Moisture Associated Microbial Communities in Antarctic Dry Valley Soils

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Rachelle Anderson

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Abstract

Climate change is having a dramatic impact on the natural environment and is one of the most imminent and important issues of the 21st century. With limited vegetation and few large terrestrial organisms, Antarctica offers a unique opportunity to understand the impact of abiotic, climatic factors on microbial ecosystems (free of many of the confounding biological variables in more complex systems).

Recent *in situ* studies indicate that microbial communities within Antarctic soils may respond to environmental changes within far shorter time frames than originally believed. In a landmark study, Tiao et al. (2012) investigated the rate at which microbial communities responded to a unique soil modification experiment. To this end, a mummified seal carcass (dated at 250 years) was shifted from its original site in Miers Valley, to a new, geomorphically similar site in close proximity. Remarkably, increased microbial biomass, decreased biodiversity, and shifts in the microbial community composition were observed within just two summers. While the seal carcass altered the underlying soil's nitrogen and organic carbon content, pH, and conductivity, statistical analysis revealed that none of these physicochemical changes could satisfactorily explain the changes in the microbial community. Instead the data suggest that the changes observed may have been caused by physical, abiotic factors induced by the seal carcass (i.e. increased and more stable relative humidity (RH), reduced UV exposure, and reduced daily temperature fluctuations). However, due to the un-replicated, observational nature of the study, this is merely speculation.

In order to verify these findings and resolve the drivers of the microbial community changes observed, a controlled, *in situ* experiment was designed to replicate the abiotic effects of the seal carcass (stabilise temperature, reduce UV exposure, and increase and stabilise RH in the underlying soil). To do this, overturned or upright black and translucent plastic trays were set up on undisturbed regions of Antarctic Dry Valley soil; soil samples were taken every January for a five-year period; and Ion Torrent sequencing of 16S rRNA gene amplicons was used to assess changes in the microbial community composition

and structure. However, based on RH data and visual observations of the site it would appear that the tray experiment was unknowingly set up in either a flat, low lying area where moisture accumulated; or in a subsurface water track. Due to the constant high moisture content within the soil on which the experiment was deployed, the effect of the tray treatments on the local environment (i.e. RH and temperature) was negligible.

The microbial community composition in the tray treatment experiment stayed quite consistent across all years and treatments, however there was a significantly greater abundance of Cyanobacteria/ Chloroplasts in the translucent overturned (TO) treatment than in the black upright (BU) or black overturned (BO) treatments. Interestingly, the moist tray treatment soil had a greater relative abundance of Cyanobacteria/ Chloroplasts, Proteobacteria, and Bacteroidetes than the drier seal control site, in keeping with the observations of other studies investigating microbial community composition within wet environments. These findings hint at the importance of small-scale topographic factors in microbial community structure, and/or highlight the potential of using microbial community composition as a bio-indicator of hidden water tracks.

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List of Abbreviations

ACC	Antarctic Circumpolar Current
ANOVA	Analysis of variance
BME	2-Mercaptoethanol, or β-mercaptoethanol
BO	Black overturned (tray treatment)
BP	Base pairs
BU	Black upright (tray treatment)
CFC	Chlorofluorocarbon
CH_4	Methane
CO_2	Carbon dioxide
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FTC	Freeze-thaw-cycle
NMDS	Non-metric multidimensional scaling
OTC	Open-top chamber
OTU	Operational taxonomic unit
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
RH	Relative humidity
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SDS	Sodium dodecyl sulfate
TE	Tris-EDTA
TO	Translucent overturned (tray treatment)
TU	Translucent upright (tray treatment)

Chapter One

1 Introductory Review

1.1 General background

Climate change is one of the most imminent issues of the 21st century (Dawson & Spannagle, 2008; Goklany, 2009). Polar ice is melting; global sea level is rising; oceans are becoming more acidic; and extreme weather events are becoming more frequent and severe (IPCC, 2014; NASA, 2015; The Royal Society, 2010). An overwhelming body of evidence indicates that climate change is occurring, and that anthropogenic carbon dioxide (CO_2) and methane (CH_4) emissions, in conjunction with deforestation, are playing a significant role (IPCC, 2007).

Antarctica's hostile environmental conditions prevent most plants and animals from surviving in the continent, and as a result the ecosystem is simple and dominated by microorganisms (Chown et al., 2015; Hogg et al., 2006). Due to their reduced complexity, simple ecosystems are relatively easy to understand (compared to temperate systems), and thus are the ideal place to study the basic foundations of ecosystem structure. This knowledge can then be applied to more complex systems and used to develop models that predict how Antarctica, and ultimately the rest of the world, may respond to changing environmental conditions (Hogg et al., 2006). Additionally, as microbial communities lie at the base of all food chains and control biogeochemical cycling, changes in microbial populations can have a massive cascading effect up to higher trophic levels (Terborgh & Estes, 2013).

Early, culture-based studies indicated that Antarctic microbial ecosystems were homogenous across the Dry Valleys, and that diversity was extremely low (Hopkins, Sparrow, Novis, et al., 2006; Johnson et al., 1978). It was widely believed that these communities were controlled by legacy carbon inputs, and consequently, that changes in microbial community structure occurred over centuries or even millennia (Barrett et al., 2006; Moorhead et al., 1999). However, this lack of diversity was later found to be an artefact of the early culture-based techniques employed, as a limited number of bacterial species are culturable

(Rappe & Giovannoni, 2003). With the advent of molecular based techniques it has become apparent that microbial diversity and abundance within Antarctic Dry Valley soil is far greater than initially anticipated (Chown et al., 2015; Smith et al., 2006). It has also been revealed that Antarctic Dry Valleys soils are spatially and temporally heterogeneous, with different ecological factors influencing microbial distribution and community composition (Bockheim, 2002; Lee et al., 2012).

Recent *in situ* studies indicate that microbial communities may respond to environmental changes within far shorter time frames than originally believed (Tiao et al., 2012; Yergeau et al., 2012). In a unique, multi-year seal carcass transplantation experiment, Tiao et al. (2012) shifted a mummified seal carcass (dated at 250 years) from its original site in Miers Valley to a new geomorphically similar location 20 metres away, and changes in the underlying microbial community were monitored via yearly sampling over a five year period. The aim of the study was to determine the rate at which the microbial community responded to a new set of environmental conditions, induced by the seal carcass (Tiao et al., 2012).

The original seal site had increased microbial biomass, decreased biodiversity relative to the surrounding soil, and significant changes in microbial community composition; with an increased relative abundance of Proteobacteria, and Firmicutes, and a decreased relative abundance of Bacteroidetes (Tiao et al., 2012). Remarkably, NMDS plots of the new site showed that there was a major shift in community structure towards the original seal site within two years, with a significant increase in Firmicutes and Proteobacteria, and a major decrease in Bacteroidetes (to < 1%) (Tiao et al., 2012).

Since all of the most abundant operational taxonomic units (OTUs) at the new site were present in the control soil, it would appear that the seal carcass itself was not responsible for species introduction (Tiao et al., 2012). Instead it is likely that the microbial community changes observed were due to differences in a specific species' adaption to the new microclimate imposed by the carcass. These well-adapted microorganisms increased rapidly in abundance, outcompeting the less well-adapted species, resulting in decreased microbial diversity (Tiao et al., 2012).

The seal carcass at the original site altered the underlying soil's nitrogen and organic carbon content, pH, and conductivity (Tiao et al., 2012). Surprisingly however, statistical analysis revealed that none of these physicochemical changes could account for the changes in microbial community structure observed. Instead the researchers hypothesised that the changes observed may have been caused by physical, abiotic factors induced by the seal carcass – namely increased and more stable RH, reduced UV exposure, and more stable temperature (Tiao et al., 2012).

In the Dry Valleys, water availability appears to be one of the primary factors driving microbial community structure (Aislabie et al., 2006; Niederberger et al., 2008). Water is available only on a seasonal basis, and its availability is influenced by relative humidity (RH) (a measure of the amount of moisture in the air, relative to what it can hold before reaching saturation), temperature and the presence of permafrost (Noy-Meir, 1973). The dehydrating Antarctic winds generate a RH gradient between the soil surface and deeper down that causes continuous sublimation of underlying permafrost. With an object (such as the seal carcass) on top of the soil, this sublimation is significantly reduced, allowing RH to increase beneath the object (Cary et al., 2010). This then provides a new set of environmental conditions which select for different microbial species. However, due to strict environmental regulations in Antarctica the seal study was un-replicated and purely observational, and thus this is merely speculation. This study called for a controlled, multi-year *in situ* experiment to be conducted to verify these findings and resolve the drivers of the observed changes.

1.2 Climate change

Climate change is arguably one of the most critical issues of the 21st century (Dawson & Spannagle, 2008; Goklany, 2009). An overwhelming body of evidence indicates that climate change is occurring, and that anthropogenic CO_2 and CH_4 emissions, in conjunction with deforestation, are playing a significant role (Liverman, 2007). The sun is the ultimate driver of Earth's climate. Satellite data suggest that ~30% of the energy that reaches the Earth from the sun is reflected back into space (The Royal Society, 2010), while the remainder is absorbed, and re-emitted, by molecules in the Earth's atmosphere in the form of infrared radiation (The U.S. National Academy of Sciences and The Royal Society, 2014). These molecules, termed 'greenhouse gases', include water vapour, CO_2 and CH_4, and their presence in the atmosphere is essential for our survival on the planet. If the heat emitted from the Earth's surface was entirely reflected back into space without absorption from molecules in the Earth's atmosphere, the average surface temperature would be too low to sustain life as we know it (Dawson & Spannagle, 2008).

The carbon cycle is the process in which carbon is exchanged between three main reservoirs: land, ocean and atmosphere (Gautier, 2008). Prior to 1750, the amount of carbon released into the atmosphere was in balance, with the ocean and land absorbing roughly equivalent amounts. Historical data indicate that for the last 10,000 years atmospheric CO_2 concentration has been resting stably between 260-280 ppm (Solomon & Miller, 2007). Recently however, anthropogenic perturbations have thrown this cycle out of balance, and this is having dramatic impacts on our climate. Atmospheric CO_2 concentrations have increased by about 40% since the dawn of the industrial revolution in the 18th century, primarily due to the combustion of fossil fuels, driven by economic demand, rampant deforestation, and an ever-increasing population (IPCC, 2014; Stern & Treasury, 2007).

Based on historical CO_2 measurements, estimated using 'ancient bubbles' trapped within ice cores taken deep beneath Greenland and Antarctica, it would appear that today's CO_2 concentrations (at ~400 ppm) (NASA, 2015) are greater than those observed over the last 800,000 years (which fluctuated between 180 and 300

ppm) (IPCC, 2014). Methane is another major greenhouse gas contributing to climate change, second only to CO_2 (Dawson & Spannagle, 2008). Methane is 60 times stronger than CO_2 in terms of its global warming potential, however due to its short residency in the atmosphere its contribution to climate change is not as great (Dawson & Spannagle, 2008). Anthropogenic sources of methane emission come mainly from agricultural practices (farming of ruminant livestock), primary energy production, and landfills (Dawson & Spannagle, 2008).

Human activity in the form of land use changes (particularly deforestation for agricultural purposes), is also thought to contribute to climate change by altering surface albedo (the amount of radiation reflected back into space), as well as reducing terrestrial CO_2 absorption by plants (Betts, 2000; IPCC, 2014). As a result of these land use changes and increased greenhouse gas concentration, slightly more of the sun's thermal energy is being trapped in the atmosphere, causing a rise in global temperature (The U.S. National Academy of Sciences and The Royal Society, 2014).

Climatic warming models all indicate that natural contributions to warming (i.e. variations in the Sun's radiative output, El Niño, La Niña, and volcanic eruptions) are not able to account for the climatic changes observed since the start of the industrial revolution (Lean & Rind, 2008). Indeed, when only these contributions are included, the models predict very limited warming, or even a slight cooling, over the last 100 years. In contrast, when human contribution is accounted for, the models are able to accurately predict the climatic changes observed today (The U.S. National Academy of Sciences and The Royal Society, 2014). Additionally, carbon isotope measurements from the atmosphere reveal a clear distinction between ^{14}C depleted 'ancient' carbon arising from fossil fuels, and 'modern' carbon, arising from natural sources (Lichtfouse et al., 2011).

1.2.1 Consequences of climate change

Since 1980 the Earth's surface has warmed by about 0.8 ^{o}C (NASA, 2015; The Royal Society, 2010). Climatic warming has been concentrated primarily during two periods: from ~ 1910 to 1940, and from ~1975 to 2000. These warming trends have been observed in three independent temperature records: above land,

above sea, and at the ocean's surface. This warming is not uniform across all regions, and includes considerable variability from one year to the next. However, when surface temperatures are averaged over each decade to account for some of this variability, it is evident that each decade since 1970 has been warmer than the decade prior (The Royal Society, 2010).

Indeed, of the 10 warmest years on record (extending 134 years), nine have occurred since the turn of the century (NASA, 2015). As a result, September Arctic sea ice is currently decreasing at a rate of 13.4% per decade, with 2012 levels being the lowest on record. Similarly, Antarctica has been losing ~134 gigatonnes of land ice per year since 2002, while Greenland has been losing ~ 287 gigatonnes of sheet ice per year (a figure that doubled from 1996 to 2005) (NASA, 2015).

Large quantities of methane are stored in permafrost within the Arctic and Antarctic. As the planet warms more permafrost will thaw, releasing the trapped methane and triggering a positive feedback loop (Dawson & Spannagle, 2008). These climatic processes are resulting in more frequent and severe extreme weather events (storms, heavy rain and snow fall, flooding, and drought) (IPCC, 2014). With warmer temperatures, more ocean water vaporises into the atmosphere. Additionally, for every degree that air temperature rises, its capacity to hold water vapour increases by about seven percent (according to the Clausius-Clapeyron relation). As a result, clouds are able to hold more moisture, producing heavier precipitation events (IPCC, 2007). Since water is a greenhouse gas, this will amplify global warming in a positive feedback loop.

The melting of Arctic and Antarctic ice sheets, in addition to thermal expansion of the ocean, is causing sea levels to rise (Rignot et al., 2011). Satellite data and long-term tide gauges indicate that over the last two decades, global sea level has risen an average of 3.2 mm per year, with an overall increase of 20 cm since 1901 (The Royal Society, 2010). Sea level is predicted to continue rising over the next few decades, which is particularly concerning for low-lying coastal regions (Cabanes et al., 2001). Projected sea level rises of ~1.2 m are predicted to affect

~4.6% of the world's population, and annual global gross domestic product is estimated to decrease by 9.3% (Hinkel et al., 2014).

Ocean acidification is another significant issue associated with climate change. CO_2 dissolves in water to form weak carbonic acid, according to the equation (Doney et al., 2009):

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

The oceans have absorbed approximately a third of the anthropogenic CO_2 released into the atmosphere, resulting in a global decrease in ocean pH (The Royal Society, 2010). This is a significant concern as a number of marine organisms (namely shellfish and coral) have calcium carbonate shells, which dissolve readily under acidic conditions. As ocean acidity increases it is becoming more difficult for these organisms to maintain their shells, and as a result their numbers are steadily declining (Doney et al., 2009).

Another threat associated with global warming is the potential for the thermohaline circulation to experience a complete or partial shutdown (Broecker, 1997). The thermohaline circulation is an ocean circulation system that is driven by temperature, wind and salinity gradients (Dawson & Spannagle, 2008). It is involved in the warming of regions in North America, Europe and the North Atlantic, as well as in the transport of dissolved carbon, salt, and oxygenated water to the deep ocean. Any climatic changes affecting ocean salinity or temperature are likely to influence the thermohaline circulation, as climatic records indicate that changes in thermohaline circulation have occurred in the past, and are associated with periods of cooling in regions within Europe and the North Atlantic (Link & Tol, 2004). Aside from causing significant cooling in these regions, a collapse in the thermohaline circulation could lead to changes in rainfall and the length of the growing season (and hence crop production); a reduction in the number of marine animals as their habitat undergoes rapid changes; and in the worst case scenario, a large scale anoxic event (Dawson & Spannagle, 2008).

While climate change is no new occurrence, with gradual changes in Earth's orbit (i.e. Milankovitch cycles) inducing natural cycles of cooling and warming over

~100,000 year intervals (Berger, 1988), none have occurred at the current rate. All major climatic shifts, natural or otherwise, have dramatic impacts on the environment and the organisms within it. In the past climatic changes have caused mass migrations, species extinctions, and significant changes in ocean and atmospheric patterns (Thomas et al., 2004). With environmental changes occurring at such a drastic rate it is more difficult for species to adapt to the new environmental conditions, and ultimately the resulting effects are likely to be more extreme (The U.S. National Academy of Sciences and The Royal Society, 2014).

If climate change goes unchecked and CO_2 and CH_4 emissions continue at the current rate, then a further 2.6-4.8 ^{o}C warming is predicted by the end of the century (The U.S. National Academy of Sciences and The Royal Society, 2014). Given the recognised dramatic impacts of climate change it is important to monitor rates of global warming and introduce effective policy to mitigate its effects.

1.2.2 Antarctica and climate change

To understand modern climatic changes and the factors which contribute to these, it is essential to study past climatic trends. This will allow scientists to identify the natural ranges of climatic variability on a range of different time scales, and thus determine whether modern changes are surpassing those which occur naturally (Parrenin et al., 2013; Turner et al., 2009).

As the most remote continent on Earth, separated from the rest of the world by the Antarctic Circumpolar Current (ACC) and Southern Ocean (Pearce et al., 2009; Walton, 2013), Antarctica is the continent least influenced by human activities and settlement, and is thus the ideal location to examine global and local scale climate change and disentangle human-driven and naturally occurring processes. Climatic records extending millions of years into the past are held within Antarctic ice cores and sedimentary rocks and soil (Parrenin et al., 2013). Obtaining this geological and geochemical information is important for developing models which can help predict global and regional climatic changes, and how the continent, and ultimately planet, will respond to these changes

(Turner et al., 2009). Similarly, to predict how extant species may respond to changing environmental conditions, it is important to understand how past glaciations have influenced species extinction and diversification (Kennicutt et al., 2014).

