3.5** Partial dependence plots of all variables' relationship with degradation rate of red tea ( $k_{red}$ ) ordered by relative importance to the model performance. Black lines are means and ribbons represent the standard deviation from 150 bootstrap iterations.





**Supplementary Figure A3.6** Human land use induced effects on degradation of recalcitrant organic matter (red tea) in mangrove temperate forests. Two-variable partial dependence plots of A pH and daily temperature (25<sup>th</sup>), B %Human land use and daily temperature (25<sup>th</sup>), and C Foliar  $\delta^{15}\text{N}$  and daily temperature (25<sup>th</sup>).

6.4 Supplementary Materials to Chapter 4



**Supplementary Figure A4.1** Environmental variables measured across depths and treatments. The boxplots indicate the median with the interquartile range (IQR) between the 25<sup>th</sup> and the 75<sup>th</sup> percentile and the whiskers extend 1.5\*IQR.

**Supplementary Table A4.1** ANOVA tables for the statistical comparison of the sedimentary variables between treatments and depths. These results relate to Supplementary figure 1. The final entry to this table is the linear regression highlighting a slight grain size gradient from plot 1 to plot 18.

| <b>Analysis of Variance</b>      |           |               |                |                |                  |
|----------------------------------|-----------|---------------|----------------|----------------|------------------|
| <b>Response: TOM</b>             | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b> |
| Treatment                        | 1         | 19.987        | 19.9869        | 3.4276         | 0.07336 .        |
| Depth                            | 1         | 6.103         | 6.1030         | 1.0466         | 0.31396          |
| Treatment:Depth                  | 1         | 0.336         | 0.3358         | 0.0576         | 0.81187          |
| Residuals                        | 32        | 186.598       | 5.8312         |                |                  |
| <b>Response: Salinity</b>        | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b> |
| Treatment                        | 1         | 4.340         | 4.3403         | 2.4925         | 0.12422          |
| Depth                            | 1         | 5.840         | 5.8403         | 3.3539         | 0.07637 .        |
| Treatment:Depth                  | 1         | 0.840         | 0.8403         | 0.4826         | 0.49228          |
| Residuals                        | 32        | 55.722        | 1.7413         |                |                  |
| <b>Response: ORP</b>             | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b> |
| Treatment                        | 1         | 361.0         | 361.00         | 1.3140         | 0.2602           |
| Depth                            | 1         | 177.8         | 177.78         | 0.6471         | 0.4271           |
| Treatment:Depth                  | 1         | 58.8          | 58.78          | 0.2139         | 0.6468           |
| Residuals                        | 32        | 8791.3        | 274.73         |                |                  |
| <b>Response: pH</b>              | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b> |
| Treatment                        | 1         | 0.10240       | 0.102400       | 1.2599         | 0.2700           |
| Depth                            | 1         | 0.04551       | 0.045511       | 0.5599         | 0.4598           |
| Treatment:Depth                  | 1         | 0.01868       | 0.018678       | 0.2298         | 0.6349           |
| Residuals                        | 32        | 2.60093       | 0.081279       |                |                  |
| <b>Response: Recalcitrant OC</b> | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b> |
| Treatment                        | 1         | 5.758         | 5.7581         | 3.3725         | 0.0756 .         |
| Depth                            | 1         | 4.058         | 4.0585         | 2.3770         | 0.1330           |
| Treatment:Depth                  | 1         | 0.051         | 0.0508         | 0.0298         | 0.8641           |
| Residuals                        | 32        | 54.636        | 1.7074         |                |                  |

|                            |    |        |         |         |                  |
|----------------------------|----|--------|---------|---------|------------------|
| Response: <b>Labile OC</b> | Df | Sum Sq | Mean Sq | F value | Pr(>F)           |
| Treatment                  | 1  | 5.214  | 5.2142  | 4.2473  | <b>0.04752 *</b> |
| Depth                      | 1  | 4.178  | 4.1784  | 3.4036  | 0.07433 .        |
| Treatment:Depth            | 1  | 0.099  | 0.0995  | 0.0810  | 0.77773          |
| Residuals                  | 32 | 39.285 | 1.2277  |         |                  |

|                                |    |         |         |         |                  |
|--------------------------------|----|---------|---------|---------|------------------|
| Response: <b>Water content</b> | Df | Sum Sq  | Mean Sq | F value | Pr(>F)           |
| Treatment                      | 1  | 149.65  | 149.647 | 4.6763  | <b>0.03816 *</b> |
| Depth                          | 1  | 33.02   | 33.024  | 1.0320  | 0.31732          |
| Treatment:Depth                | 1  | 2.23    | 2.232   | 0.0697  | 0.79342          |
| Residuals                      | 32 | 1024.05 | 32.001  |         |                  |

|                       |    |        |         |         |           |
|-----------------------|----|--------|---------|---------|-----------|
| Response: <b>MB-C</b> | Df | Sum Sq | Mean Sq | F value | Pr(>F)    |
| Treatment             | 1  | 55.90  | 55.903  | 3.2876  | 0.07982 . |
| Depth                 | 1  | 29.86  | 29.860  | 1.7560  | 0.19513   |
| Treatment:Depth       | 1  | 12.69  | 12.688  | 0.7461  | 0.39456   |
| Residuals             | 30 | 510.13 | 17.004  |         |           |

|                                 |    |         |         |         |        |
|---------------------------------|----|---------|---------|---------|--------|
| Response: <b>log (MedianGS)</b> | Df | Sum Sq  | Mean Sq | F value | Pr(>F) |
| Treatment                       | 1  | 0.1973  | 0.19734 | 0.4339  | 0.5148 |
| Depth                           | 1  | 0.7921  | 0.79205 | 1.7416  | 0.1963 |
| Treatment:Depth                 | 1  | 0.00029 | 0.00029 | 0.0006  | 0.9800 |
| Residuals                       | 32 | 14.5533 | 0.45479 |         |        |

|                                       |                                       |          |            |         |                        |
|---------------------------------------|---------------------------------------|----------|------------|---------|------------------------|
| Response: <b>Mud content gradient</b> | lm(formula = Mud ~ Plot, data = meta) |          |            |         |                        |
| Residuals:                            | Min                                   | 1Q       | Median     | 3Q      | Max                    |
| Coefficients:                         | -29.217                               | -8.237   | 2.914      | 10.630  | 24.578                 |
|                                       |                                       | Estimate | Std. Error | t value | Pr(> t )               |
|                                       | Intercept                             | 50.7116  | 4.6054     | 11.011  | <b>&lt; 0.0001 ***</b> |
|                                       | Plot                                  | 1.3481   | 0.4255     | 3.168   | <b>0.00323 **</b>      |

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Residual standard error: 13.24 on 34 degrees of freedom

Multiple R-squared: 0.228, Adjusted R-squared: 0.2053

F-statistic: 10.04 on 1 and 34 DF, p-value: 0.003233

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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Supplementary Table A4.2** ANOVA tables for the statistical comparison of the porewater variables between treatments and depths. In case of a significant interaction term, the TukeyHSD test results are included below the main comparison. These results relate to Figure 2 in the main manuscript.

| <b>Analysis of Variance</b>                          |           |               |                |                |                        |
|------------------------------------------------------|-----------|---------------|----------------|----------------|------------------------|
| <b>Response: <math>\log(\text{NH}_4^+)</math></b>    | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b>       |
| Treatment                                            | 1         | 2.520         | 2.5196         | 2.2004         | 0.142397               |
| Depth                                                | 1         | 8.148         | 8.1479         | 7.1159         | <b>0.009458 **</b>     |
| Treatment:Depth                                      | 1         | 0.389         | 0.3893         | 0.3400         | 0.561687               |
| Residuals                                            | 71        | 81.297        | 1.1450         |                |                        |
| <b>Response: <math>\log(\text{NO}_x)</math></b>      | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b>       |
| Treatment                                            | 1         | 6.217         | 6.2172         | 10.7484        | <b>0.001618 **</b>     |
| Depth                                                | 1         | 0.221         | 0.2211         | 0.3822         | 0.538428               |
| Treatment:Depth                                      | 1         | 0.124         | 0.1237         | 0.2139         | 0.645177               |
| Residuals                                            | 71        | 41.069        | 0.5784         |                |                        |
| <b>Response: DOC</b>                                 | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b>       |
| Treatment                                            | 1         | 500040        | 500040         | 2.4422         | 0.1279                 |
| Depth                                                | 1         | 257052        | 257052         | 1.2554         | 0.2709                 |
| Treatment:Depth                                      | 1         | 64860         | 64860          | 0.3168         | 0.5775                 |
| Residuals                                            | 32        | 6551980       | 204749         |                |                        |
| <b>Response: <math>\log(\text{PO}_4^{3-})</math></b> | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F value</b> | <b>Pr(&gt;F)</b>       |
| Treatment                                            | 1         | 13.607        | 13.6068        | 37.141         | <b>&lt; 0.0001 ***</b> |
| Depth                                                | 1         | 13.201        | 13.2014        | 36.034         | <b>&lt; 0.0001 ***</b> |
| Treatment:Depth                                      | 1         | 6.082         | 6.0820         | 16.601         | <b>0.0001186 ***</b>   |
| Residuals                                            | 71        | 26.011        | 0.3664         |                |                        |



### Tukey multiple comparisons of means, 95% family-wise confidence level

Fit: aov(formula = log(PO4) ~ Treatment \* Depth, data = nut\_enr)

| \$`Treatment:Depth`                             | diff             | lwr              | upr              | p adj                 |
|-------------------------------------------------|------------------|------------------|------------------|-----------------------|
| Nutrient:Surface - Control:Surface              | 0.1831974        | -0.3571336       | 0.7235284        | 0.8090055             |
| Control:Subsurface - Control:Surface            | 0.2835357        | -0.2331196       | 0.8001909        | 0.4766766             |
| <b>Nutrient:Subsurface - Control:Surface</b>    | <b>1.6112624</b> | <b>1.1070585</b> | <b>2.1154663</b> | <b>&lt; 0.0001***</b> |
| Control:Subsurface - Nutrient:Surface           | 0.1003383        | -0.4399927       | 0.6406693        | 0.9614031             |
| <b>Nutrient:Subsurface - Nutrient:Surface</b>   | <b>1.4280651</b> | <b>0.8996273</b> | <b>1.9565029</b> | <b>&lt; 0.0001***</b> |
| <b>Nutrient:Subsurface - Control:Subsurface</b> | <b>1.3277268</b> | <b>0.8235229</b> | <b>1.8319307</b> | <b>&lt; 0.0001***</b> |

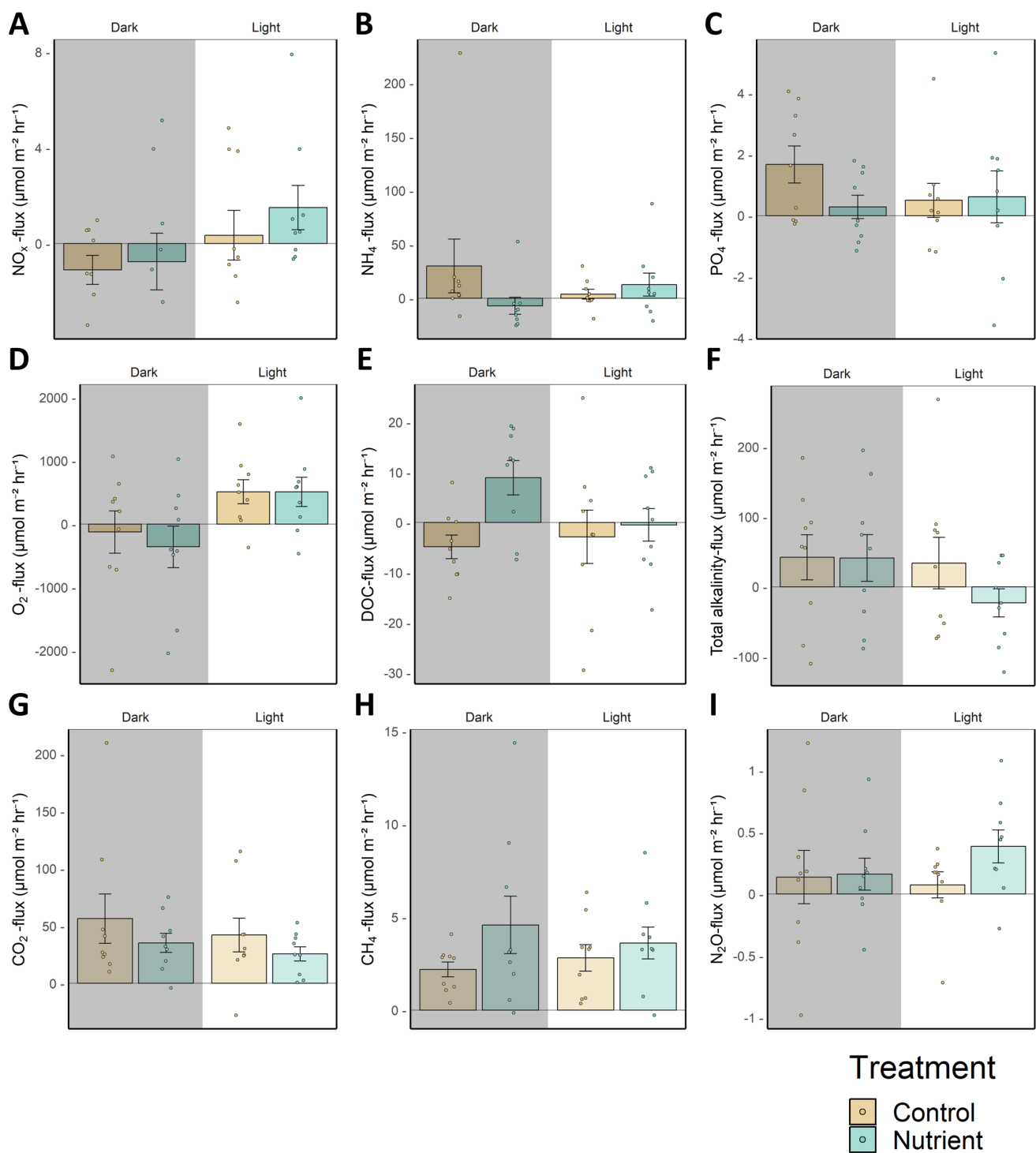
| Response: <b>Total alkalinity</b> | Df | Sum Sq  | Mean Sq | F value | Pr(>F)             |
|-----------------------------------|----|---------|---------|---------|--------------------|
| Treatment                         | 1  | 712982  | 712982  | 4.3646  | <b>0.044994 *</b>  |
| Depth                             | 1  | 2054469 | 2054469 | 12.5767 | <b>0.001265 **</b> |
| Treatment:Depth                   | 1  | 1005183 | 1005183 | 6.1533  | <b>0.018750 *</b>  |
| Residuals                         | 31 | 5064021 | 163356  |         |                    |