1.2.3 Antarctic warming

As a whole, the Antarctic continent has experienced very little climatic warming. Some regions have experienced slight temperature increases, while others have undergone mild cooling (IPCC, 2007). This is due in part to the ozone hole which, over the last 30 years, has actually been shielding the continent from much of the effects of global warming (Turner et al., 2009). As a greenhouse gas, a reduction in stratospheric ozone results in reduced temperatures as less UV and infrared radiation is absorbed and retained (Dawson & Spannagle, 2008). The ozone hole has also altered south polar winds (the polar vortex), isolating the continent from the warming experienced elsewhere (Thompson & Solomon, 2002; Turner & Overland, 2009). Reduction in stratospheric ozone has been driven largely by Chlorofluorocarbon (CFC) compounds, found in refrigeration units, propellants and some solvents (Metz et al., 2005). CFC compounds are broken down by UV radiation to form chlorine atoms which catalyse ozone destruction (Metz et al., 2005). These ozone depleted regions (commonly referred to as ‘holes’) are concentrated above the poles, primarily appear during the summer months, and can have ozone levels up to 40% lower than natural values (Dawson & Spannagle, 2008).

However, the ozone hole has been gradually shrinking since the Montreal Protocol - an international pact to phase out the use of CFC compounds - was established in 1987 (Turner et al., 2009; Velders et al., 2007). CFCs are set to be completely phased out by 2030, and it is predicted that within the next 50 years, springtime ozone concentrations will have largely recovered. While this is a huge success in terms of environmental policy, closure of the ozone hole is expected to exacerbate continental Antarctic warming (Turner et al., 2009).

If CO_2 emissions continue at their current rate, temperatures within Antarctica are expected to rise by several degrees over the next century, and sea ice is predicted

to decline by about a third (Turner et al., 2009). This will have a dramatic effect on both a global and local scale. Globally, sea level rises resulting from failure of the west and east Antarctic ice sheets will have devastating impacts on low-lying, coastal regions (Rignot et al., 2011). Collectively, the Arctic and Antarctic make up ~20% of the land surface on Earth, and environmental changes at the poles will have flow-on effects for other regions. If the Antarctic were to lose all of its ice, ocean levels would rise ~60 metres (Dawson & Spannagle, 2008).

Locally, increasing temperatures will prompt environmental changes that will undoubtedly affect marine and terrestrial biota, and may elicit dramatic alterations in Antarctic ecosystems (ASOC, 2008; Walther et al., 2002). While terrestrial animals are sparse, the Antarctic oceans support an abundance of krill, which serves an important ecological function at the base of the food chain (Dawson & Spannagle, 2008). Krill numbers have been declining in recent years, due in part to decreasing sea ice. If this continues it may have devastating flow on effects on marine biota (Dawson & Spannagle, 2008). In contrast to the animals in other regions, those inhabiting the Arctic and Antarctic have little option in terms of adapting to the new environmental conditions. As a result, many of these animals will be faced with extinction (Dawson & Spannagle, 2008). With a warmer climate and increased precipitation, more Antarctic regions will have the possibility to be colonised by vegetation, which will impact ecosystem dynamics (Dawson & Spannagle, 2008).

While it is unclear exactly how the continent will respond to this warming, great insight can be gained from studying what has already occurred on the Antarctic Peninsula. In contrast to the rest of the continent, the Antarctic Peninsula has been dramatically affected by global warming (Turner et al., 2009; Vaughan et al., 2003). Indeed, the Antarctic Peninsula is one of the most rapidly warming locations on the planet, with average annual temperatures increasing by 0.56 °C per decade, and by 1.09 °C per decade during the summer months, over the last 50 years (Turner et al., 2005). Ice shelves on the peninsular have been retreating (Abram et al., 2013), partially due to stronger westerly winds caused by changes in the Southern Hemisphere annular mode, for which the ozone hole is ultimately responsible (Thompson & Solomon, 2002; Turner et al., 2005). Sea ice along the

Western Antarctic Peninsula decreased by ~40% from 1979 to 2004 (ASOC, 2008), and the Antarctic Peninsula's annual contribution to global sea-level rise has been estimated to be ~0.16 ± 0.06 mm (Turner et al., 2009). Air temperature has increased far above freezing and, during some years, sea ice in the region is completely absent (ASOC, 2008). Precipitation rates have also increased, which, in conjunction with increased temperatures, has been linked to changes in the physical environment and overlying vegetation (Fowbert & Smith, 1994).

An overwhelming body of evidence indicates that this warming has triggered significant changes in marine and terrestrial Antarctic ecosystems. Emperor and Adelie penguins, krill and Weddell seals have declined in number (Atkinson et al., 2004); and two native, flowering plants, Antarctic pearlwort (*Colobanthus quitensis*) and Antarctic hair grass (*Deschampsia antarctica*), have increased in both distribution and abundance along the western coast of the Antarctic Peninsula (Fowbert & Smith, 1994). A number of Antarctic birds – particularly those that rely on tundra and ice - have experienced a decrease in home range and changes in food availability and feeding season that may have a devastating impact on colony numbers and, consequently genetic diversity (Dawson & Spannagle, 2008).

Microorganisms are also being affected by this climate change. A recent study looking at soil microbial activity and moss growth in a 150-year old moss bank on the Antarctic Peninsula has shown that both have experienced a dramatic increase since ~1960, in keeping with temperature and climate change trends (Royles et al., 2013). These increases were attributed to the longer growing season, triggered by warmer temperatures and earlier thawing periods (Royles et al., 2013). It is possible that with the forecast closure of the ozone hole, the rest of Antarctica may experience a rapid acceleration in warming, and subsequent ecological effects, similar to those observed within the Antarctic Peninsula.

1.3 The Antarctic environment

Antarctica is a continent of extremes, being the coldest, driest, and windiest continent on the planet. The majority of the continent lies south of the Antarctic Circle, experiencing 24 hours of sunlight during the summer, and 24 hours of darkness in winter (Turner et al., 2009). While the Antarctic ice sheet permanently covers most of the continent, ~0.35% remains ice free for the entirety, or majority, of the year (Hopkins, Sparrow, Novis, et al., 2006). The soils exposed in these ice-free regions, while constituting only a minor fraction of the continent, provide important terrestrial refuges for unique microbial ecosystems (Chown et al., 2015; Rogers et al., 2012).

1.3.1 The Dry Valleys

The McMurdo Dry Valley system is the largest ice-free region on Antarctica, at ~4500 km^2 (Figure 1.1) (Levy, 2013). With high levels of UV radiation, average annual precipitation ranging from 10-100 mm per year, and average annual temperatures in the region of −15 to −30 °C, the McMurdo Dry Valleys are among the most hostile environments on the planet. Soils within the Dry Valleys also have high salt concentrations, further reducing water availability for the inhabiting organisms (Bockheim & McLeod, 2008).

With the exception of areas exposed to legacy, or contemporary, organic input, Dry Valley soil is also extremely low in nitrogen and carbon content (Selby, 1989). Based on stable isotope data, it would appear that ancient lake sediments provide the soil with much of its contemporary nitrogen and carbon, particularly in low-lying regions (Moorhead et al., 1999). Mummified penguin and seal carcasses provide additional sources of organic input, but the impact of these is restricted to a small, localised area (Cary et al., 2010; Hopkins, Sparrow, Novis, et al., 2006).

The landscape of the McMurdo Dry Valleys is tightly controlled by climatic processes (Fountain et al., 2014). Field observations within the area indicate that over the last decade the region has experienced drastic, unprecedented changes in morphology. These changes appear to be driven by the melting of buried glaciers, and are ultimately caused by greater levels of solar radiation and rising

temperatures (Fountain et al., 2014). Several streams are expected to have their courses diverted, leading to soil erosion that will ultimately be deposited in lakes (increasing nutrient availability and water level) (Fountain et al., 2014).

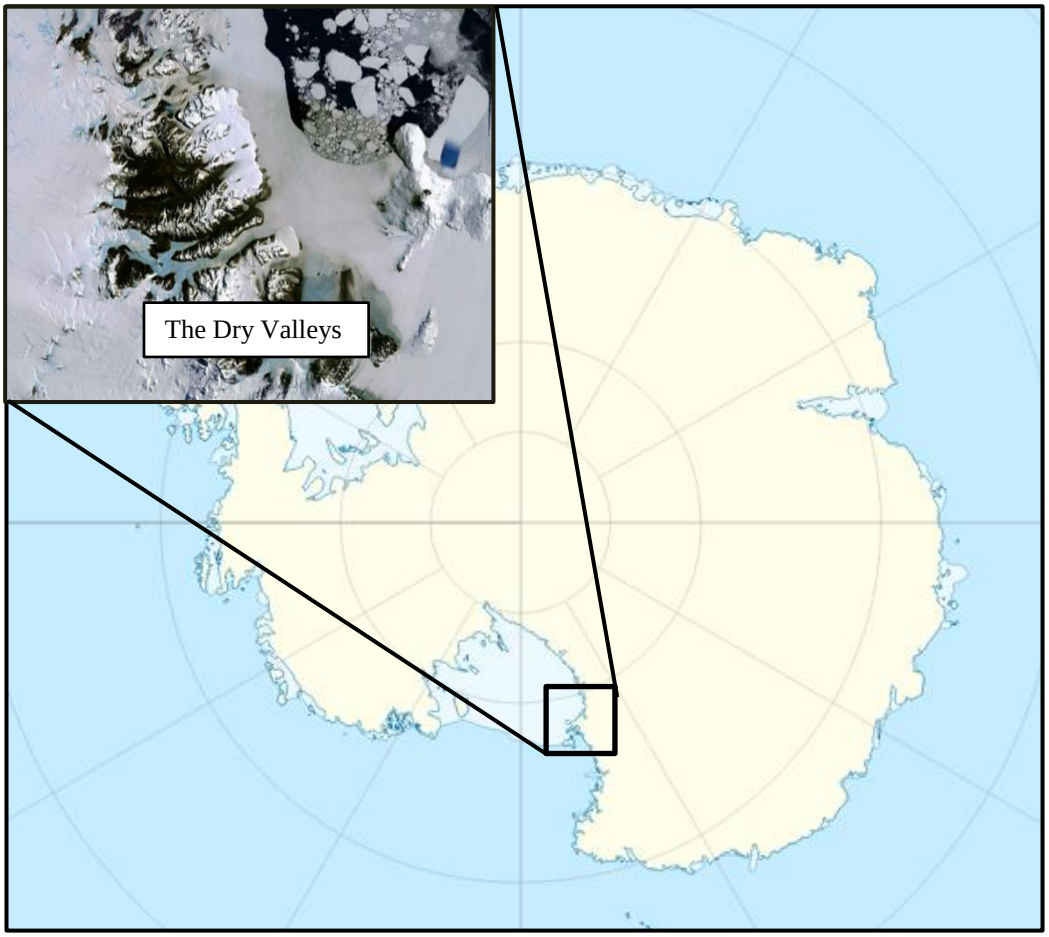


Figure 1.1. Map of the Dry Valleys, Antarctica

Upper image from Google Maps, Antarctic map from Wikipedia, the free encyclopedia (Wikipedia, 2010).

1.4 Antarctic microbiology

Microorganisms are fundamental components of the biosphere, with roles as both primary producers and as decomposers (Naeem et al., 2000). Autotrophic bacteria (along with green plants and algae) produce the organic material used by all the other organisms within an ecosystem, while decomposers recycle essential nutrients which allows life to be sustained (Naeem et al., 2000). Scientists widely acknowledge that microorganisms play a central role in determining the

concentrations of prominent greenhouse gases in the atmosphere (CH_4, CO_2 and N_2O) (Singh et al., 2010). Several lines of evidence suggest that current rates of climate change (particularly changes in temperature and soil moisture content) will have significant effects on edaphic microbial communities and the processes they control (Singh et al., 2010). Microbial communities lie at the base of all food chains and are responsible for establishing the biogeochemical processes that allow other organisms to survive, facilitating succession. Thus changes in microbial populations can have a massive cascading effect up to higher trophic levels (Terborgh & Estes, 2013). Microbial ecologists are currently very interested in how changes in microbial community composition may influence ecosystem functioning at a larger scale (Logue et al., 2015).

Due to the severe abiotic conditions imposed by the Antarctic environment (i.e. limited water and nutrient availability, high levels of UV radiation, prolonged periods of darkness, and extreme temperatures), the continent is unable to support the majority of plant and animal species found in temperate regions (Vincent, 1989). As a result, Antarctica has simple terrestrial ecosystems dominated by microorganisms, and almost exclusively controlled by abiotic factors (due to the near absence of biological influences) (Chown et al., 2015; Hogg et al., 2006).

Simple ecosystems are easier to understand than more complex systems, and thus are the ideal place to study the basic foundations of ecosystem structure. This knowledge can then be applied to more complex systems and used to develop models that predict how Antarctica, and ultimately the rest of the world, may respond to changing environmental conditions forecast under the current rate of continental warming (Hogg et al., 2006).

Microbial communities are very responsive to changing environmental conditions. This is thought to be due to fast generation time, ready dispersal, and genomic plasticity (horizontal gene transfer) (Logue et al., 2015). Thus there is the potential to use microbial abundance, diversity, and community composition as an early indication of how climate change is influencing the environment. This is advantageous in that it may be possible to monitor environmental perturbations using a single variable, rather than a raft of chemical and physical factors (Allen et al., 2011).

Furthermore, from a conservational perspective, Antarctica harbours a unique range of microbes with several seemingly endemic species that haven't been observed elsewhere (Lee et al., 2012). Under the Antarctic Treaty it is our responsibility to protect these unique ecosystems and species, and minimise human impact in one of the few regions that remain largely untouched by human activity and settlement (Antarctic Treaty Consultative Parties, 1960).

1.4.1 Major bacterial phyla

While over 80 phyla of bacteria are known, >90% of all formally described species fall within just four of these: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Madigan et al., 2014). Of these four phyla, Proteobacteria is the largest and most metabolically diverse (with phototrophic, chemoorganotrophic, and chemolithotrophic species). The group was first established by Carl Woese in 1987, but was named 'purple bacteria and relatives', due to the purple colouration of many of its members (Gupta, 2000; Woese, 1987). However as more species within the phylum were discovered it became apparent that photosynthesis was common to only a few members, and the group was ultimately renamed after the Greek God Proteus, who could take on a range of forms (Stackebrandt et al., 1988). The vast majority of Proteobacteria are Gram-negative, and include a number of medically significant pathogens (Gupta, 2000). The phylum has been further subdivided into five subclasses: α, β, γ, δ and ε (Woese, 1987). The majority of α-Proteobacteria are obligate or facultative aerobes, and a number are oligotrophic (surviving in environments with low nutrient levels) (Madigan et al., 2014). β-Proteobacteria are extremely diverse metabolically and include heterotrophs, photoautotrophs, and chemolithoautotrophs. γ-Proteobacteria are the most diverse and largest class within the Proteobacteria phylum, and include a number of human-associated pathogens (Madigan et al., 2014; Woese, 1987).

Bacteroidetes are Gram-negative, non-sporulating rod-shaped bacteria. They can be obligate or facultative aerobes, or obligate anaerobes, and are commonly associated with moist environments (Ley et al., 2006; Madigan et al., 2014). Bacteroidetes are a significant phylum in the human gut, where they act as mutualists (Wexler, 2007).

The Firmicutes are predominantly a Gram-positive phylum and include endospore-forming bacteria (resistant to desiccation), and lactic acid producing bacteria which play a role in alcohol spoilage (Galperin, 2013). They, together with Bacteroidetes, are also the dominant phylum associated with the human gut (Jandhyala et al., 2015).

Actinobacteria are a large group of Gram-positive, rod-shaped to filamentous bacteria. They are predominantly aerobic soil bacteria, and generally have genomes with a high GC content (Ventura et al., 2007).

Cyanobacteria, or historically "blue-green algae", are a group of prokaryotes that carry out photosynthesis using chlorophyll a (Woese, 1987). Cyanobacteria are among the oldest known phyla, and are of great evolutionary interest as they are believed to be the modern ancestor of the Chloroplasts found within eukaryotes (via endosymbiosis) (Löffelhardt & Bohnert, 1994). They are also believed to have played a role in 'The Great Oxygenic Event' which made life as we know it possible (Kaufman, 2014). Cyanobacteria are commonly found in aquatic environments where they play a major role in carbon, nitrogen and oxygen exchange processes (Havens, 2008). In the Dry Valleys, Cyanobacteria activity (measured by carbon fixation) is driven largely by temperature and irradiance (Novis et al., 2007).

1.4.2 Dry Valley microbial communities

Early, culture-based studies into the microbial communities within Antarctic Dry Valley soil found microbial abundance and diversity to be extremely low, and indicated that microbial communities were homogenous across the Dry Valleys (Vincent, 2004). The widely distributed *Corynebacterium*, *Arthrobacter*, *Micrococcus* and *Brevibacterium* genera were found to be highly dominant (Johnson et al., 1978) and this, in conjunction with evidence of long-range aeolian dispersal (i.e. the presence of *Beijerinckia*, which seldom exists outside tropical soils), led researchers to believe that Antarctic microbes may be cosmopolitan species, similar to those found in distant continents (Hopkins, Sparrow, Novis, et al., 2006; Moorhead et al., 1999; Smith et al., 2006).

However, this lack of diversity was later found to be an artefact of the early culture-based techniques employed, as a limited number of bacterial species are culturable (Rappe & Giovannoni, 2003). With the advent of molecular based techniques it has become apparent that microbial diversity and abundance within Antarctic Dry Valley soil is far greater than initially believed, with an estimated 106−108 prokaryotic cells g−1 (Cowan et al., 2002) (Smith et al., 2006). Phylogenetic studies from a range of locations within the Dry Valleys indicate that at the phylum level microbial diversity rivals that of temperate systems (Bockheim, 2002; Lee et al., 2012). At least 14 phyla have been identified, including Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes and Cyanobacteria (Aislabie et al., 2006; Smith et al., 2006). Figure 1.2 presents a summary of the major phyla in the McMurdo Dry Valleys (based on 16S rRNA gene sequences from Luther Vale, Miers Valley, and Wright Valley).

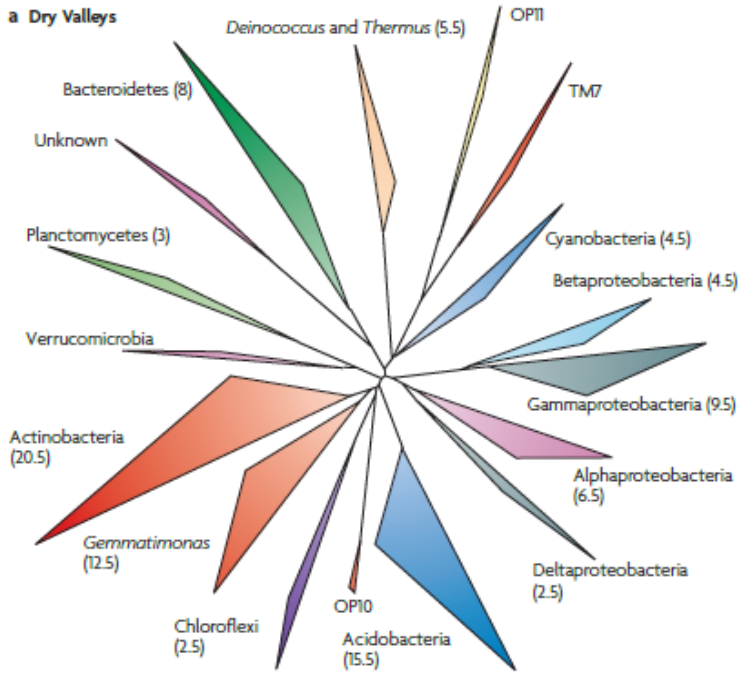


Figure 1.2. Major phyla in the McMurdo Dry Valleys, Antarctica

Phylogenetic diversity in the McMurdo Dry Valleys, Antarctica (based on 426 bacterial 16S rRNA gene sequences). Studies from Miers Valley, Vanda and Bull Pass in the Wright Valley, and Luther Vale were included. Sequence percentages of each phylum are shown in brackets. Reprinted from "On the rocks: the microbiology of Antarctic Dry Valley soils", by S.C. Cary, I.R. McDonald, J.E. Barrett, & D.A. Cowan, 2010, *Nature Reviews Microbiology, 8*, p. 134. Copyright 2010 by Macmillan Publishers Limited. Reprinted with permission.

Smith et al. (2006) aimed to update findings from historical, culture-dependent studies and provide a "snapshot" insight into the bacterial diversity within three environmentally distinct dry Antarctic soils: beneath a seal carcass on Bratina Island; on the slopes of Miers Valley; and in fine gravels from a high altitude location between Shangri La and Miers Valleys. The team found that a number of phylotypes fell into the "unculturable" class, and many of the sequences identified had little homology to extant species, supporting the case for endemism within Antarctica (Smith et al., 2006). Community composition based on DNA sequence data varied considerably between the different sites (Figure 1.3). Cyanobacteria were identified in only the high-altitude soils, where they were the most abundant phylum (comprising 40% of sequences), followed by uncultured, Bacteroidetes, and Actinobacteria (each at 15%). Actinobacteria were detected at all of the surveyed sites, and were most abundant in the slopes of Miers Valley (40%), followed by Acidobacteria and uncultured (both at 20%). Uncultured phylotypes were the most abundant beneath the seal carcass (at 30%), followed by Acidobacteria and Actinobacteria (at 25%) (Smith et al., 2006). These findings provide strong evidence that microbial communities within Antarctica are not homogenous across the Dry Valleys, and that different local selection pressures may select for different microbial communities.

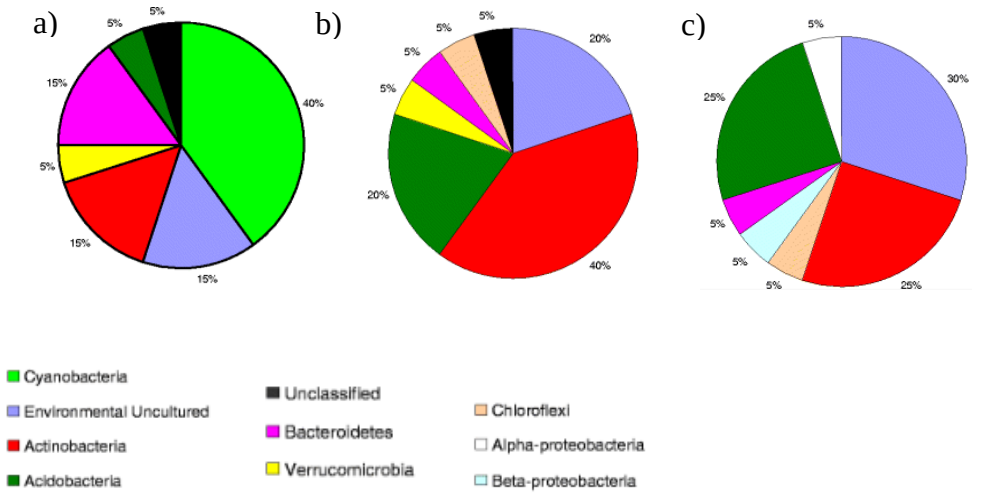


Figure 1.3. Relative abundances of bacterial phyla in three distinct Antarctic Dry Valley soil types

a) high altitude soil, b) slopes of Miers Valley, and c) beneath a seal carcass on Bratina Island. Based on 16S rRNA gene sequences from the Ross Dependency region.

Reprinted from "Bacterial diversity in three different Antarctic Cold Desert mineral soils" by J. J. Smith, L. A. Tow, W. Stafford, S. C. Cary & D. A. Cowan, 2006, *Microbial Ecology*, 51(4), 413-421. Reprinted with permission.

1.5 Spatial and temporal heterogeneity in Antarctica

Although the Dry Valley environment is extremely harsh and not conducive to life, there are still some microorganisms capable of carrying out metabolic activity. This metabolic activity is sensitive to environmental variables such as water and nutrient availability (Barrett, Virginia, Wall, & Adams, 2008; Hopkins, Sparrow, Elberling, et al., 2006; Tiao et al., 2012).

While average annual Antarctic temperatures are extremely cold, during the austral summer surface soils are exposed to significant fluctuations in temperature (± 20^{o}C), with maximum daily temperatures commonly exceeding 0^{o}C (Aislabie et al., 2006). Lake and subsurface ice melt during these months leads to greater water availability within the soil, and this results in increased microbial activity (Barrett, Virginia, Wall, Doran, et al., 2008).

Antarctic soils are spatially heterogeneous, with differences in moisture availability, salinity, elemental composition, altitude, weathering progression, and

surface geology (Bockheim, 2002; Lee et al., 2012). These ecological factors influence microbial distribution and community composition within the Dry Valleys, with different environmental conditions selecting for different bacterial species (Geyer et al., 2014). The Dry Valleys contain localised 'hot spots' of activity peppered throughout the low productivity landscape (Schlesinger et al., 1996). In these regions of high productivity liquid water and organic carbon are more readily available, and pH and salinity are less extreme.

1.6 Factors influencing the microbial communities within Antarctica

There are a raft of ecological factors, working in combination, that influence microbial community distribution, composition and diversity within Antarctica. These include salinity (Zeglin et al., 2009), latitude (Yergeau et al., 2008), UV exposure (for surface dwelling bacteria) (Tosi et al., 2005), soluble salt and nutrient concentration (Pointing et al., 2009), pH, depth (Stomeo et al., 2012), slope, drainage, exposure, water availability and temperature (Van Horn et al., 2013). Small-scale topographical positioning also has the potential to influence microbial diversity and abundance, with soils that receive more sunlight having greater biotic potential due to the longer period spent at temperatures which support metabolic activity (Seybold et al., 2010; Stomeo et al., 2012). Identifying the physical and biological factors controlling microbial distribution, activity and diversity is important in order to predict how these microbial communities may respond to changing environmental conditions. There is no general consensus about which ecological factors are the most important in determining microbial community structure and diversity within Antarctic Dry Valley soil (indeed it would appear that the driving factors vary depending on a complex interplay between variables in the location sampled), but temperature and water availability are thought to play a major role (Cary et al., 2010; Kennedy, 1993; Zeglin et al., 2009).

Water availability within Dry Valley soil varies on both a spatial and a temporal scale and is controlled by temperature, proximity to nearby lakes, and the presence of permafrost (Noy-Meir, 1973). Permafrost is found in all but high

altitude Dry Valley soils, and is overlaid by the 'active' zone (10-25 cm deep) (Gilichinsky et al., 2007), which is a layer of soil which undergoes seasonal freeze-thaw cycles (FTC). Due to sublimation, water trapped in the permafrost layer does not exist in the liquid form (Gilichinsky et al., 2007), but in the active zone water is available on a seasonal basis for three months of the austral summer (during which time the requirements for metabolic activity are presumably met) (Stomeo et al., 2012). Within the active layer water content increases with increasing depth, and is influenced by RH and temperature (Seybold et al., 2010). Soil in the active zone receives moisture from both irregular precipitation events, and from the underlying permafrost via an upwards entrainment (Barrett et al., 2007; Bockheim, 2002). This upwards entrainment is driven by a high RH gradient between surface and deep soil, causing water to sublime from permafrost to the atmosphere and providing the microbial communities with a semi-continuous supply of water (Stomeo et al., 2012). Water is also available during the summer months from glacier runoffs and outflows from Dry Valley Lakes (Baker, 1967).

Aislabie et al. (2006) employed both culture-dependent and sequencing methods to investigate microbial diversity (and the factors driving this diversity) in soils from four environmentally distinct locations within Victoria land, Antarctica: coastal regions along Marble Point; in Wright Valley near Lake Vanda and at Bull Pass; and on Mt. Fleming (Aislabie et al., 2006). The team found a few bacterial species to dominate soils within Wright Valley and Marble Point, with the most dominant species differing between locations. Water availability and organic carbon content were key factors driving microbial community differences in the sites examined. The most diverse microbial communities were found in the moister soils along the coast of Marble Point, while the least diverse were found in the drier Mt. Fleming soils. Desiccation tolerant species such as *Rubrobacter* (Actinobacteria phylum) and *Deinococcus* (Deinococcus-Thermus) were prevalent in drier regions of the Wright Valley, presumably due to this adaptive feature (Aislabie et al., 2006).

Similarly, to determine how Dry Valley microbial populations are affected by soil properties, Niederberger et al. (2008) looked at the microbial communities within

soil broadly categorised as ‘high’ or ‘low’ productivity along transects within Luther Valley. High productivity soil was defined as having high water, carbon and nutrient content; while low productivity soil was defined as having low water, carbon and nutrient content. Examination of 16S rRNA clone libraries revealed significant differences between the microbial communities of the two soil types, despite only being 200 metres apart (Niederberger et al., 2008). Species from Deinococcus/Thermus (which are resistant to ionising radiation and dehydration) were identified exclusively in low-productivity soil, while *Xanthomonas* (of the γ-Proteobacteria phylum), β-Proteobacteria, Cyanobacteria, and Verrucomicrobia were associated exclusively with high-productivity soils. These differences were linked to nutrient and water availability, and add further evidence to the case that microorganisms within Antarctic Dry Valley soil are not cosmopolitan, but controlled by environmental factors (Niederberger et al., 2008).

These findings were in keeping with those of Van Horn et al. (2013) who investigated the factors controlling bacterial abundance, diversity, and community composition within the Taylor and Wright Valleys. To do this, samples were collected from dry mineral soils in close proximity to “snow patches” (which form local-scale, natural biogeochemical gradients). Interestingly, the team found Proteobacteria and Firmicutes to be the most prevalent phyla in the drier, high elevation sites, while Actinobacteria and Acidobacteria were most dominant in the low elevation, moister sites, and these differences were attributed to differences in moisture availability (Van Horn et al., 2013).

Likewise, to investigate how local selection pressures influence microbial communities, Geyer et al. (2014) identified and sampled three soil types within the Wright and Taylor Valleys: soils with extreme pH (>9.4) and conductivity (> 1000 μS cm−1); mesic soils with relatively high moisture availability and organic carbon content; and low pH/ salinity soils. The aim of the study was to determine how microbial community structure and diversity varied between sites, and which environmental factors were responsible for microbial diversity (Geyer et al., 2014). In keeping with previous findings, different soil conditions selected for distinct microbial communities, and that certain phyla were associated with particular soil properties. Acidobacteria and Actinobacteria were associated with

high pH soils, Firmicutes with soils high in carbon and conductivity, and the *Nitrospira* genus with soils high in carbon. High pH regions were also associated with reduced bacterial community diversity and richness at the genus, order and phylum level, while moist and high conductivity soils had similar levels of bacterial richness (Geyer et al., 2014). At the genus and order levels, moisture availability was strongly correlated with species richness, while no such association was seen at the phylum level (Geyer et al., 2014). The poor explanatory power of the environmental variables at phylum level makes sense in light of the huge extent of bacterial diversity at this level, as it is extremely difficult to find a single factor that reliably and consistently explains community composition (Geyer et al., 2014). This highlights the importance of selecting the appropriate taxonomic scale when investigating differences in microbial community composition.

In line with this research, Lee et al. (2012) examined microbial communities from four distinct regions within the Dry Valleys (Beacon Valley, Upper Wright Valley, Battleship Promontory and Miers Valley) to determine whether physicochemical factors play a major role in shaping microbial communities within Antarctic soil. The results showed that at the OTU level, microbial communities from the four Valleys differed in terms of diversity, structure and phylogeny, while at the phylum level, the microbial communities were similar in composition (Figure 1.4) (Lee et al., 2012). Remarkably, of 214 phylotypes detected in the study, only two were detected in all four valleys, which suggests that aeolian transport may not play as large a role in determining community composition as previously thought, or that local selection pressures are greater than originally believed (Lee et al., 2012; Moorhead et al., 1999). Altitude, and salt and copper content, were significantly correlated with microbial community structure. These findings add to the growing body of evidence that Dry Valley microbial ecosystems are highly localised, and that physicochemical factors may play a significant role in structuring community composition (Lee et al., 2012).

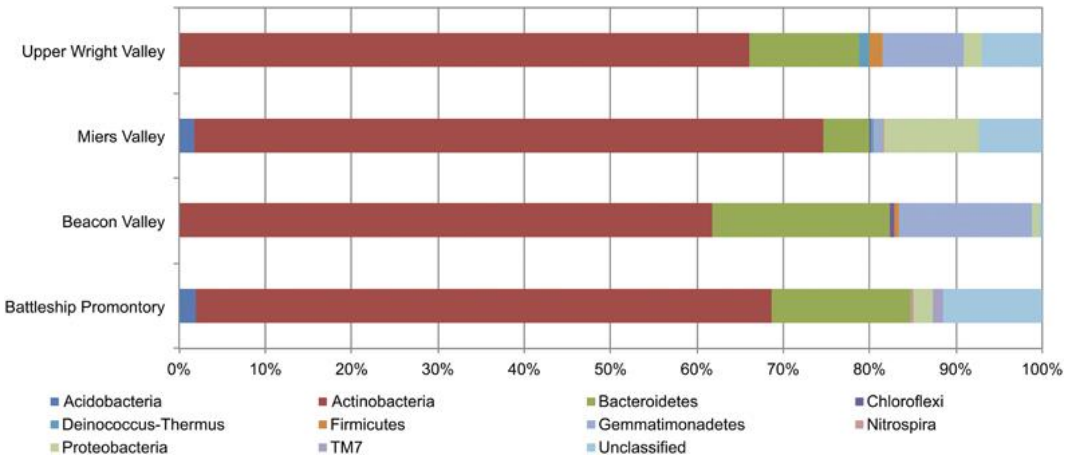


Figure 1.4. Phylum-level distribution of OTUs in four Dry Valley locations

Phylogenetic assignments were made using the Ribosomal Database Project Classifier. Reprinted from "The Inter-Valley Soil Comparative Survey: the ecology of Dry Valley edaphic microbial communities", by C.K. Lee, B.A. Barbier, E.M. Bottos, I.R. McDonald, & S.C. Cary, 2012, *The ISME Journal*, 6(*5*), p.1046–1057. Copyright 2012 by the International Society for Microbial Ecology. Reprinted with permission.

1.7 Antarctic microbial communities and climate change

Microbial communities tend to respond to temperature increases in a non-linear manner, leading to speculation that climate change may have a larger impact on microbial communities than originally predicted (Scherm & Van Bruggen, 1994; Yergeau & Kowalchuk, 2008). Climate change will not only increase temperatures within the continent, but also increase soil moisture availability, extend the growing season, vegetation distribution, and number and severity of Freeze Thaw Cycles (FTC) (Fowbert & Smith, 1994). Since the majority of Antarctic microorganisms are thought to be psychotropic, rather than psychrophilic, it is believed that the secondary effects of temperature increases will be more significant than the temperature increase itself (Kerry, 1990).

Freeze-thaw events are a prominent feature of the Antarctic Dry Valleys (Aislabie et al., 2006). These cycles of freezing and thawing have the ability to rupture microbial cells and break apart large soil aggregates, resulting in a release of nutrients that are then available to the surviving bacteria (Skogland et al., 1988). Freeze-thaw events are predicted to deliver >15% of the total organic matter to edaphic microbes in Antarctic soils (Tearle, 1987), ten times more than

is released via the decomposition of vegetation. Hence periods of thaw are often accompanied by subtle micro niche alterations that affect the microbial community. While little is known about the impact of warming on microbial ecosystems and the nutrient cycling processes that they control, it is likely to be significant (Yergeau & Kowalchuk, 2008; Zogg et al., 1997).

To gain a deeper understanding of the rate at which microbial communities may respond to changing environmental conditions, several multi-year *in situ* investigations have been carried out (Tiao et al., 2012; Yergeau et al., 2012). These studies have allowed changes in Antarctic microbial communities to be tracked on a temporal scale, generating invaluable datasets and enhancing our understanding of the impact of contemporary environmental conditions on microbial community dynamics.