### Tukey multiple comparisons of means, 95% family-wise confidence level

Fit: aov(formula = TA ~ Treatment \* Depth, data = meta[-8, ])

| \$`Treatment:Depth`                             | diff             | lwr              | upr              | p adj               |
|-------------------------------------------------|------------------|------------------|------------------|---------------------|
| Nutrient:Surface - Control:Surface              | -78.35557        | -611.37922       | 454.6681         | 0.9781094           |
| Control:Subsurface - Control:Surface            | 155.87210        | -361.23679       | 672.9810         | 0.8454567           |
| <b>Nutrient:Subsurface - Control:Surface</b>    | <b>756.27198</b> | <b>239.16308</b> | <b>1273.3809</b> | <b>0.0021417**</b>  |
| Control:Subsurface - Nutrient:Surface           | 234.22768        | -298.79597       | 767.2513         | 0.6359579           |
| <b>Nutrient:Subsurface - Nutrient:Surface</b>   | <b>834.62755</b> | <b>301.60390</b> | <b>1367.6512</b> | <b>0.0009944***</b> |
| <b>Nutrient:Subsurface - Control:Subsurface</b> | <b>600.39987</b> | <b>83.29098</b>  | <b>1117.5088</b> | <b>0.0178885*</b>   |

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



**Supplementary Figure A4.2** Submerged (A-F) and emerged (G-I) fluxes from mangrove sediments across treatments and light exposures. Bars and errors represent means and standard errors while the points represent the actual data.

**Supplementary Table A4.3** ANOVA tables for the statistical comparison of the submerged fluxes between treatments and light exposures. These results relate to supplementary figure 2A-F.

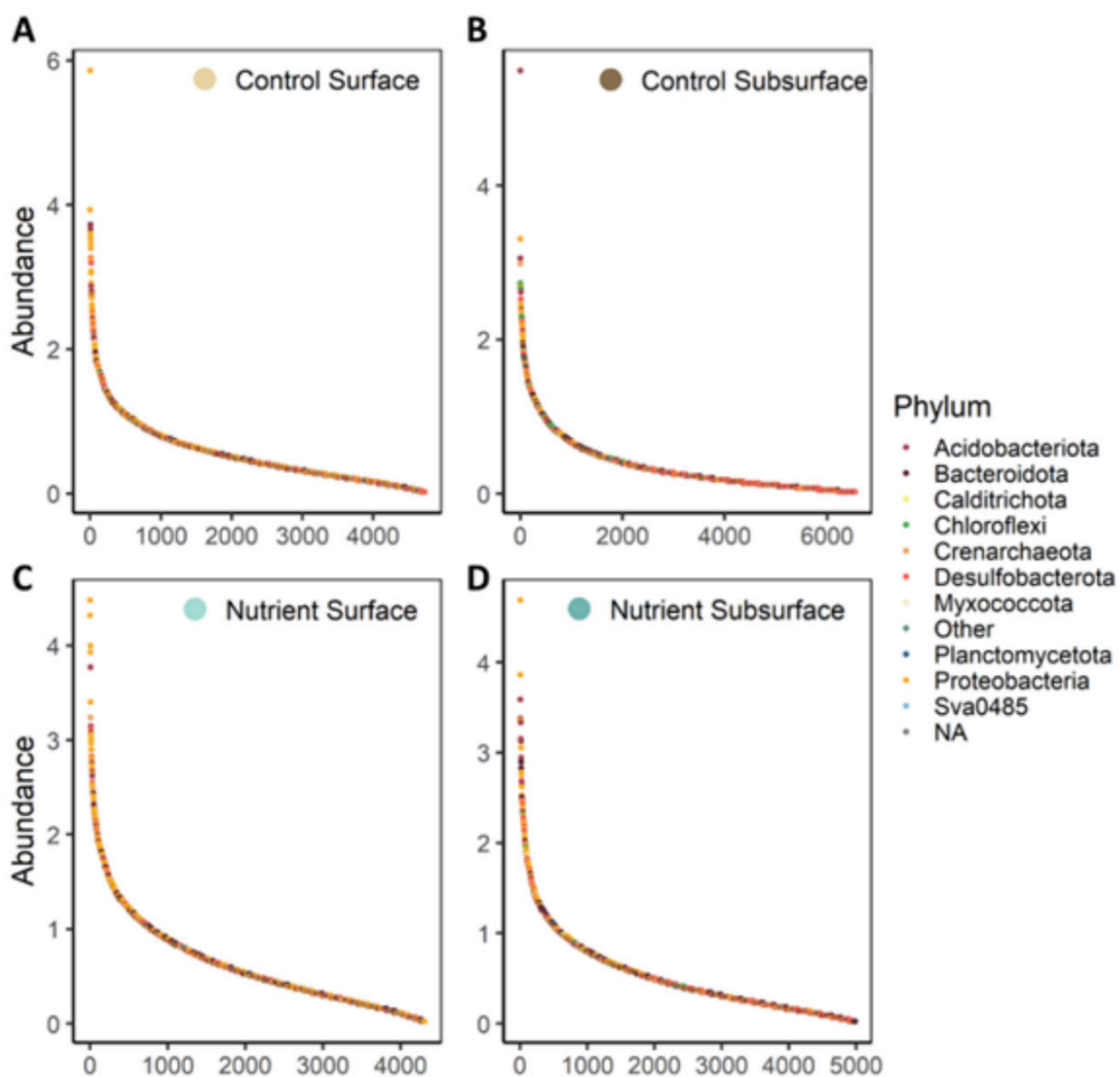
| Analysis of Variance                         |    |          |         |         |                  |
|----------------------------------------------|----|----------|---------|---------|------------------|
| Response: <b>O<sub>2</sub> Flux</b>          | Df | Sum Sq   | Mean Sq | F value | Pr(>F)           |
| Treatment                                    | 1  | 128834   | 128834  | 0.1852  | 0.66982          |
| Exposure                                     | 1  | 5075023  | 5075023 | 7.2956  | <b>0.01096 *</b> |
| Treatment:Exposure                           | 1  | 118147   | 118147  | 0.1698  | 0.68300          |
| Residuals                                    | 32 | 22260066 | 695627  |         |                  |
| Response: <b>DOC Flux</b>                    | Df | Sum Sq   | Mean Sq | F value | Pr(>F)           |
| Treatment                                    | 1  | 554.9    | 554.94  | 4.3522  | <b>0.04502 *</b> |
| Exposure                                     | 1  | 130.3    | 130.25  | 1.0215  | 0.31974          |
| Treatment:Exposure                           | 1  | 310.2    | 310.16  | 2.4325  | 0.12868          |
| Residuals                                    | 32 | 4080.2   | 127.51  |         |                  |
| Response: <b>Total alkalinity flux</b>       | Df | Sum Sq   | Mean Sq | F value | Pr(>F)           |
| Treatment                                    | 1  | 7545     | 7545.1  | 0.8552  | 0.3620           |
| Exposure                                     | 1  | 11824    | 11824.1 | 1.3402  | 0.2556           |
| Treatment:Exposure                           | 1  | 7054     | 7054.5  | 0.7996  | 0.3779           |
| Residuals                                    | 32 | 282320   | 8822.5  |         |                  |
| Response: <b>NH<sub>4</sub><sup>+</sup></b>  | Df | Sum Sq   | Mean Sq | F value | Pr(>F)           |
| Treatment                                    | 1  | 21.7     | 21.65   | 0.0444  | 0.8345           |
| Exposure                                     | 1  | 765.8    | 765.77  | 1.5698  | 0.2196           |
| Treatment:Exposure                           | 1  | 986.0    | 985.99  | 2.0212  | 0.1651           |
| Residuals                                    | 31 | 15122.4  | 487.82  |         |                  |
| Response: <b>NO<sub>x</sub></b>              | Df | Sum Sq   | Mean Sq | F value | Pr(>F)           |
| Treatment                                    | 1  | 5.010    | 5.0096  | 0.6018  | 0.4436           |
| Exposure                                     | 1  | 30.591   | 30.5908 | 3.6751  | 0.0642           |
| Treatment:Exposure                           | 1  | 1.457    | 1.4574  | 0.1751  | 0.6784           |
| Residuals                                    | 32 | 266.361  | 8.3238  |         |                  |
| Response: <b>PO<sub>4</sub><sup>3-</sup></b> | Df | Sum Sq   | Mean Sq | F value | Pr(>F)           |
| Treatment                                    | 1  | 3.698    | 3.6981  | 1.0637  | 0.3101           |
| Exposure                                     | 1  | 1.353    | 1.3532  | 0.3892  | 0.5371           |
| Treatment:Exposure                           | 1  | 1.353    | 5.0935  | 1.4650  | 0.2350           |
| Residuals                                    | 32 | 111.257  | 3.4768  |         |                  |