Over a two year period Deslippe et al. (2012) monitored the bacterial and fungal communities of Antarctic mineral and organic soil in a controlled, greenhouse warming experiment that had been running for 18 years. The warming treatment reduced bacterial community evenness, while increasing evenness in the fungal community. The effects of the warming treatment on the bacterial community were particularly prominent in the organic soil, where temperature changes were associated with a decrease in the relative abundance of Gemmatimonadaceae and Proteobacteria, and an increase in Actinobacteria. However, in this experiment it was not just the microbes, but the whole community ecosystem, that changed in response to the greenhouse warming. It was suggested that the observed changes in microbial community structure were more strongly associated with the secondary effects of warming (i.e. changes in plant and fungi abundance), than with the warming itself (Deslippe et al., 2012).

Likewise, Yergeau et al. (2012) carried out a three year *in situ* warming experiment (of 0.5 to 2 °C) using open-top chambers (OTCs) in three maritime Antarctic and sub-Antarctic environments. At each location both vegetated and non-vegetated sampling sites were selected, with vegetated locations having greater nitrogen and carbon contents. The OTCs caused average annual soil temperature (5 cm below the surface) to increase approximately 0.8 °C compared

to control sites (Yergeau et al., 2012). However, this temperature increase varied between sampling locations, vegetated and non-vegetated plots, and was not consistent throughout the year (Yergeau et al., 2012). Quantitative PCR and 16S rRNA gene 454-pyrosequencing revealed a significant increase in the α-Proteobacteria to Acidobacteria ratio, as well as an increase in fungal abundance in OTC soil (relative to control soil). However, given the small temperature increase (which was smaller than several of the naturally occurring differences between locations), it is more likely that vegetation cover and sampling location were the main driving forces in shaping bacterial communities.

These *in situ* manipulation studies have demonstrated that, contrary to the long-held belief that Antarctic microbial communities take thousands of years to respond to environmental changes (Moorhead et al., 1999), microorganisms can respond to new environmental conditions (induced by OTCs) within very short timeframes (< 3 years). However, a major issue with these induced warming studies is that while the microbial community changes observed were attributed to temperature changes and reduced FTC frequency, the OTCs also altered a number of abiotic, physical variables (including RH, temperature fluctuations, snow cover, air flow, UV exposure, and fungal and plant abundance/ community composition) that are likely to be overriding the effect of warming itself. Indeed, given that the OTC-induced warming (of 0.5 to 2 °C) was less than site to site variation in temperature, the warming effect is likely to be negligible. In light of this, drivers of the microbial community changes could not be confidently identified. To address this knowledge gap, and to resolve the drivers of the microbial community changes observed, controlled *in situ* investigations should be conducted.

A number of studies have aimed to understand microbial community dynamics by studying them in natural systems or in multifactor experiments (Yergeau et al., 2012; Yergeau & Kowalchuk, 2008). While this provides a holistic picture of ecosystem dynamics (i.e. the complex interactions between microbes, plants, climate and the physical environment), it is incredibly difficult to identify cause-and-effect relationships as different variables can often be in opposition and can obscure one another (i.e. a mild elevation in soil moisture content may be offset

by temperature changes). A wiser approach when trying to understand a complex system is to first understand its components, and then piece the components together to form a more complete picture. In a natural system this means isolating variables and removing some of the confounding interactions. Once this ground work has been laid it is then easier to recognise trends and disentangle interactions in larger, more complex systems.

1.8 Unique microbial communities

Microbial community composition and diversity in the Antarctic Dry Valleys are influenced by a number of abiotic factors working in conjunction (Stomeo et al., 2012). A valuable approach in determining which of these abiotic factors are most influential in shaping the microbial communities is studying model communities where these factors can be isolated and examined individually (Cary et al., 2010).

Naturally occurring protective environments, in the form of translucent rocks (hypoliths) and mummified seal remains, are peppered throughout the Dry Valleys (Cary et al., 2010). These sporadically distributed micro-niches reduce UV exposure, stabilise temperature, and protect the underlying soil from the dehydrating effects of the wind. This prevents sublimation of the subsurface permafrost, increasing and stabilising RH beneath the object and generating environmental conditions more ideal for microbial growth (Cary et al., 2010).

Mummified seal carcasses (that have been in place for over 100 years), in addition to stabilising environmental conditions, offer a rare opportunity to investigate the impact of long-term, localised nutrient input on microbial communities (Cary et al., 2010). Translucent quartz rocks allow light to penetrate to the underlying soil, supporting the growth of photoautotrophs (i.e. Cyanobacteria) while acting to retain moisture. This allows more complex microbial ecosystems to develop, and as a result microbial productivity and diversity in these hypolithic communities is significantly higher than that of surrounding soils (Smith et al., 2000). Consequently, these two naturally occurring phenomena provide a unique insight into how microbial communities may respond to a new set of environmental conditions, induced by global warming.

1.9 Seal carcass transplantation experiment

In a landmark study Tiao et al. (2012) conducted a unique, *in situ* experiment to investigate the rate at which microbial communities respond to a new set of environmental conditions. To achieve this, a mummified seal carcass (dated at 250 years) (Figure 1.5) was shifted from its original site in Miers Valley to a new, geomorphically similar location 20 metres away, and changes in the underlying microbial community were monitored via yearly sampling over a five year period (Tiao et al., 2012).

The edaphic microbial community beneath the original seal carcass had greater biomass, lower biodiversity, and a significantly different community composition compared with the microbial community at the control site (with a higher abundance of Firmicutes and γ-Proteobacteria, and a lower abundance of Bacteroidetes). Of the seven most abundant phyla found at the control site, only two were also observed in significant numbers beneath the seal carcass*:* Bacteroidetes and Actinobacteria (Figure 1.6). Indeed, the seal-covered soil was dominated by two OTUs that were less than 0.5% abundant in the control soil.

Figure 1.5. Seal carcass used in the seal transplantation experiment

Reprinted from "Rapid microbial response to the presence of an ancient relic in the Antarctic Dry Valleys" by G. Tiao, C.K. Lee, I.R. McDonald, D.A. Cowan and S.C. Cary, 2012, *Nat Comms,* 3, p. 660-668. Reprinted with permission.

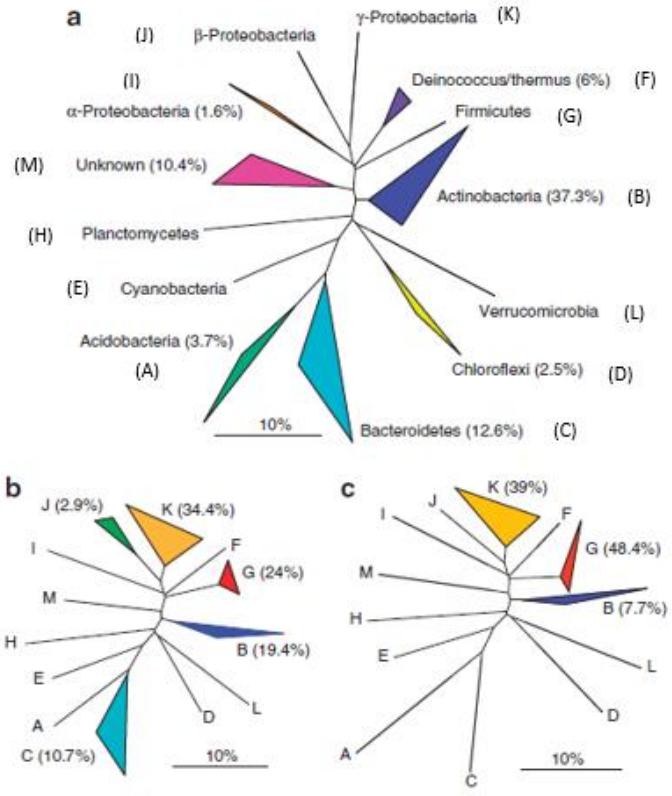


Figure 1.6. Relative abundance of different phyla in seal movement study

(a) control site, (b) original seal-covered site, (c) new seal-covered site. Samples a-c were collected in January 2005, 2007, and 2009 respectively.

Reprinted from "Rapid microbial response to the presence of an ancient relic in the Antarctic Dry Valleys" by G. Tiao, C.K. Lee, I.R. McDonald, D.A. Cowan and S.C. Cary, 2012, *Nat Comms*, 3, p. 660-668. Reprinted with permission.

Remarkably, MDS plots of the new seal site (directly beneath the seal abdomen) revealed a shift in microbial community composition toward that of the original site within just two summers (Figure 1.7). The two most prominent OTUs at the original seal site, one from the *Planococcaceae* family (Firmicutes), and the other from the *Psychrobacter* genus (γ-Proteobacteria), also dominated the new site (constituting 48 and 39% of sequences, respectively). The microbial community at the new site was significantly less diverse than at both the control and original site. Bacteroidetes were less than 1% abundant at the new site, despite being fairly abundant at both the original and control site (10 and 13% abundant, respectively) (Tiao et al., 2012).

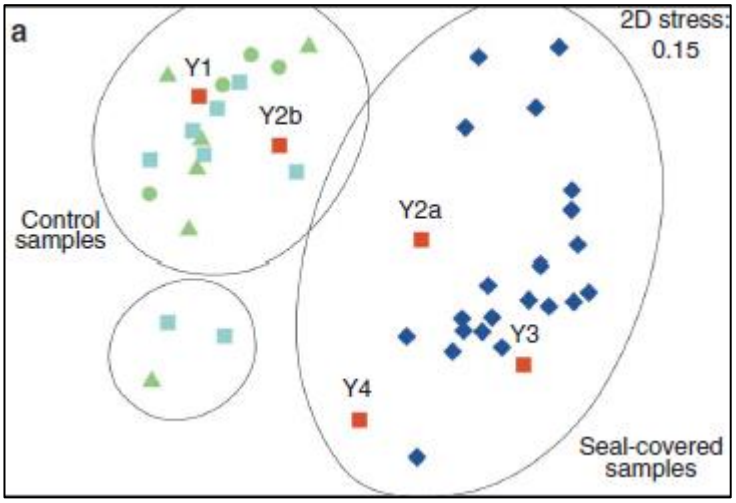


Figure 1.7. NMDS plots of microbial communities in control, original, and new seal-covered sites

Control sites are green circles (year 0); and green triangles (years 1-4). Original seal-covered sites are blue diamonds. The new seal-covered site samples that were not in contact with the carcass are light blue, while the new seal covered sites in contact with the carcass are red squares (with labels Y1-Y4 to indicate the year of sampling).

Reprinted from "Rapid microbial response to the presence of an ancient relic in the Antarctic Dry Valleys" by G. Tiao, C.K. Lee, I.R. McDonald, D.A. Cowan and S.C. Cary, 2012, *Nat Comms,* 3, p. 660-668. Reprinted with permission.

The sample taken from the new site before seal transplantation had a microbial community composition that grouped strongly with samples taken from the control site, indicating that it was a true representation of the surrounding area. Additionally, since all of the most abundant OTUs at the new site were present in the control soil (albeit in low abundance), it would appear that the seal carcass itself was not responsible for species introduction (Tiao et al., 2012). Instead it is likely that the microbial community changes observed were due to differences in a specific species' adaption to the new environmental conditions imposed by the carcass (Tiao et al., 2012). These better adapted microorganisms increased rapidly in abundance, outcompeting the less well-adapted species, resulting in decreased microbial diversity. Interestingly, the bacterial cell count at the original site decreased rapidly following seal transplantation, while the microbial community composition remained the same (Tiao et al., 2012). This indicates that removal of the seal carcass altered the physiochemical conditions that were promoting the high bacterial abundance (relative to control soil).

The seal carcass altered a number of the underlying soil's physical and chemical properties including pH, conductivity, nitrogen and organic carbon content, and elemental composition (Tiao et al., 2012). However, Biota-Environment STepwise matching (BEST) analysis of DNA community profiles revealed that none of these physicochemical factors satisfactorily explained differences in microbial community structure observed (Tiao et al., 2012). Based on this, the authors hypothesised that the shifts in microbial community structure may have been caused by physical, abiotic factors induced by the seal carcass – namely reduced UV exposure, more stable temperature, and increased/ more stable RH (Figure 1.8) (Tiao et al., 2012).

The dehydrating Antarctic winds generate a RH gradient between the surface and deep soil that causes sublimation of underlying permafrost (Bockheim, 2002). With an object (such as the seal carcass) on top of the soil, this sublimation is significantly reduced, and RH increases beneath the object (Figure 1.9) (Cary et al., 2010; Tiao et al., 2012). This provides a new set of environmental conditions which select for different microbial species.

This study demonstrates that microbial communities within Dry Valley soils can respond to environmental changes far quicker than previously believed, experiencing an increase in microbial biomass, and rapid decrease in biodiversity, within just two summers. It offers the first direct experimental evidence that, contrary to widely held beliefs (Barrett et al., 2006; Moorhead et al., 1999), contemporary environmental conditions may have a significant influence on microbial community dynamics (Tiao et al., 2012). However, due to the strict environmental regulations surrounding the Antarctic, the study was un-replicated and purely observational in nature, and consequently the researcher's hypothesis is merely speculation. The study calls for a controlled, multi-year, *in situ* experiment to be conducted to verify these findings and resolve the drivers of the microbial community changes observed.

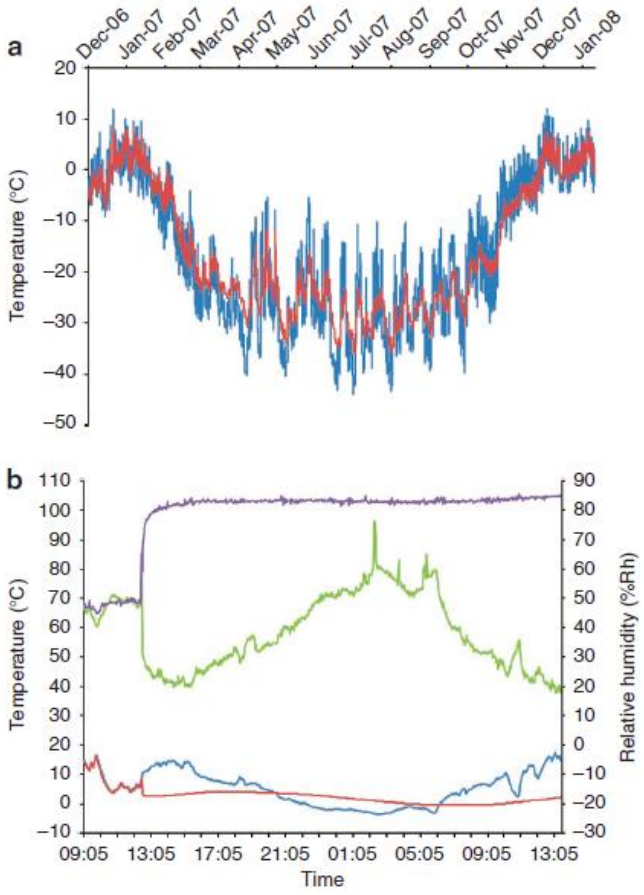


Figure 1.8. RH and soil temperature readings in control and new seal-covered sites

(a) Temperatures beneath the transported seal carcass (red) and in control soil (blue); (b) Relative humidity beneath the seal carcass (purple) and in control soil (green), and temperature measurements beneath the carcass (red) and in the control soil (blue).

Reprinted from "Rapid microbial response to the presence of an ancient relic in the Antarctic Dry Valleys" by G. Tiao, C.K. Lee, I.R. McDonald, D.A. Cowan and S.C. Cary, 2012, *Nat Comms*, 3, p. 660-668. Reprinted with permission.

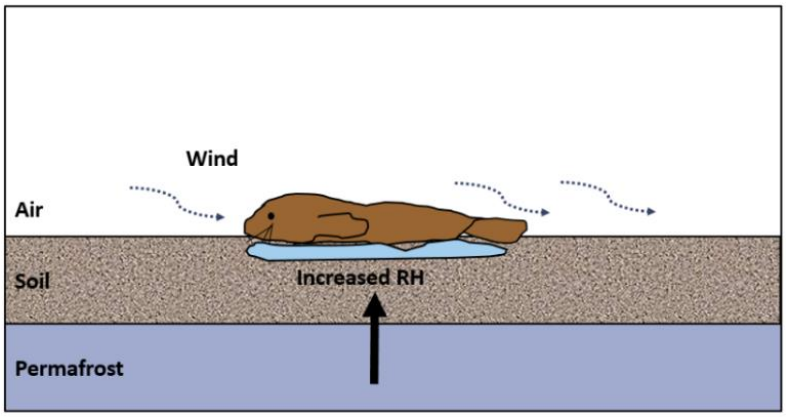


Figure 1.9. Mechanism of increased RH beneath the seal carcass

Adapted from "On the rocks: the microbiology of Antarctic Dry Valley soils" by S.C. Cary, I.R. McDonald, J.E. Barrett and D.A. Cowan, 2010 *Nat Rev Micro, 8*(2), 129-138. Reprinted with permission.

1.10 Aims, approach and significance of the thesis

The current study aims to shed light on these unexpected findings and verify whether changes in RH and temperature (caused by overlying objects) are indeed driving the changes in microbial community structure observed. If so it is possible that other landscape features – such as large rocks and boulders – are influencing the microbial populations in the underlying soil (Tiao et al., 2012). This would provide strong evidence that contemporary environmental conditions are having a significant effect on microbial communities.

To do this, a controlled, *in situ* experiment was designed to replicate the abiotic effects of the seal carcass (stabilise temperature, reduce UV exposure, and increase humidity in the underlying soil). Plastic polyethylene trays were set up on undisturbed regions of Antarctic Miers Valley soil, and soil samples and physicochemical measurements were taken every summer over a five-year period. Ion Torrent sequencing of 16S rRNA gene amplicons was used to assess changes in the microbial community. Experimental set up and sample collection was carried out by Dr Charles Lee (University of Waikato), and Ion Torrent sequencing was performed by John Longmore (University of Waikato).

The objectives of the study were to assess how (and to what extent) the trays influence abiotic factors in the soil (temperature and RH); and determine how/

whether alpha diversity and microbial community composition were affected by these abiotic factors over the five-year period. The results of the study are outlined in the second chapter of this thesis.

Chapter Two

Moisture Associated Microbial Communities in Antarctic Dry Valley Soils

2 Introduction

Climate change is having a dramatic impact on the natural environment, causing more frequent extreme weather events, increased global temperatures, and rising sea levels (NASA, 2015; The Royal Society, 2010). With limited vegetation and few large terrestrial organisms, Antarctica offers a unique opportunity to examine the impact of abiotic, climatic factors on microbial ecosystems (free of many of the confounding biological variables found in more complex systems). This knowledge can then be extended to more complex systems and used to develop models that predict how Antarctica, and ultimately the rest of the world, may respond to the changing environmental conditions forecasted under the current rate of continental warming (Hogg et al., 2006). Since microbial communities lie at the base of all food chains and have fundamental roles in biogeochemical processes (i.e. nitrogen cycling), subtle changes in microbial populations can have a massive cascading effect up to higher trophic levels (Terborgh & Estes, 2013).

The McMurdo Dry Valleys (the largest ice-free region on Antarctica) are among the most hostile environments on the planet, with high levels of UV radiation, extremely low average annual temperatures ranging from −15 to −30 °C; and low average precipitation of just 10-100 mm per year (Bockheim & McLeod, 2008; Levy, 2013). Although the Dry Valley environment is extremely harsh and not conducive to life, there are still some edaphic microorganisms capable of carrying out metabolic activity. Several studies have demonstrated that this metabolic activity is extremely sensitive to environmental variables such as water and nutrient availability (Barrett, Virginia, Wall, & Adams, 2008; Hopkins, Sparrow, Elberling, et al., 2006; Tiao et al., 2012).

In the Dry Valleys water availability varies on both a spatial and a temporal scale, and is thought to be one of the main factors determining microbial community structure and diversity (Cary et al., 2010; Kennedy, 1993; Zeglin et al., 2009). Soil in the active zone (a layer which undergoes seasonal freeze-thaw cycles (FTC)) receives moisture from irregular precipitation events, seasonal glacier/ lake water melt, and from the underlying permafrost via an upwards entrainment (driven by a high relative humidity (RH) gradient between the surface soil and deeper down) (Barrett et al., 2007; Bockheim, 2002; Stomeo et al., 2012). Antarctic warming is predicted to increase nutrient and water availability, stimulate the growth of vegetation, and reduce ice cover (Fowbert & Smith, 1994). Given the sensitivity of microorganisms to these factors, these changes are likely to alter the activity, diversity and community structure of edaphic Dry Valley microbial communities.

Contrary to the long-held belief that Antarctic microbial ecosystems were homogenous and controlled by legacy carbon inputs (with changes in microbial community structure occurring over centuries or even millennia) (Barrett et al., 2006; Moorhead et al., 1999), recent molecular and *in situ* studies have shown that Antarctic Dry Valley soils are spatially heterogeneous (Bockheim, 2002; Lee et al., 2012), and that edaphic microbial communities may respond to environmental changes within far shorter time frames than originally believed (Tiao et al., 2012; Yergeau et al., 2012).

In a unique, multi-year *in situ* experiment Tiao et al. (2012) investigated the rate at which Dry Valley microbial communities responded to a new set of environmental conditions, induced by a seal carcass. Mummified seal remains are distributed sporadically throughout the Dry Valleys and act as protective micro-niches, stabilising RH and temperature, and reducing UV and wind exposure. In the experiment a mummified seal carcass (dated at 250 years) was shifted from its original location in Miers Valley to a nearby, geomorphically similar site; soil samples were taken every year over a five year period; and changes in the underlying microbial community were monitored via 454-Pyrosequencing (Tiao et al., 2012).

Remarkably, increased microbial biomass, decreased biodiversity and shifts in the microbial community composition were observed within just two summers (Tiao

et al., 2012). The seal carcass altered the underlying soil's nitrogen and organic carbon content, pH, and conductivity; yet surprisingly statistical analysis revealed that none of these physicochemical changes satisfactorily explained the changes in microbial community structure observed. Instead, it was determined that the changes observed were likely caused by physical, abiotic factors induced by the seal carcass (i.e. increased and more stable RH, reduced UV exposure, and more stable temperature) (Tiao et al., 2012). With an object (such as a seal carcass) on top of the soil, sublimation of the underlying permafrost would be significantly reduced, and RH beneath the object would increase. This would generate a new set of environmental conditions which select for different microbial species (Cary et al., 2010; Tiao et al., 2012). However, due to the un-replicated, observational nature of the study, this is merely speculation.

The aim of the current study was to determine whether changes in RH and temperature (caused by overlying objects) are driving the changes in microbial community structure observed previously (Tiao et al., 2012). If so it is possible that other naturally occurring landscape features (i.e. large rocks) are also influencing microbial populations in Dry Valley soil, and would offer strong evidence that present-day environmental conditions are having a significant effect on microbial communities. To do this an *in situ* experiment was designed to emulate the seal experiment (stabilise temperature, reduce UV exposure, and increase relative humidity in the underlying soil). However, it would appear that the experiment was unknowingly deployed in a subsurface water track (or in a low-lying area where moisture accumulated), and consequently the effect of the experiment on RH and temperature was negligible. Interestingly however, the moist tray treatment soil had a greater relative abundance of Cyanobacteria/ Chloroplasts, Proteobacteria, and Bacteroidetes than the drier seal control site, in keeping with the observations of previous studies investigating microbial community composition within wet environments. These findings hint at the possibility of using microbial community composition as a bio-indicator of hidden water tracks.

2.1 Methods

2.1.1 Experimental design

A controlled, *in situ* experiment was designed to simulate the placement of the seal on the soil surface (stabilise temperature, reduce UV exposure, and increase relative humidity in the underlying soil). To do this, overturned or upright black and translucent plastic trays were set up on undisturbed areas of Antarctic Dry Valley soil (78.09343° S, 163.83001° E) close to Lake Miers (Figure 2.1 and Figure 2.2).

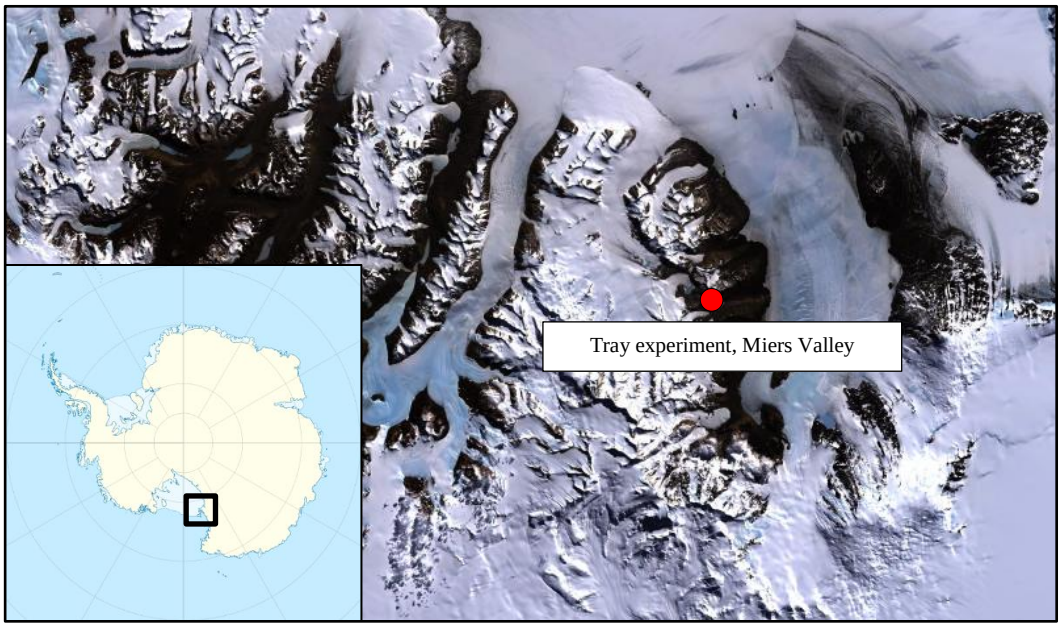


Figure 2.1. Location of the tray experiment in Miers Valley, Antarctica

(Image by Robert Simmon courtesy of NASA; Antarctic map from Wikipedia (Wikipedia, 2010))

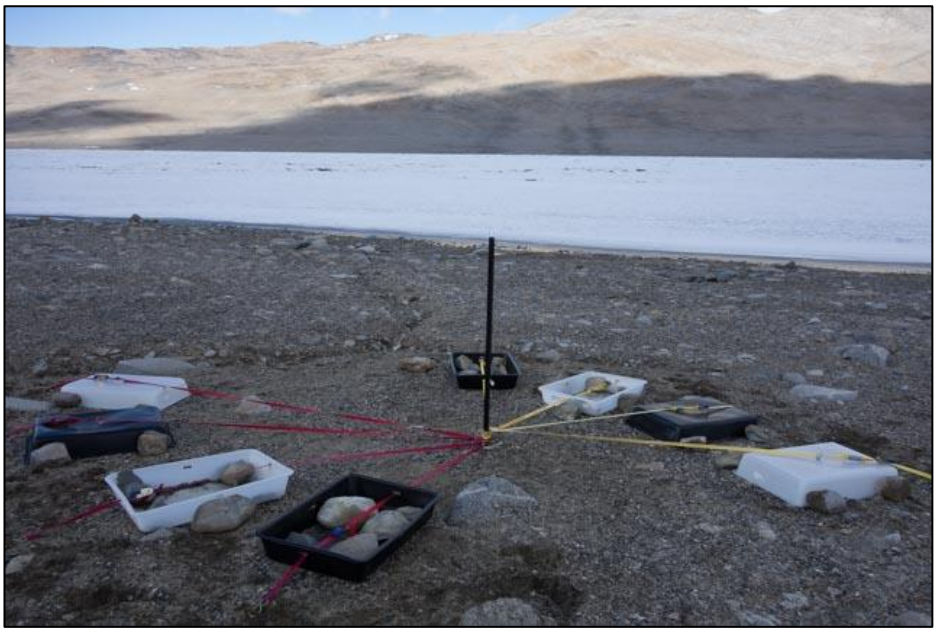


Figure 2.2. Photo of the tray treatment experimental set up
(Image by Dr. Charles Lee)

2.1.2 Hypothesised experimental effects

All of the tray treatments were hypothesised to reduce daily temperature fluctuations and provide protection from the dehydrating wind. Two main parameters were investigated: light and RH. Black and translucent trays were used to investigate the effect of light on microbial communities; black trays blocked UV radiation from reaching the underlying soil, while translucent trays allowed some light to pass through. Tray orientation (upright or overturned) was designed to investigate RH. The bases of the upright trays were in direct contact with the soil, which was anticipated to prevent sublimation and stabilise and increase RH in the underlying soil. The overturned trays were expected to form a protective dome over the soil, sheltering the underlying soil from the harsh Antarctic winds without greatly altering RH. From these two factors, four combinations were established in duplicate: upright black tray (BU); overturned black tray (BO), upright translucent tray (TU); and overturned translucent tray (TO) (Table **2.1**).

The BU trays were designed to exactly mimic the effects of the seal carcass (reduce UV exposure, stabilise yearly temperature, and stabilise and increase RH). The TU tray treatment was designed to have the same effect on temperature and RH, but also allowed light to pass through and reach the underlying soil. The BO

dome trays were expected to protect the underlying soil from the harsh environmental conditions, reduce UV exposure, and stabilise temperature. The TO dome trays were anticipated to act as mini greenhouses, protecting the underlying soil from the harsh environmental conditions, allowing light to pass through, increasing temperature, and reducing RH.

Table 2.1. Predicted effects of the tray treatments

	Upright	Overturned
Black Tray	• More stable temperature • Wind protection • All UV radiation blocked • Increased RH	• More stable temperature • Wind protection • All UV radiation blocked
Translucent Tray	• More stable temperature • Wind protection • Some light allowed to pass through • Increased RH	• More stable temperature • Wind protection • Some light allowed to pass through • Reduced RH • Increased temperature

All trays
Exclusive to translucent trays
Exclusive to TO trays
Exclusive to black trays
Exclusive to upright trays

2.1.3 Sampling

Black and translucent plastic trays were set up on an untouched area (78.09343° S, 163.83001° E) close to Lake Miers, and soil samples were taken every January over a five-year period (from 2012-2016). The trays were weighed down with large rocks, and secured in place with tent pegs and strops extending from a central waratah (Figure **2.3**). Soil samples (<10 g) were collected aseptically from three to five randomly selected locations beneath each tray at a depth of 0-5 cm (the experimental region was ~4-5 m in diameter). Two control samples were collected, one from inside the experimental set up area, and one from outside the experimental set up (to control for snow accumulation within the experiment). Samples were stored in sterile plastic tubes and kept at -80 ^{o}C prior to analysis.

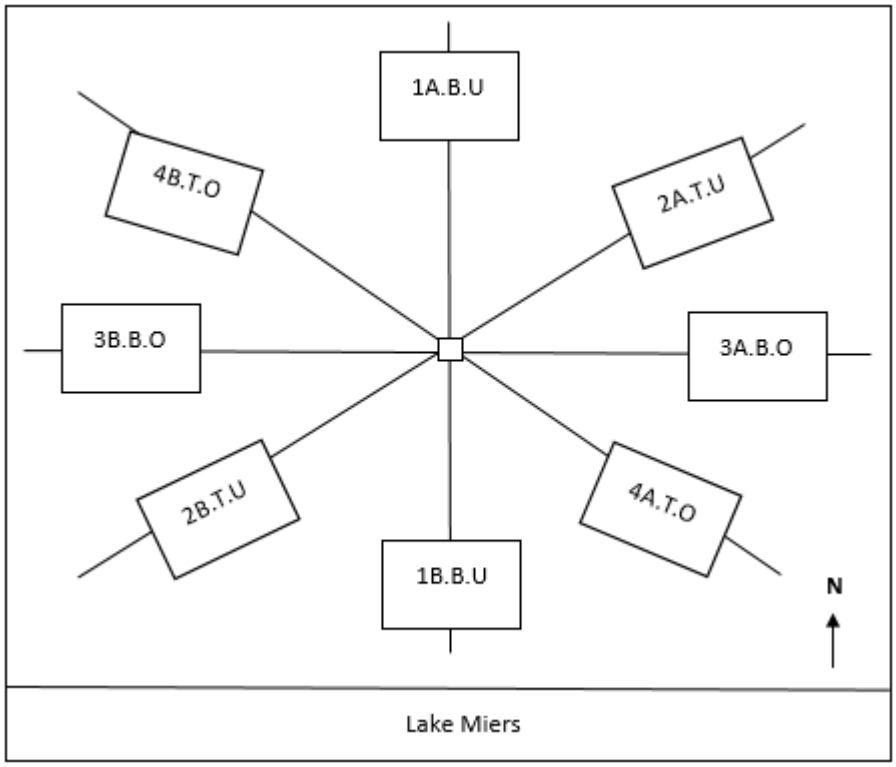


Figure 2.3. Tray treatment experiment layout

2.1.4 Relative humidity and temperature readings

RH and temperature readings were taken every four hours over a two year period (from January 2012 to November 2013) using iButton relative humidity and temperature loggers (model DS1923; Maxim Integrated Products, Sunnyvale, CA, USA). These were placed beneath the trays and were in direct contact with the soil.

2.1.5 Soil pH and conductivity analysis

Soil pH and conductivity readings were taken via a slurry method (Lee et al., 2012) on a Thermo Scientific Orion 4-Star Plus pH/Conductivity Meter (Thermo Scientific, Auckland, NZ). Soil (3 g) was aseptically transferred to a sterile Falcon tube, and MilliQ water (15 mL) was added. The tubes were vortexed for 10 s, and inverted 20 times to mix. The tubes were then left for half an hour to allow the soil to settle prior to reading.

2.1.6 DNA extraction

Soil (0.7 g) was aseptically placed in a sterile 1.5 mL tube containing 0.1 mm (0.5 g) and 2.5 mm (0.5 g) silica-zirconia beads, and phosphate buffered saline (PBS) (100 mM NaH_2PO_4, 270 µL) and sodium dodecyl sulfate (SDS) lysis buffer (100 mM NaCl, 500 mM Tris pH 8.0, 10% SDS; 270 µL) were added to each tube. The samples were bead beaten for 15 s, and shaken on a vortex genie for 10 min to lyse the cells. The samples were centrifuged at 13,200 rpm for 30 s, 180 µL cetyltrimethylammonium bromide (CTAB) extraction buffer (100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2% CTAB, 1% PVP, 0.4% β-mercaptoethanol (BME)) was added, and the tubes were vortexed for 10 s. The samples were then incubated at 60 °C at 300 rpm for 30 min, and centrifuged for 30 s at 13,200 rpm. Chloroform:isoamyl alcohol (24:1, 350 µL) was added, and each tube was vortexed for 15 s. The samples were then centrifuged at 13,200 rpm for 5 min, and the upper aqueous layer was transferred to a sterile 1.5 mL Eppendorf tube. A second 500 µL of chloroform:isoamyl alcohol (24:1) was added to each sterile Eppendorf tube, the samples were vortexed for 15 s, and then placed on a rocking bed for 20 min. The tubes were centrifuged for 5 min at 13,200 rpm, the upper aqueous layer was transferred to another sterile 1.5 mL Eppendorf tube, and the volume was recorded. Ammonium acetate (10 M) was added to achieve a final concentration of 2.5 M, and the samples were vortexed for 10 s. The samples were then centrifuged for 5 min at 13,200 rpm, the upper aqueous layer was transferred to a sterile 1.5 mL Eppendorf tube, and the volume was recorded. Isopropyl alcohol (to a volume 0.54 times of that recorded in the previous transfer step) was added to each tube, the tubes were mixed via repeated inversion (20 times), and stored overnight at -20 °C to precipitate the DNA.