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Supplementary Table A4.4** ANOVA tables for the statistical comparison of the emerged fluxes between treatments and light exposures. These results relate to supplementary figure 2G-I.

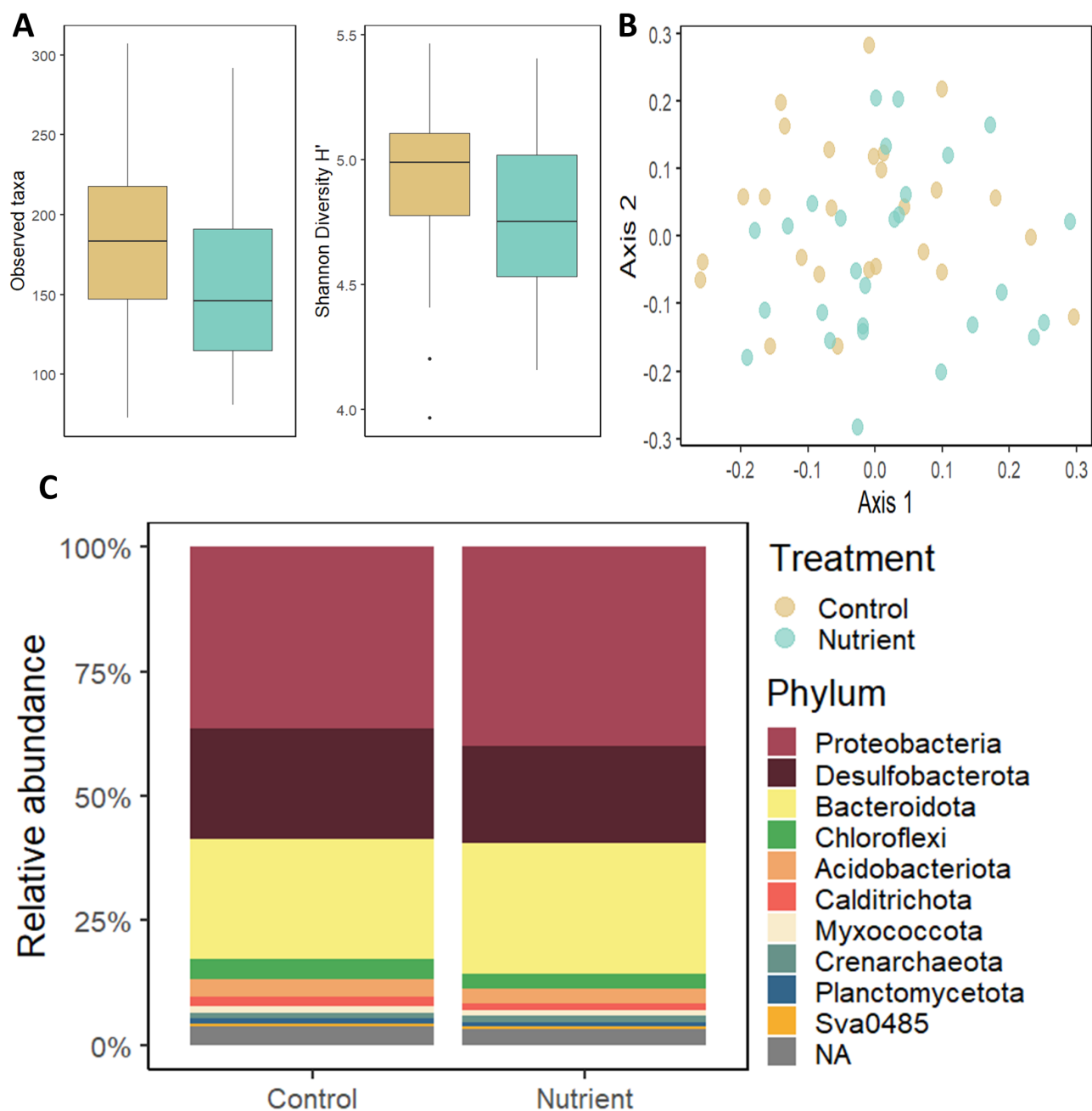
| <b>Analysis of Variance</b>          |    |         |          |         |        |
|--------------------------------------|----|---------|----------|---------|--------|
| Response: <b>CO<sub>2</sub> flux</b> | Df | Sum Sq  | Mean Sq  | F value | Pr(>F) |
| Treatment                            | 1  | 769.2   | 769.21   | 0.7902  | 0.3809 |
| Exposure                             | 1  | 59.3    | 59.31    | 0.0609  | 0.8067 |
| Treatment:Exposure                   | 1  | 458.5   | 458.53   | 0.4711  | 0.4976 |
| Residuals                            | 31 | 30175.0 | 973.39   |         |        |
| Response: <b>CH<sub>4</sub> flux</b> | Df | Sum Sq  | Mean Sq  | F value | Pr(>F) |
| Treatment                            | 1  | 22.498  | 22.4980  | 2.6373  | 0.1142 |
| Exposure                             | 1  | 0.337   | 0.3366   | 0.0395  | 0.8438 |
| Treatment:Exposure                   | 1  | 6.987   | 6.9870   | 0.8191  | 0.3722 |
| Residuals                            | 32 | 272.979 | 8.5306   |         |        |
| Response: <b>N<sub>2</sub>O flux</b> | Df | Sum Sq  | Mean Sq  | F value | Pr(>F) |
| Treatment                            | 1  | 0.2313  | 0.231259 | 1.1130  | 0.2993 |
| Exposure                             | 1  | 0.0578  | 0.057755 | 0.2779  | 0.6017 |
| Treatment:Exposure                   | 1  | 0.1915  | 0.191468 | 0.9215  | 0.3443 |
| Residuals                            | 32 | 6.6493  | 0.207789 |         |        |