The samples were centrifuged at 13,200 rpm at 4 ^{o}C for 20 min, and the supernatant carefully removed and discarded via pipette (taking care not to disrupt the pellet). The pellet was washed with cold 70% analytical-reagent grade ethanol (1 mL), and centrifuged for 1 minute at 13,200 rpm. The ethanol was removed and discarded via pipette, and the residual ethanol dried from the tubes using a Speedivac drying vacuum set on medium heat (~15 min). Sterile LO-TE (20 µL) was used to re-suspend the DNA, and the DNA was quantified using a Qubit

dsDNA HS assay kit and Qubit 2.0 Fluorometer (Life Technologies, Auckland). The samples were then stored at -80 °C until ready for PCR.

2.1.7 PCR

To identify bacterial tax within the soil samples, the V4 region of the 16S rRNA gene was amplified. PCR was performed on each sample in duplicate using the Ion Torrent primer set 515F (5'CCATCTCATCCCTGCGTGTCTCCGACTCAG-unique IonXpress barcode-GATGTGCCAGCMGCCGCGGTAA-3') and 806R (5'CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATGGACTA CHVGGGTWTCTAAT-3') (Caporaso et al., 2011). Each PCR reaction contained bovine serum albumin (BSA) (0.4 mg/mL, 1 µL), dNTPs (Roche Diagnostics, NZ) (2 mM, 3 µL), 10X PCR Buffer (200 mM Tris-HCl, 3 µL, pH 8.4), KCl (500 mM, 3 µL), MgCl2 (50 mM, 3 µL), forward and reverse primer (10 mM, 0.5 µL each), 5 U/µL Platinum Taq polymerase (Life Technologies) (0.12 µL), 1 ng of DNA (2 µL total), and the reaction was made up to 25 µL with sterile MilliQ water (11.88 µL). The PCR master mix was treated with ethidium monoazide (EMA) to inhibit any DNA contamination prior to the addition of DNA template (Rueckert & Morgan, 2007).

PCR was run on the Ion Torrent (ITags) program. The thermal cycling conditions were: 94 °C for 3 min, then 30 cycles of 94 °C for 45 s, 50 °C for 1 minute, 72°C for 90 s, and finally 72 °C for 10 min. After PCR the duplicate reactions were pooled and run on an agarose gel (stained with 1% SYBR safe) with a 1 Kb ladder (15 µL) at 75 V for 40 min. The gel was visualised using and AlphaImager to verify that the PCR had worked correctly. PCR products were stored at -80 °C until ready for clean-up.

Ion-Torrent PCR products were cleaned up and the concentration normalised using the SequalPrep™ Normalization Plate Kit following the manufacturer's protocols (Life Technologies, Auckland). This kit facilitates high throughput normalization and purification of amplicons in a one-step process, without the need for quantification. The SequalPrep™ Normalization Plate Kit employs ChargeSwitchR Technology. In a low pH buffer, the negatively charged DNA backbone is bound to the ChargeSwitchR plate coating, and proteins and other contaminants are washed away. PCR products (25 µL) were added to separate wells of the SequalPrep™ Normalization Plate, mixed with an equal volume of

the Binding Buffer, and left to incubate at room temperature for 1 h. The Binding Buffer was removed via pipette, and the wells were then washed with Wash Buffer (50 µL) to remove contaminants. The Wash Buffer was then removed, and Elution Buffer (20 µL) was added to each well to purify, normalise and elute the PCR products. The PCR products were then stored at -20°C until ready for sequencing.

2.1.8 Sequencing

Sequencing was performed on the Ion Torrent Personal Genome Machine (PGM) (ThermoFisher Scientific) at the University of Waikato DNA sequencing facility. Reads from Ion Torrent sequencing ranged from 275 to 308 base pairs, and were quality filtered and trimmed using Mothur software (Schloss et al., 2009). Sequences which contained 6 or more consecutive homopolymers were removed from the data set (using the 'maxhomop=6' command). The primer sequences and barcodes were removed, sequences with estimated error rates >1% were excluded, and the reads were cut down to 250 bp. The USEARCH UPARSE-OTU algorithm (using the 'cluster_otus' command) (UPARSE 9) was used to generate OTUs (clustered to ≥97% pair-wise identity) from the 16S rRNA gene sequences, and to filter chimeras (Edgar, 2013). A second round of chimera filtering was carried out using the uchime2_ref command in UPARSE 9 (against a SILVA_128_SSURef_Nr99_tax_silva_trunc.fasta reference file) (Quast et al., 2013).

2.1.9 Data analysis

2.1.9.1 QIIME

The Ribosomal Database Project (RDP) classifier was used to generate a FASTA file with taxonomic information for each OTU, and this taxonomic information was then loaded into the Biom file. Sequences were aligned in QIIME, using RDP classifier sequences as an input file. The alignments were then filtered in QIIME to remove any regions with gaps in each sequence, and a phylogenetic reference tree was generated. Relative abundance bar graphs were created at the phylum, class, order, family, genus, and species level. A 'Fast Map' file was created that contained pertinent metadata. Different manipulations were conducted to isolate samples and carry out year-by year analyses.

2.1.9.2 'R'

The R 'Phyloseq' package (designed to analyse/ visualise microbial community phylogenetic sequencing data) was used to calculate and display alpha and beta diversity (McMurdie & Holmes, 2013). Variance stabilisation was carried out using DESeq2.

Non-metric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA) plots were created in R. NMDS and PCoA are ordination techniques used to visualise the level of similarity between groups in a data set. In NMDS, the more similar two samples, the closer they are in the NMDS plot (Kenkel & Orloci, 1986). PCoA involves the generation of a distance matrix, which is then used to assign each data point a position that minimises strain. The PCoA plot includes axes which represent the amount of variability captured within the data set (the higher the percentage, the more variation is explained by the axis) (Kenkel & Orloci, 1986).

2.1.9.3 Statistica

Conductivity, pH, RH and microbial relative abundance data (recorded in separate Microsoft Excel spreadsheets) were imported into Statistica (Statsoft Inc.), and descriptive statistics were calculated where appropriate (mean, standard deviation). One-way ANOVA analyses were carried out on parametric data, and a Kruskal-Wallis ANOVA was performed for non-parametric data. Newman-Keuls and Duncan post-hoc tests were conducted when ANOVA analyses produced statistically significant p values (< 0.05) to determine which means were different.

2.2 Results

2.2.1 Temperature

Average temperature over the two year measuring period was greatest for the translucent overturned (TO) treatment (-18.58 and -18.21 ºC for 4A.TO and 4B.TO respectively), and very similar for the other tray treatments and the control (Table 2.2 and Figure 2.4). A one-way ANOVA conducted on the average temperature between plots revealed statistically significant differences ($p < 0.05$) between plots. To determine which means were different, Newman-Keuls and Duncan post-hoc tests were conducted. Both tests revealed that there were statistically significant ($p < 0.05$) differences between the TO tray treatments and all of the other control/ tray treatments. There were no statistically significant differences between any of the replicates.

The temperature and RH readings from the southern-positioned black upright (1B.BU) replicate were not taken as the iButton reader was dislodged post set up. RH and temperature readings were taken for the south-west positioned translucent upright (2B.TU) replicate, but were not included in the RH or temperature analysis as sampling dates did not start on the same day for this sample as for the other treatments (and thus an accurate day-by-day comparison with the control sample could not be carried out).

Table 2.2. Average temperature for each plot in the tray experiment

Plot	Temp (ºC)	Temp Standard Deviation
1A.BU	-19.78	10.83
2A.TU	-19.96	10.90
3A.BO	-20.02	12.37
3B.BO	-19.25	12.80
4A.TO	-18.58	15.22
4B.TO	-18.21	14.41
Control Inside	-19.83	12.36
Control Outside	-20.06	11.99

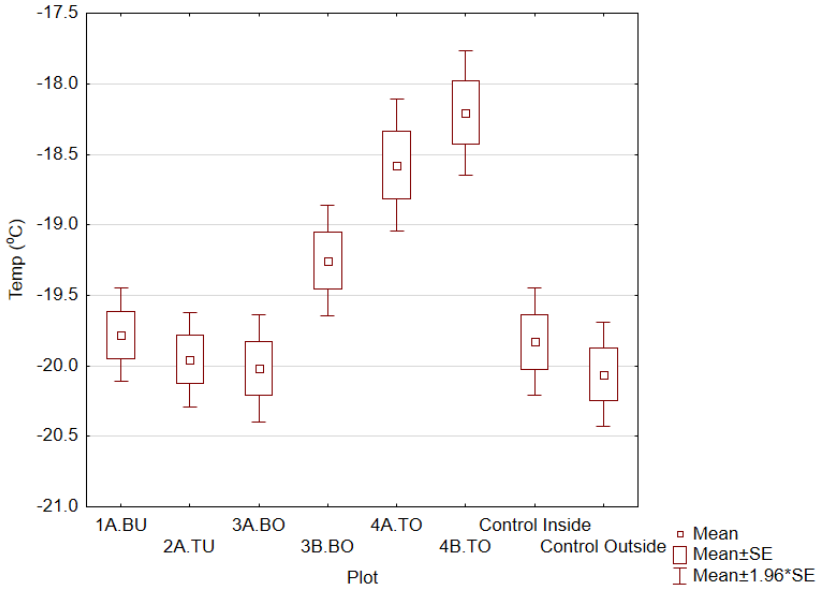


Figure 2.4. Average temperature for each plot in the tray experiment (2012-2013)

2.2.1.1 January temperature

Temperature readings from the tray treatments and outside and inside control were then compared for the month of January. Summer was the season of interest in the experiment as it is this season (when temperatures can exceed zero) that microbial activity is the greatest (Niederberger et al., 2015). The tray treatments may have modified the temperature during the winter period, but if the temperature was below zero then limited microbial activity would be occurring regardless of whether the temperature was elevated/ stabilised (Niederberger et al., 2015). January was selected for comparison as this was the month in which the yearly soil sampling was conducted.

The black upright (BU), translucent upright (TU), and black overturned (BO) trays stabilised, but did not increase daily temperature in January 2012 relative to the control (Figure 2.5; and Figure 3.1, Figure 3.2, and Figure 3.3 in Appendix 1). This temperature stabilisation was most prominent in the BU trays, and least prominent in the BO trays. In contrast to the three other tray treatments, the translucent overturned (TO) trays increased (but did not stabilise) daily temperature readings in January (Figure 2.6; and Figure 3.4 in Appendix 1). The same trends were seen in January 2013 (Figure 3.5, Figure 3.6, Figure 3.7, Figure 3.8, Figure 3.9, and Figure 3.10 in Appendix 1).

The BU and TU trays decreased the number of days (hourly recordings) above zero degrees during the summer months (January 2012 to March 2012), relative to the control (with 73 hourly readings above zero for the BU treatment; 99 for the TU treatment; and 112 readings for the outside control). The BO and TO trays on the other hand increased the number of days (hourly recordings) above zero degrees during the summer months (January 2012 to March 2012) (with an average of 133 hourly readings above zero for the BO treatments, and 189 readings for the TO treatments).

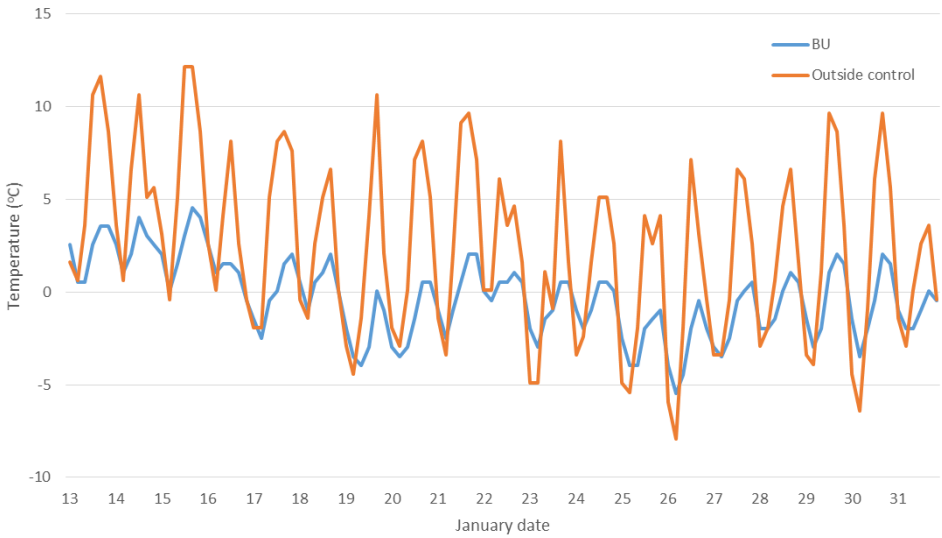


Figure 2.5. Temperature readings for the 1A.BU tray treatment (January 2012)

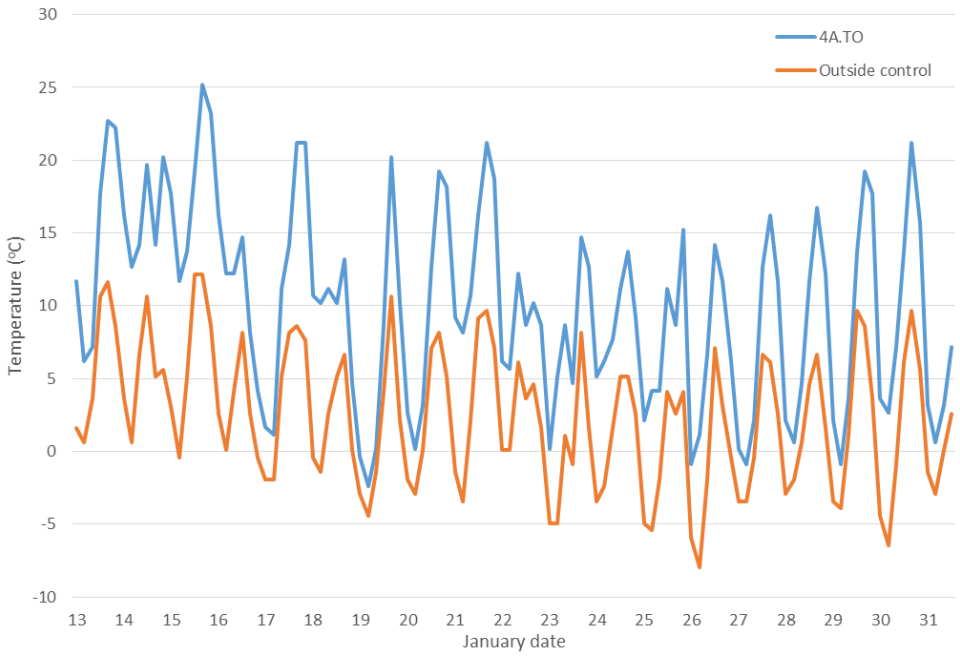


Figure 2.6. Temperature readings for the 4A.TO tray treatment (January 2012)

2.2.2 Relative humidity

Contrary to the hypothesised experimental effects, average RH over the two year measuring period was lowest for the TO treatments (at 82.65% and 87.88% for 4A.TO and 4B.TO respectively), and highest for the inside and outside control (where it was completely saturated) (Table 2.3 and Figure 2.7). A one-way ANOVA was carried out on the RH between plots and revealed that there were statistically significant differences ($p < 0.05$) between plots. Newman-Keuls and Duncan post-hoc tests found average RH differences to be statistically significant ($p < 0.05$) between all but 3B.BO and 1A.BU treatments, and the outside and inside controls.

The RH readings for the BU treatment were lower than for the control from April to December in 2012, and from April to November in 2013. RH readings for the January to March (summer) period were similar for the BU and control treatments (Figure 2.8). The same trends were seen for the TU and BO treatments (Figure 3.11, Figure 3.12, and Figure 3.13 in Appendix 1). The RH readings for the TO treatment were lower than for the control across the entire two year period (Figure 2.9; and Figure 3.14 in Appendix 1).

Table 2.3. Average relative humidity by plot in the tray experiment

Plot	RH (%)	RH Standard Deviation
1A.BU	92.32	10.408
2A.TU	96.66	11.938
3A.B.O	93.64	12.124
3B.B.O	92.52	11.004
4A.T.O	82.65	15.139
4B.T.O.	87.88	17.806
Control Inside	100.71	10.228
Control Outside	100.41	5.155

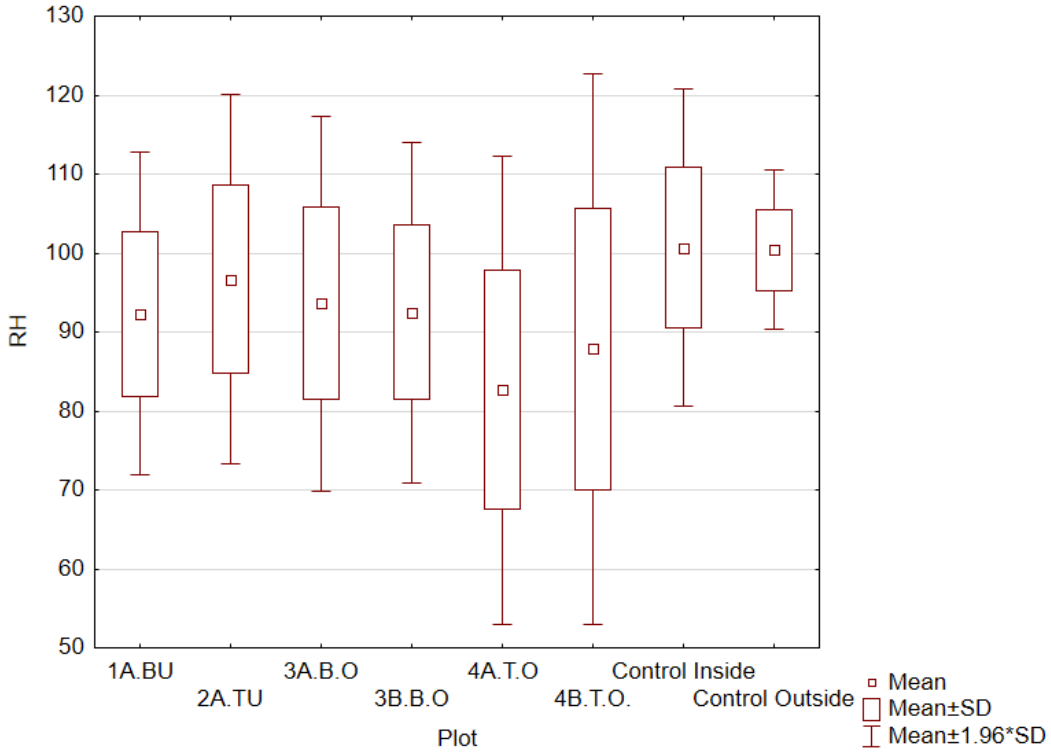


Figure 2.7. Box and whisker graph of RH in plots in the tray experiment (2012-2013)

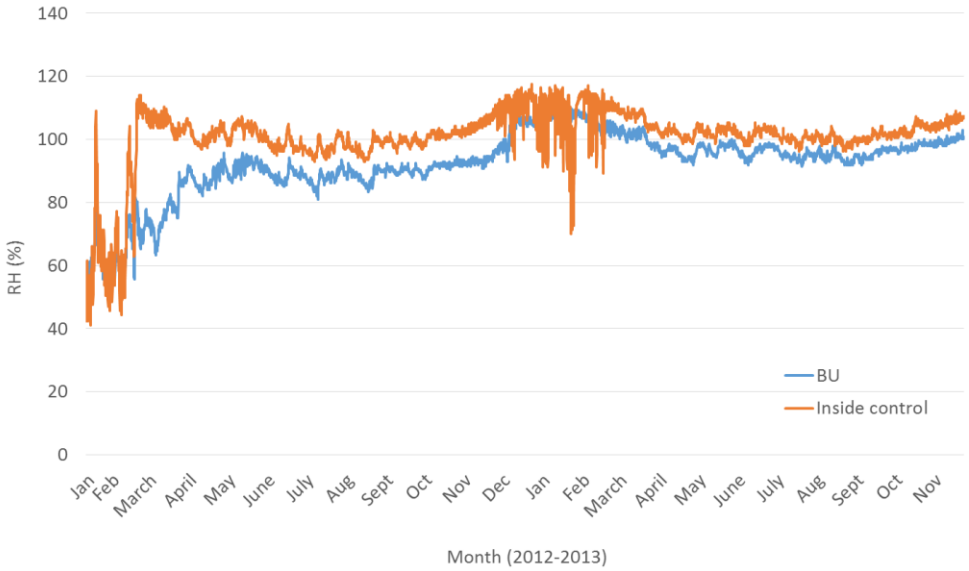


Figure 2.8. RH for the BU tray treatment (2012-2013)

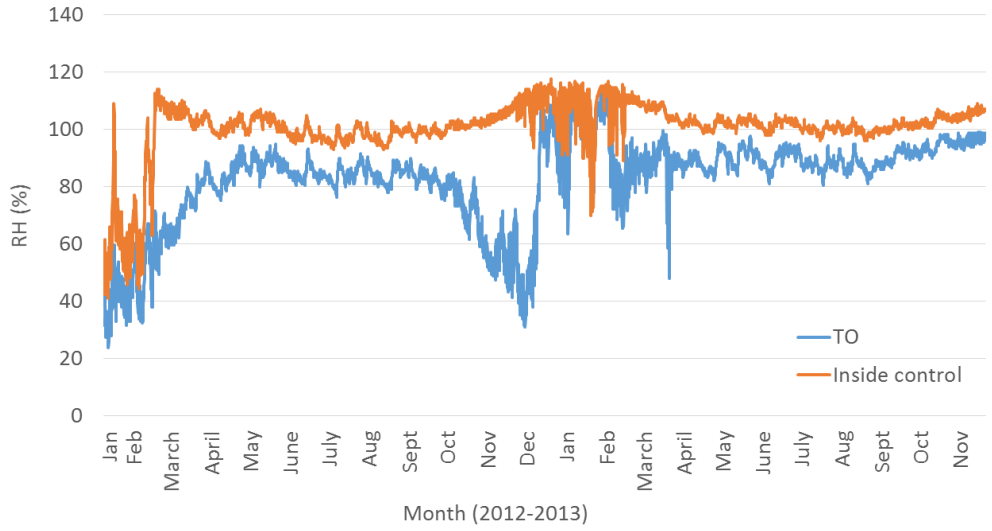


Figure 2.9. RH for the 4A.TO tray treatment (2012-2013)

2.2.2.1 January RH

The RH readings during the month of January were then examined in isolation. January RH (in 2012) was greatest in the outside control (90.03%), and lowest in the TO treatments (40.71% and 34.84% for 4A.TO and 4B.TO respectively) (Table 2.4). A one-way ANOVA of the average January 2012 RH values revealed statistically significant ($p < 0.05$) differences in RH between plots. Newman-Keuls and Duncan post-hoc tests showed that there were statistically significant

differences between average January 2012 RH values for all but the inside control and BU, and 3B.BO and 2A.TU treatments. The BU, TU and BO tray treatments stabilised (but did not increase) RH during the month of January (relative to the control), while the TO tray treatment decreased RH (Figure 2.10 and Figure 2.11; and Figure 3.15, Figure 3.16, Figure 3.17, and Figure 3.18 in Appendix 1). The same trends were seen in January 2013 (Figure 3.19, Figure 3.20, Figure 3.21, Figure 3.22, Figure 3.23, and Figure 3.24 in Appendix 1).

Table 2.4. Average RH by plot in the tray experiment (January 2012)

Plot	RH (%)	RH Standard Deviation
1A.BU	61.48	6.13
2A.TU	58.23	7.45
3A.B.O	51.99	5.91
3B.B.O	8.27	6.16
4A.T.O	40.71	7.3
4B.T.O.	34.84	8.40
Control Inside	62.55	13.66
Control Outside	90.03	10.93

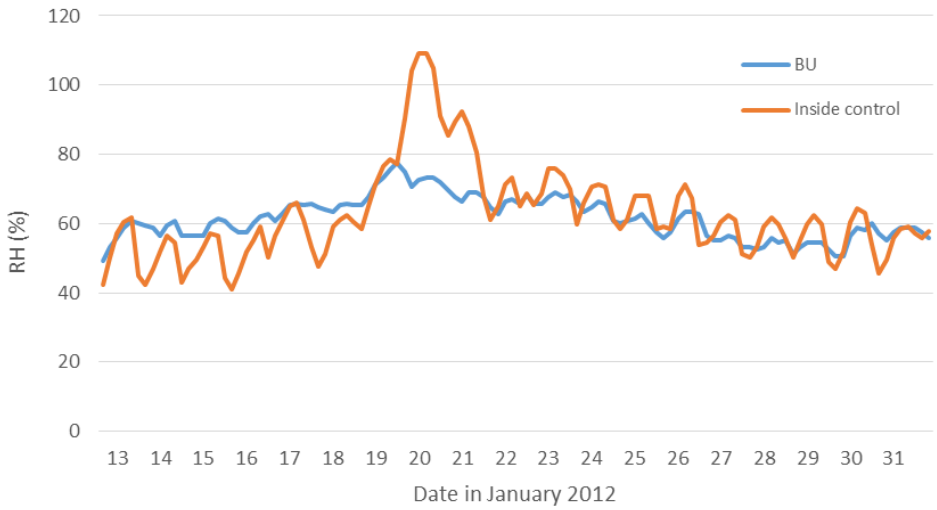


Figure 2.10. RH for the BU tray treatment in January 2012

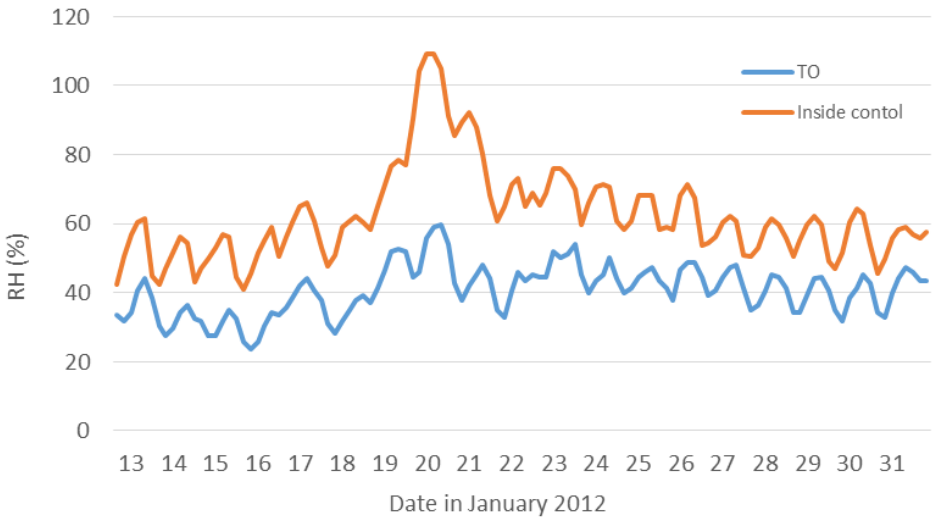


Figure 2.11. RH for the 4A.TO tray treatment in January 2012

2.2.3 pH

pH was very similar for all of the treatments, but was greatest for the control treatment at 9.59, and lowest for the TO treatment at 9.40 (Table 2.5). A one-way ANOVA on the pH between years and treatments was conducted, and the resulting p value was 0.10 (> 0.05). Thus the null hypothesis that there was no statistically significant differences in pH between years and treatments could not be rejected. However, there were statistically significant differences in pH between plots (i.e. 1A.BU, 1B.BU, 2A.BO, 2B.BO etc.).

Table 2.5. pH values for tray treatments

Treatment	pH	Standard deviation
BU	9.56	0.27
TU	9.58	0.10
BO	9.45	0.13
TO	9.40	0.14
Control	9.59	0.15

2.2.4 Conductivity

Conductivity was greatest in the TO treatment (at 266.16 μS/cm) and lowest in the TU treatment (at 143.41 μS/cm). Standard deviation values were quite large for each of the treatments, indicating significant within-treatment variation (Table 2.6). A Kruskal-Wallis ANOVA revealed that there were no statistically significant differences ($p > 0.05$) in conductivity between years or treatments. However, there were statistically significant differences ($p < 0.05$) in conductivity values between the plots.

Table 2.6. Conductivity values for tray treatments

Treatment	Conductivity (μS/cm)	Standard deviation
BU	154.52	63.88
TU	143.41	63.57
BO	210.62	101.62
TO	266.16	162.65
Control	170.14	71.75

2.2.5 Microbial community rarefaction curves

DNA sequence rarefaction curves were generated for each sample using Chao 1 and the Shannon index. In all cases, these curves plateaued, indicating that adequate sampling depth had been achieved for all samples (Figure 3.25 and Figure 3.26 in Appendix 2).

2.2.6 Alpha and beta diversity

Average alpha diversity was greatest in the TU tray treatments (with 807 observed OTUs, and a Chao1 value of 992.76), and lowest in the control soil (with 696 observed species, and a Chao1 value of 871.01) (Table 2.7, Table 2.8, and Figure 2.12). However, the differences in alpha diversity between treatments and plots were not statistically significant ($p > 0.05$). There were, however, statistically significant differences in the number of observed OTUs and Chao1 values between years ($p < 0.05$) (Figure 2.13). Newman-Keuls and Duncan post-hoc tests revealed that these statistically significant differences were between 2014 and years 2012, 2013, and 2015 for the number of observed OTUs; and between 2014 and 2015 for the Chao1 values. To identify trends in microbial diversity free of year-to-year variation, each year was then examined individually (Figure 3.27, Figure 3.28, Figure 3.29, Figure 3.30, and Figure 3.31 in Appendix 2). This year-by-year analysis revealed that alpha diversity varied quite substantially for each of the treatments across years, and no common trends could be identified from one year to the next.

Table 2.7. Chao1 estimates for each tray treatment

Treatment	Chao1 value
BU	899.04
TU	992.76
BO	969.67
TO	909.14
Control	871.01

Table 2.8. Average number of observed OTUs for each tray treatment

Treatment	Observed OTUs
BU	736.00
TU	807.70
BO	790.00
TO	747.80
Control	696.90

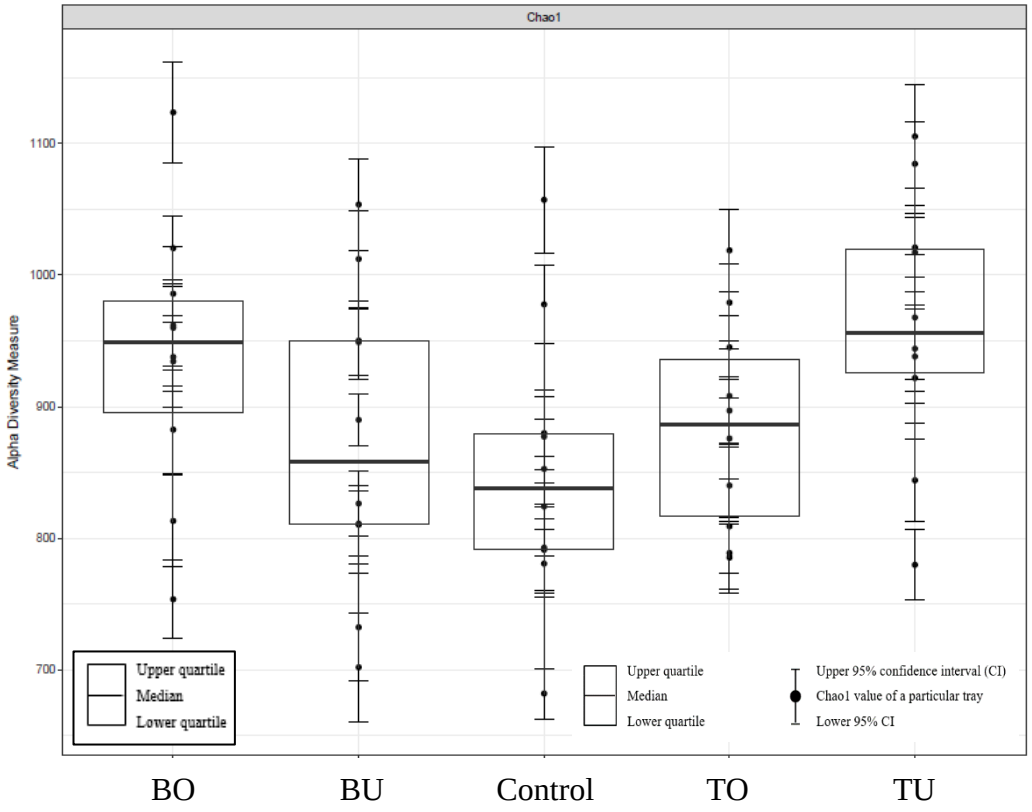


Figure 2.12. Alpha diversity across tray treatments (2012-2016)

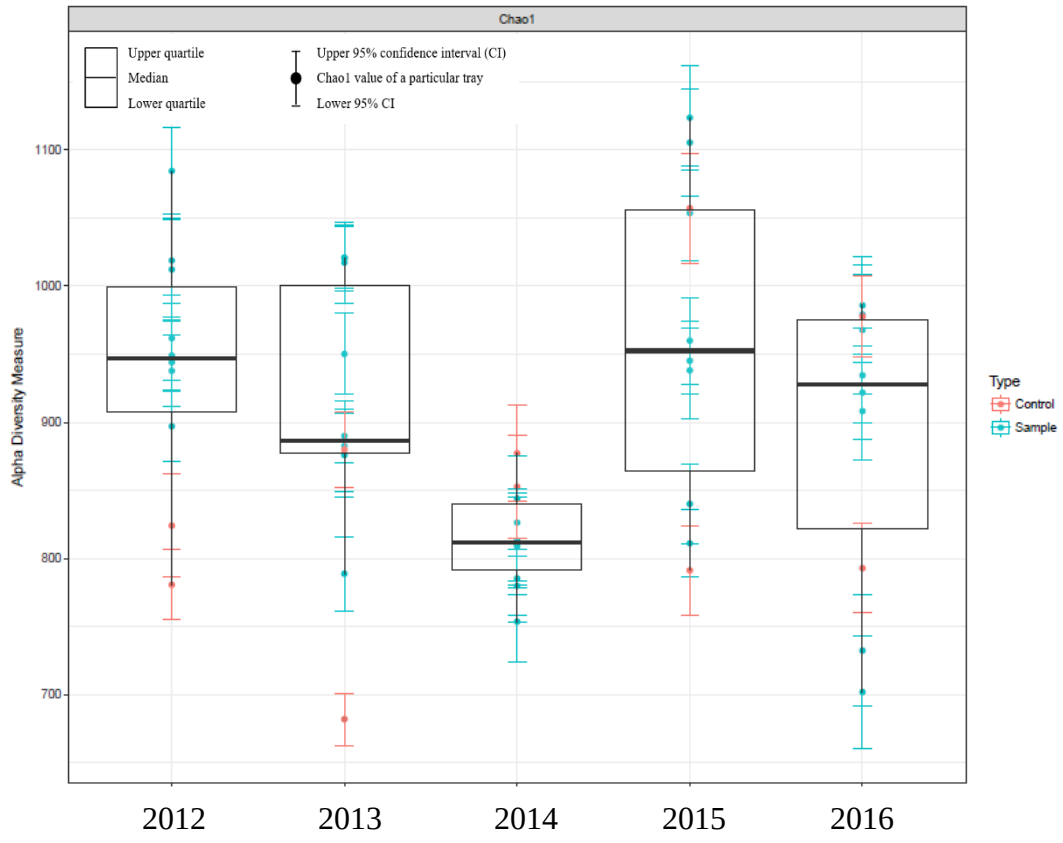


Figure 2.13. Alpha diversity across years in the tray experiment

2.2.7 Community composition

Relative abundances of the different phyla for each duplicate treatment **(**Figure 2.14**)** identified no major changes in community composition across the five year period for any of the treatments. Actinobacteria were at ~3% relative abundance across the control and tray treatments, and Proteobacteria were at ~15-17% relative abundance. Bacteroidetes were at ~19% relative abundance in the tray treatments and 17% abundant in the control. In the BU, TU, BO and control treatments Acidobacteria were at ~14% relative abundance, while in the TO treatment they were lower at 11%. Cyanobacteria/ Chloroplasts were at the greatest relative abundance (8.32%) in the TO treatment, and lowest in the black tray treatments (at 2.84% and 3.61% in the BU and BO trays respectively). Relative abundances of the TU and control treatment were similar at 5.33 and 4.79% respectively.