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



**Supplementary Figure A4.3** Rank abundance curves of the bacterial community at each depth and treatment, with points are coloured by phylum.





**Supplementary Figure A4.4** Diversity assessment between the treatments in the surface samples. **A** ASV richness and Shannon diversity index. **B** RPCA plot of the beta diversity between both treatments. **C** Relative abundances of Phyla in both treatments.

**Supplementary Table A4.5** ANOVA tables for the statistical comparison of the alpha diversity of the bacterial communities between treatments and depths. These results relate to figure 3B.

| <b>Analysis of Variance</b>        |    |         |         |         |          |
|------------------------------------|----|---------|---------|---------|----------|
| Response: <b>log(Observed)</b>     | Df | Sum Sq  | Mean Sq | F value | Pr(>F)   |
| Treatment                          | 1  | 1.1346  | 1.13462 | 6.9858  | 0.009552 |
| Depth                              | 1  | 1.76836 | 1.76836 | 10.8878 | 0.001346 |
| Treatment:Depth                    | 1  | 0.1915  | 0.14864 | 0.9152  | 0.341078 |
| Residuals                          | 99 | 16.0793 | 0.16242 |         |          |
|                                    |    |         |         |         |          |
| Response: <b>Shannon diversity</b> | Df | Sum Sq  | Mean Sq | F value | Pr(>F)   |
| Treatment                          | 1  | 0.8172  | 0.8172  | 5.5671  | 0.020264 |
| Depth                              | 1  | 1.1081  | 1.10814 | 7.5490  | 0.007135 |
| Treatment:Depth                    | 1  | 0.0956  | 0.09557 | 0.6510  | 0.421674 |
| Residuals                          | 99 | 14.5325 | 0.14679 |         |          |

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Supplementary Table A4.6** Anova table and pairwise comparison of the multivariate statistic based on general linear models generated by the mvabund algorithm.

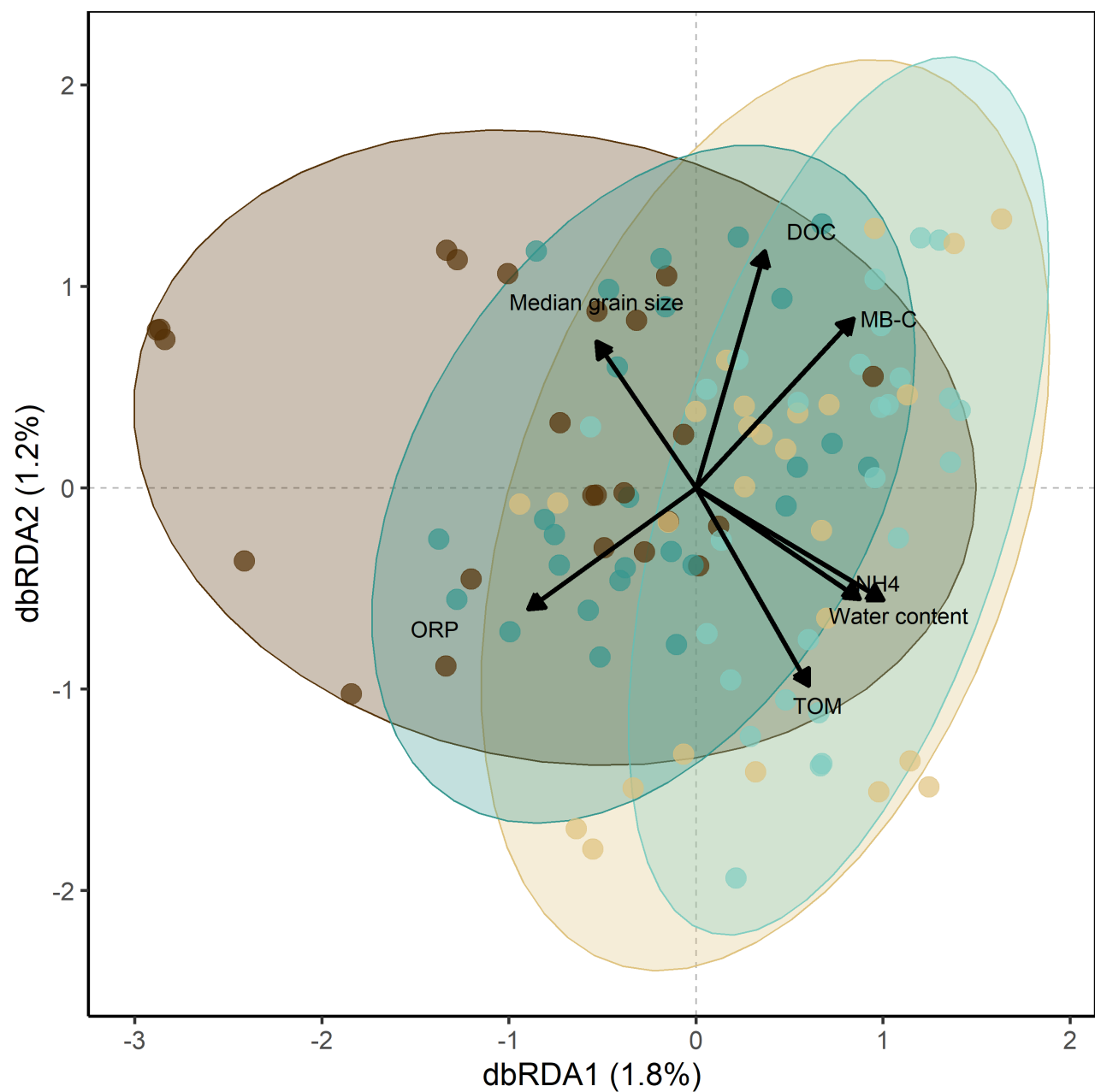
|                                           | <b>Res.D</b> |                               |                                               |
|-------------------------------------------|--------------|-------------------------------|-----------------------------------------------|
|                                           | <b>F</b>     | <b>Deviance</b>               | <b>p.adj.</b>                                 |
| Intercept                                 | 102          | NA                            | NA                                            |
| Treatment                                 | 101          | 6857.461                      | 0.001                                         |
| Depth                                     | 100          | 8590.417                      | 0.001                                         |
| Treatment:Depth                           | 99           | 4802.943                      | 0.001                                         |
|                                           |              | <b>Observed<br/>Statistic</b> | <b>Free stepdown<br/>adjusted<br/>p-value</b> |
| <b>Contrast</b>                           |              |                               |                                               |
| Control Surface vs Control Subsurface     |              | 7192.555                      | 0.001                                         |
| Nutrient Surface vs Nutrient Subsurface   |              | 5856.126                      | 0.002                                         |
| Control Subsurface vs Nutrient Subsurface |              | 6571.621                      | 0.002                                         |
| Control Surface vs Nutrient Surface       |              | 5066.004                      | 0.032                                         |

**Supplementary Table A4.7** Anova tables for the statistical comparison of the Nearest taxon index (NTI) and the net relatedness index (NRI) of the bacterial communities between treatments and depths. These results relate to figure 4.5A,B.

| Analysis of Variance                                                  |             |             |            |                  |                           |
|-----------------------------------------------------------------------|-------------|-------------|------------|------------------|---------------------------|
| Response: NTI                                                         | Df          | Sum Sq      | Mean Sq    | F value          | Pr(>F)                    |
| Treatment                                                             | 1           | 1.876       | 1.8756     | 1.3865           | 0.24182                   |
| Depth                                                                 | 1           | 8.977       | 8.9771     | 6.6363           | 0.01147*                  |
| Treatment:Depth                                                       | 1           | 1.090       | 1.0900     | 0.8058           | 0.37155                   |
| Residuals                                                             | 99          | 133.920     | 1.3527     |                  |                           |
| Response: sqrt(NRI+1.5)                                               | Df          | Sum Sq      | Mean Sq    | F value          | Pr(>F)                    |
| Treatment                                                             | 1           | 3.6098      | 3.6098     | 15.540           | 0.0001507 ***             |
| Depth                                                                 | 1           | 6.2697      | 6.2697     | 26.991           | 1.097e <sup>-06</sup> *** |
| Treatment:Depth                                                       | 1           | 1.0646      | 1.0646     | 4.583            | 0.0347454 *               |
| Residuals                                                             | 99          | 22.9965     | 0.2323     |                  |                           |
| Tukey multiple comparisons of means, 95% family-wise confidence level |             |             |            |                  |                           |
| S`Treatment:Depth`                                                    | diff        | lwr         | upr        | p adj            |                           |
| Nutrient:Surface - Control:Surface                                    | -0.182329   | -0.52839276 | 0.163735   | 0.5168056        |                           |
| Control:Subsurface - Control:Surface                                  | 0.7032475   | 0.34673102  | 1.059764   | 0.0000077**<br>* |                           |
| Nutrient:Subsurface - Nutrient:Surface                                | 0.2962122   | -0.04985165 | 0.6422761  | 0.1205223        |                           |
| Nutrient:Subsurface - Control:Subsurface                              | ▼ -0.589364 | -0.94588061 | -0.2328477 | 0.000215***      |                           |

**Supplementary Table A4.8** Statistical results from the iCAMP analysis comparing the differences between bacterial community assembly mechanisms between depths and treatments.

|                                    |                | <b>Control<br/>Subsurface<br/>vs Surface</b> | <b>Nutrient<br/>Subsurface vs<br/>Surface</b> | <b>Surface<br/>Control vs<br/>Nutrient</b> | <b>Subsurface<br/>Control vs<br/>Nutrient</b> |
|------------------------------------|----------------|----------------------------------------------|-----------------------------------------------|--------------------------------------------|-----------------------------------------------|
| <b>Homogenising<br/>dispersal</b>  | relative diff. | 0.091                                        | -0.098                                        | -0.062                                     | 0.126                                         |
|                                    | Cohen's d      | 0.661                                        | -0.885                                        | -0.522                                     | 0.917                                         |
|                                    | effect size    | medium                                       | large                                         | medium                                     | large                                         |
|                                    | p-value        | 0.316                                        | 0.262                                         | 0.350                                      | 0.254                                         |
| <b>Dispersal<br/>limitation</b>    | relative diff. | 0.070                                        | -0.119                                        | 0.007                                      | 0.186                                         |
|                                    | Cohen's d      | 1.840                                        | -2.845                                        | 0.223                                      | 4.966                                         |
|                                    | effect size    | large                                        | large                                         | small                                      | large                                         |
|                                    | p-value        | 0.099                                        | 0.020                                         | 0.433                                      | 0.000                                         |
| <b>Drift and others</b>            | relative diff. | -0.128                                       | -0.040                                        | -0.034                                     | -0.122                                        |
|                                    | Cohen's d      | -3.840                                       | -1.698                                        | -1.550                                     | -3.601                                        |
|                                    | effect size    | large                                        | large                                         | large                                      | large                                         |
|                                    | p-value        | 0.003                                        | 0.115                                         | 0.138                                      | 0.005                                         |
| <b>Homogeneous<br/>selection</b>   | relative diff. | 0.293                                        | 0.354                                         | 0.194                                      | 0.119                                         |
|                                    | Cohen's d      | 3.569                                        | 5.495                                         | 2.579                                      | 1.427                                         |
|                                    | effect size    | large                                        | large                                         | large                                      | large                                         |
|                                    | p-value        | 0.001                                        | 0.000                                         | 0.035                                      | 0.156                                         |
| <b>Heterogeneous<br/>selection</b> | relative diff. | 0.265                                        | 0.414                                         | -0.195                                     | -0.358                                        |
|                                    | Cohen's d      | 2.166                                        | 1.911                                         | -1.183                                     | -1.701                                        |
|                                    | effect size    | large                                        | large                                         | large                                      | large                                         |
|                                    | p-value        | 0.064                                        | 0.078                                         | 0.202                                      | 0.111                                         |
| <b>Stochasticity</b>               | relative diff. | -0.058                                       | -0.066                                        | -0.022                                     | -0.014                                        |
|                                    | Cohen's d      | -3.783                                       | -5.957                                        | -2.544                                     | -0.823                                        |
|                                    | effect size    | large                                        | large                                         | large                                      | large                                         |
|                                    | p-value        | 0.001                                        | 0.000                                         | 0.038                                      | 0.287                                         |



**Supplementary Figure A4.5** Constrained ordination showing the relationship between environmental variables and the bacterial community across all samples. Variables selected by stepwise forward selection are displayed as arrows. Ellipses represent the 95% confidence intervals around the group centroids. Light brown = Control Surface; dark brown = Control Subsurface; light green = Nutrient Surface; dark green = Nutrient Subsurface



**Supplementary Table A4.9** Summary tables of the dbRDA and permutational ANOVA assessing environmental variable association with the bacterial communities. These results relate to figure 6 in the main text.

| <b>All samples</b>                                     |          |               |               |                 |            |
|--------------------------------------------------------|----------|---------------|---------------|-----------------|------------|
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| Ordination                                             | 7        | 3.164         | 1.171         | <b>0.001</b>    | <b>***</b> |
| Residual                                               | 95       | 36.667        |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| Water content                                          | 1        | 0.517         | 1.3389        | <b>0.009901</b> | <b>**</b>  |
| NH <sub>4</sub>                                        | 1        | 0.496         | 1.2847        | <b>0.009901</b> | <b>**</b>  |
| ORP                                                    | 1        | 0.456         | 1.1821        | <b>0.009901</b> | <b>**</b>  |
| MB-C                                                   | 1        | 0.468         | 1.2133        | <b>0.009901</b> | <b>**</b>  |
| TOM                                                    | 1        | 0.422         | 1.0931        | 0.079208        | .          |
| Median grain size                                      | 1        | 0.392         | 1.0167        | 0.316832        |            |
| Total alkalinity                                       | 1        | 0.412         | 1.0684        | 0.099010        | .          |
| Residual                                               | 95       | 36.667        |               |                 |            |
| <b>Surface samples (control and nutrient combined)</b> |          |               |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| Ordination                                             | 3        | 1.2931        | 1.0677        | <b>0.009</b>    | <b>**</b>  |
| Residual                                               | 49       | 19.7819       |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| DOC                                                    | 1        | <b>0.4495</b> | <b>1.1135</b> | <b>0.0396</b>   | <b>*</b>   |
| ORP                                                    | 1        | <b>0.4295</b> | <b>1.0639</b> | <b>0.0495</b>   | <b>*</b>   |
| MBC                                                    | 1        | 0.4140        | 1.0256        | 0.2277          |            |
| Residual                                               | 49       | 19.7819       |               |                 |            |
| <b>Control Subsurface</b>                              |          |               |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| Ordination                                             | 3        | 1.5633        | 1.3669        | <b>0.001</b>    | <b>***</b> |
| Residual                                               | 20       | 7.6244        |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| <b>PO<sub>4</sub></b>                                  | <b>1</b> | <b>0.5985</b> | <b>1.5700</b> | <b>0.009901</b> | <b>**</b>  |
| ORP                                                    | 1        | 0.4520        | 1.1857        | 0.069307        | .          |
| <b>MB-C</b>                                            | <b>1</b> | <b>0.5127</b> | <b>1.3450</b> | <b>0.009901</b> | <b>**</b>  |
| Residual                                               | 20       | 7.6244        |               |                 |            |
| <b>Nutrient Subsurface</b>                             |          |               |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| Ordination                                             | 3        | 1.4106        | 1.1444        | <b>0.001</b>    | <b>**</b>  |
| Residual                                               | 22       | 9.0395        |               |                 |            |
| variable                                               | DF       | SumOfSqs      | F             | Pr(>F)          |            |
| <b>MB-C</b>                                            | <b>1</b> | <b>0.4946</b> | <b>1.2037</b> | <b>0.009901</b> | <b>**</b>  |
| DOC                                                    | 1        | <b>0.4552</b> | <b>1.1078</b> | <b>0.039604</b> | <b>**</b>  |
| NO <sub>x</sub>                                        | 1        | <b>0.4609</b> | <b>1.1216</b> | <b>0.039604</b> | <b>**</b>  |
| Residual                                               | 22       | 9.0395        |               |                 |            |

## 6.5 Summer school project

**Short term responses of inorganic carbon to varying modes of nutrient delivery in mangrove sediments.**

I conceptualised and conducted a summer school project with a bachelor student (Caleb J. Crosby) in 2021, in which we aimed to identify the short term (5 day) response of mangrove sediments to nutrient enrichment. We found an enrichment effect only in the porewater of the sediments but not in the gas fluxes from the sediments suggesting dissolved mechanisms for detecting metabolic activity in waterlogged sediments. He presented this work at the New Zealand Marine Sciences Society (NZMSS) in 2022. A copy of that poster is presented below.

# The effect of nutrient enrichment on inorganic CARBON CYCLING in temperate mangrove soils

Caleb Crosbie, Timothy Thomson, Terry Isson, Natalie Prinz, Joanne Ellis



THE UNIVERSITY OF  
WAIKATO  
Te Whare Wānanga o Waikato

## AIM:

To investigate the effect of pulse versus chronic nutrient enrichment on inorganic carbon cycling within *Avicennia marina* soil.

## INTRODUCTION:

Mangrove soils are major global carbon sinks due to their unique physiochemical properties such as anoxia, high salinity and low pH. These properties control and limit the respiration rates of microbial soil communities and their ability to breakdown incoming forms of carbon<sup>1</sup>. This results in the partial decomposition of soil organic matter and the formation of recalcitrant carbon species.<sup>1</sup> However, high yielding labile carbon produced through photosynthesis by microphytobenthos may allow for greater rates of activity and respiration by heterotrophic microbial communities (HMC)<sup>2</sup>. This relationship between the influx and efflux of carbon in a soil system is determined through remineralisation and photosynthetic fixation rates of carbon. Many coastal ecosystems are experiencing increased nutrient loads due to run-off from the land. This has occurred largely due to land use change and unsustainable management practices. Observing this relationship is essential to understand the influence of disturbance, through nutrient enrichment, that may cause a system to either emit or sequesters carbon.

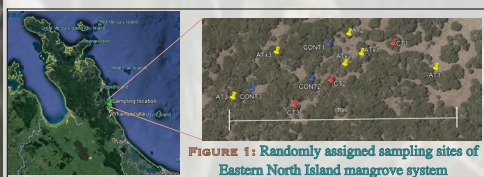


FIGURE 1: Randomly assigned sampling sites of Eastern North Island mangrove system

## HYPOTHESIS:

We hypothesise that increased availability of inorganic and organic nutrients would stimulate growth rates of photosynthetic communities resulting in higher carbon flux into the atmosphere due to HMC activity.

## METHODOLOGY:

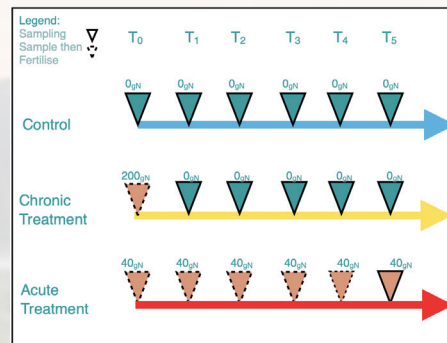


FIGURE 2: Diagram displaying the sampling methodology across the three treatment types.

**PROCESSING:** Light and dark chamber gas measurements ( $\text{CO}_2$ ) were conducted in situ through the use of an adapted IRGA. The difference between light and dark measurements illustrated the effect primary production had on  $\text{CO}_2$  flux, the isolated dark chamber measurements represented the total soil respiration. The extracted and filtered pore water was titrated to find total alkalinity in order to calculate DIC.

## RESULTS:

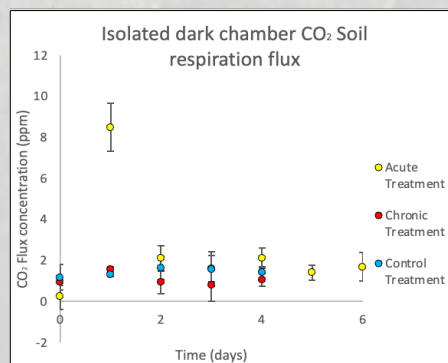


FIGURE 3: Evolution in dissolved inorganic carbon collected from pooled porewater samples over time.

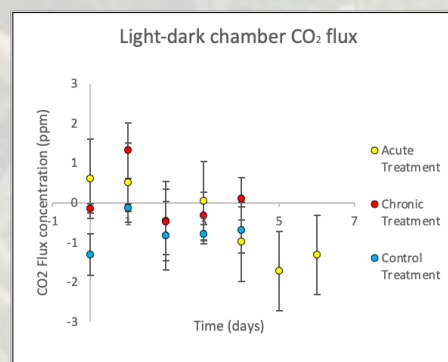


FIGURE 4: Soil respiration  $\text{CO}_2$  flux recorded in situ through dark chamber measurements.

## DISCUSSION:

The resulting  $\text{CO}_2$  flux rates show minimal variation (Fig 3 and 4). The acute treatment sites experienced greater rates of both soil respiration and photosynthesis. Conversely, chronic treatments experienced reduced levels of overall activity and greater efflux rates of  $\text{CO}_2$  compared to the control treatments. Spikes visible at  $T_1$  could be explained by the outgassing of residual fertiliser from the previous application at  $T_0$ . The delayed DIC response varied depending on the level of the disturbance, suggesting compensation for the presence of elevated nutrients (Fig 2). The acute treatment exhibited increased porewater DIC concentrations, theoretically due to microbial respiration. The chronic treatment only exhibited signs of increase relative to the control treatment at the final sampling interval.

## CONCLUSION:

There were minimal short-term effects on the overall  $\text{CO}_2$  flux rates by both continuous and single disturbance events. The most substantial deviation from the control was experienced by the acute treatment sites, whereas chronic treatments experienced a seemingly dampened flux rate. The porewater DIC concentrations however, experienced a visible delayed increase in response to varying nutrient enrichment levels. With the acute treatment presenting the greatest increase, suggesting a net increased fixation rate of  $\text{CO}_2$  and coupled respiration. This provides evidence of increased microbial activity and short-term carbon turnover in eutrophied mangrove soils.

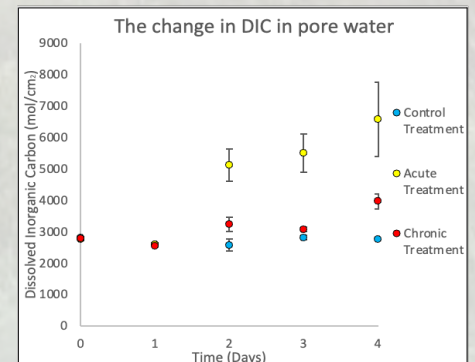


FIGURE 5: Photosynthetic effect on  $\text{CO}_2$  system flux calculated from light - dark chamber fluxes.

## REFERENCES:

- 1- Alongi, D. M. (2020). Carbon cycling in the world's mangrove ecosystems revisited: Significance of non-steady state diagenesis and subsurface linkages between the forest floor and the coastal ocean. *Estuaries and Coasts*, 43(1), 1-17.
- 2- Philip M. Riekenberg, Joanne M. Oakes, Bradley D. Eyre. (2020). A shift in the pool of retained microphytobenthos nitrogen under enhanced availability. *Water Research*, Volume 187.

## 7 Appendix II List of publications

### 7.1 Publications arising directly from this thesis

Each of the thesis's main chapters forms the basis for one publication (listed below in the respective order from Chapter 2 to Chapter 4):

**Thomson, T.**, Ellis, J. I., Fusi, M., Prinz, N., Lundquist, C. J., Bury, S. J., et al. (2024). Effects of catchment land use on temperate mangrove forests. *Sci. Total Environ.* 940, 173579. doi:10.1016/J.SCITOTENV.2024.173579.

**First published 31 May 2024 in Science of the Total Environment volume 940,173579.**

**Thomson, T.**, Pilditch, C.A., Prinz, N., Fusi, M., Lundquist, C., Cary, S.C., Ellis, J.I. (in preparation). Eutrophication enhances labile organic matter decomposition in mangrove ecosystems.

**This article is currently in preparation for submission.**

**Thomson, T.**, Fusi, M., Prinz, N., Cary, S.C., Pilditch, C. A., Ellis, J.I. (in preparation). Legacy of nutrient enrichment on subsurface bacterial community in temperate mangrove sediments.

**This article is currently in preparation for submission.**

### 7.2 Publications arising from work related to this thesis

**Thomson, T.**, Ellis, J. I., Fusi, M., Prinz, N., Bennett-Smith, M. F., Aylagas, E., ... & Jones, B. H. (2022). The right place at the right time: seasonal variation of bacterial communities in arid *Avicennia marina* soils in the Red Sea is specific to its position in the intertidal. *Frontiers in Ecology and Evolution*, 10, 845611

**First published 7 April 2022 in Frontiers in Ecology and Evolution 10, 845611**

**Thomson, T.**, Fusi, M., Bennett-Smith, M. F., Prinz, N., Aylagas, E., Carvalho, S., ... &



Ellis, J. I. (2022). Contrasting effects of local environmental and biogeographic factors on the composition and structure of bacterial communities in arid monospecific mangrove soils. *Microbiology spectrum*, 10(1), e00903-21.

**First published 5 January 2022 in Microbiology spectrum 10 (1), e00903-21**

### 7.3 Other publications during the candidature

Prinz, N, **Thomson, T.**, Ellis, J.I., Gladstone-Gallagher, R. V., Savage, C., Pilditch, C.A. (in preparation). The taonga *Austrovenus stutchburyi* as a potential bioremediation species to combat hysteresis and aid estuarine soft-sediment recovery.

**This article is currently in preparation for submission.**

Buck-Wiese, H., Hellige, I., Bligh, M., **Thomson, T.**, Arnosti, C., Asmala, E., ... & Hehemann JH., (in preparation). Brown algal fucoidan secretion and stability around the globe.

**This article is currently in preparation for submission.**

Hulver, A. M., Steckbauer, A., Ellis, J. I., Aylagas, E., Roth, F., Kharbatia, N., **Thomson, T.**, ... & Berumen, M. L. (2022). Interaction effects of crude oil and nutrient exposure on settlement of coral reef benthos. *Marine Pollution Bulletin*, 185, 114352.

**First published 1 December 2022 in Marine Pollution Bulletin 185, 114352**

Saderne, V., Fusi, M., **Thomson, T.**, Dunne, A., Mahmud, F., Roth, F., ... & Duarte, C. M. (2021). Total alkalinity production in a mangrove ecosystem reveals an overlooked Blue Carbon component. *Limnology and oceanography letters*, 6(2), 61-67.

**First published 16 October 2020 in Limnology and oceanography letters 6 (2), 61-67**

Roth, F., Karcher, D. B., Rädicker, N., Hohn, S., Carvalho, S., **Thomson, T.**, ... & Wild, C. (2020). High rates of carbon and dinitrogen fixation suggest a critical role of benthic pioneer communities in the energy and nutrient dynamics of coral reefs. *Functional Ecology*, 34(9), 1991-2004.

**First published 02 July 2020 in Functional Ecology 34 (9), 1991-2004**