To determine whether these differences were statistically significant, a one-way ANOVA was conducted on the percentage abundance of the five most dominant phyla in each of the samples: Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Cyanobacteria/ Chloroplasts. There were no statistically significant differences in the relative abundances of Actinobacteria or Bacteroidetes between treatments, plots or years. There were no statistically significant differences in the relative abundances of Acidobacteria or Proteobacteria between treatments or plots, but there were statistically significant differences between years ($p < 0.01$). To determine which years were different, Newman-Keuls and Duncan post-hoc tests were conducted. Both tests revealed that there were statistically significant ($p < 0.05$) differences between samples from 2012 and all other years (2013, 2014, 2015 and 2016). For Proteobacteria there were also statistically significant differences between samples from 2014 and samples from 2015 and 2016.

One point of interest is that, unlike for other phyla, there were statistically significant differences in the relative abundances of Cyanobacteria/Chloroplasts between treatments and plots; but not between years. This suggests that it was the treatments themselves that were associated with the relative abundance differences, not year to year variation. Newman-Keuls and Duncan post-hoc tests were conducted, and both tests revealed statistically significant ($p < 0.05$)

differences between TO and the BO and BU treatments. Of all the tray treatments and control samples, the black tray treatments had the lowest Cyanobacteria/ Chloroplast abundance. There were statistically significant differences in Cyanobacteria/ Chloroplast relative abundances between 4A.TO and all of the other plots. However it should be noted that there were also statistically significant differences between the 4A and 4B.TO replicates, indicating significant within-treatment variability.

Community composition was examined at finer levels of taxonomic resolution (class, family, genus, and species level). As for phylum level, no major changes in community composition were seen across years for the BU, TU, or BO treatments. However, based on a visual inspection, the 2014 4A.TO tray treatment appeared to have a different microbial community composition than the other treatments (Figure 2.15; and Figure 3.32, Figure 3.33, Figure 3.34, and Figure 3.35 in Appendix 3).

One-way ANOVA analyses at the species level showed a statistically significant difference ($p < 0.05$) in the abundance of an unknown species from the *Bacillariophyta* family between treatments. Newman-Keuls and Duncan post-hoc tests revealed that these significant differences were between the TO trays and all other treatments. Post-hoc analysis revealed that there were also statistically significant differences between the 4A.TO plot and all other plots/ controls (including the 4B.TO replicate). There was also a statistically significant difference between plots and treatments in the abundance of a Chloroplast of an unknown family. Post-hoc tests revealed that this statistical difference was between plot 2A.TU and plots 1A.BU, 1B.BU, 3A.BO, 3B.BO and the outside and inside control; as well as between plot 4A.TO and plots 1A.BU, 1B.BU, 3A.BO, 3B.BO and the outside control.

Statistically significant differences were also found between 4A.TO and all other plots (including the 4B.TO replicate) for an unknown species of *Chlorophyta* and an unknown species of *Lysobacter* (γ-Proteobacteria), and between plots 4A.TO and 1B.BU and 2B.TU for the relative abundance an unknown species of *Actinomycetales* (Actinobacteria). However, there were no statistically significant differences between treatments for these species.

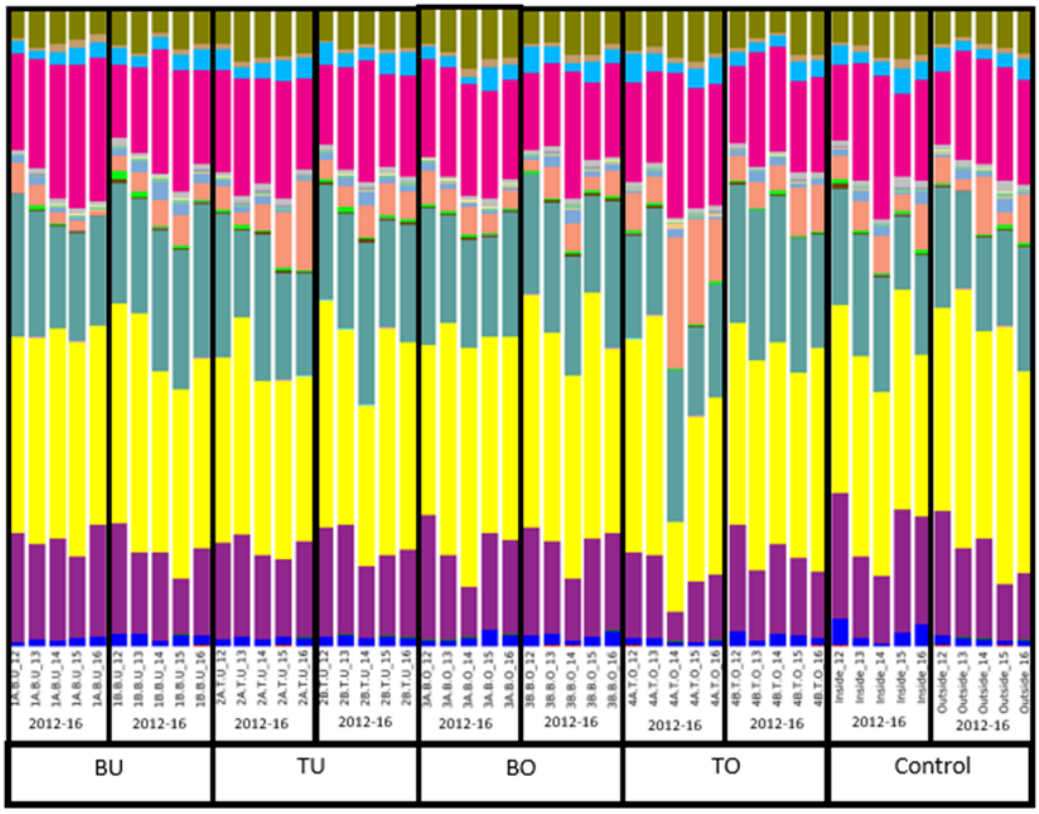
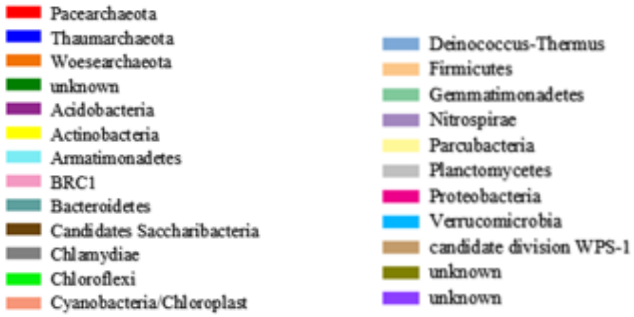


Figure 2.14. Relative abundance of different phyla in the tray experiment

Years 2012-2016 reading from left to right in each quadrant for each duplicate treatment.



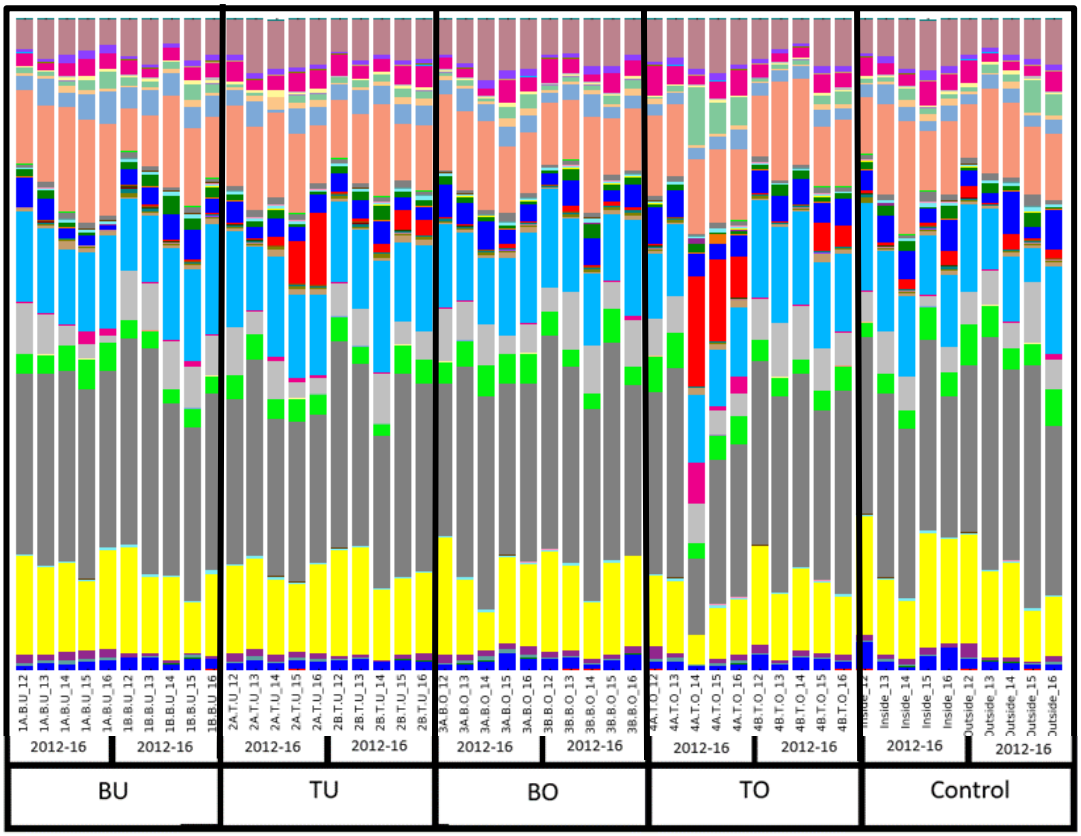


Figure 2.15. Relative abundance of different classes in the tray experiment

Years 2012-2016 reading from left to right in each quadrant for each duplicate treatment.

2.2.8 Beta diversity

NMDS plots were generated and overlaid with treatment, plot, colour, orientation or year. Little to no clustering patterns were observed in the NMDS plots (Figure 2.16), indicating a high degree of variation between treatments (i.e. samples within a treatment group were not more similar to one another than they were to samples in other treatment groups). Variance stabilisation was carried out using DESeq2, however, no clustering patterns were seen using either the transformed or non-transformed data, so the non-transformed data were selected

for display. A Principal Coordinates Analysis (PCoA) plot was also generated, and like for the NMDS plot, no clustering patterns were observed (Figure 3.36 in Appendix 4). Samples appear to group more strongly by year than by treatment in the NMDS plots, however these clustering patterns are still quite weak (Figure 2.17).

In light of the lack of clustering, pairwise comparisons were carried out on the BO and BU treatments to isolate the effect of RH (free of the additional light variable). However, clustering remained weak (Figure 3.37 and Figure 3.38 in Appendix 4), indicating that either RH was not influencing the microbial communities, or that the tray treatment was not able to sufficiently replicate the stabilised, increased RH experienced in the seal carcass experiment. The BU (seal carcass mimic) and TO ('greenhouse') tray treatments were then compared as these two treatments were opposite in terms of RH and light (the two experimental variables). However the NMDS and PCoA plots again displayed no clustering patterns (Figure 3.39 and Figure 3.40 in Appendix 4). Samples from the final year of the experiment (2016) were also examined individually to try and identify trends free of year-to-year variation. The final year was selected as it allowed time for changes in the microbial communities to become established. However, samples within a treatment again showed no clustering patterns (Figure 3.41 in Appendix 4).

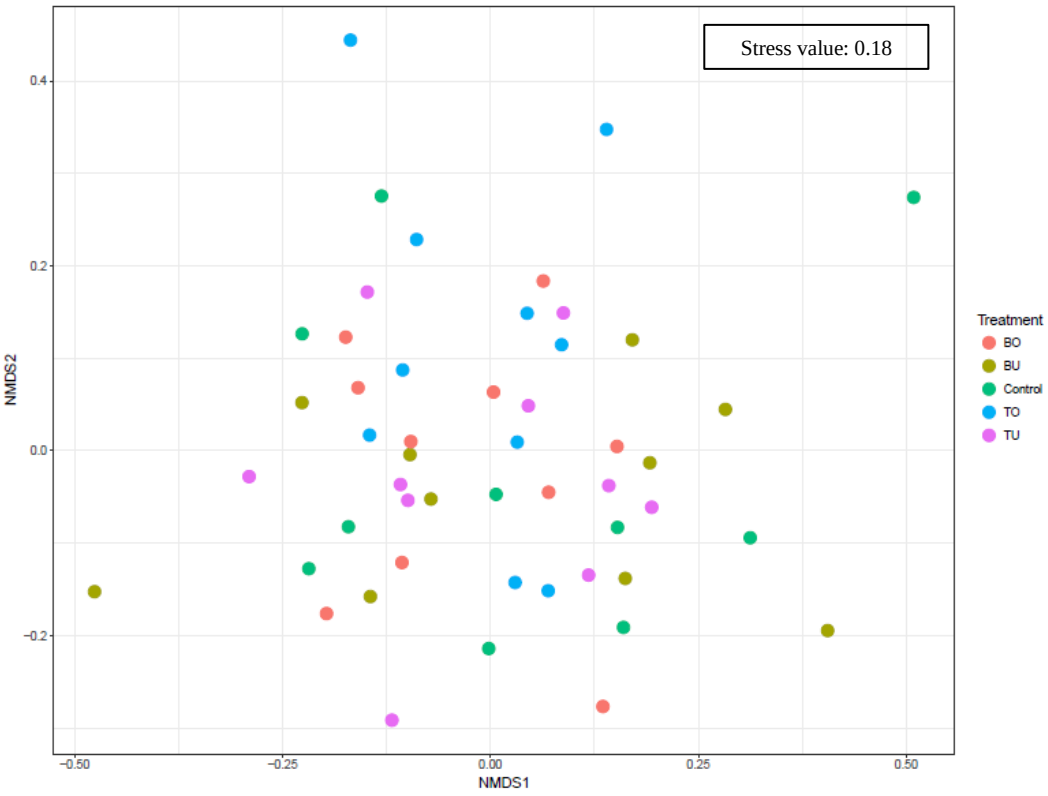


Figure 2.16. NMDS plot of tray treatments (2012-2016)

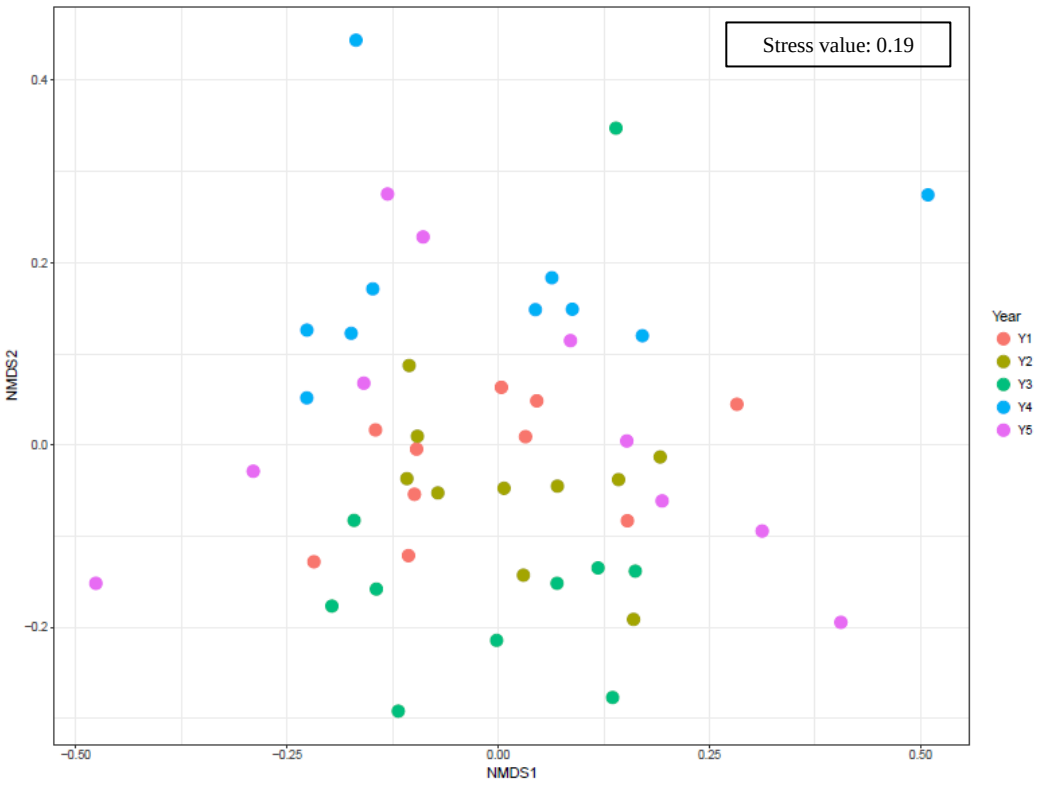


Figure 2.17. NMDS plot of tray treatments overlaid with the year

Y1 = 2012, Y2 = 2013, Y3 = 2014, Y4 = 2015, Y5 = 2016.

2.3 Discussion

The hypothesis generated from the seal movement study was that the changes induced by the seal carcass were due to secondary, abiotic factors (namely increased, more stable relative humidity (RH); more stable temperature; and reduced UV exposure) (Tiao et al., 2012). This experiment was designed to replicate the effects of the seal carcass (stabilising temperature, reducing UV exposure, and increasing and stabilising RH in the underlying soil) and test the hypothesis that localised changes in RH and temperature were driving changes in the underlying microbial community.

In the seal movement study there was a significant decrease in alpha diversity, as well as a major shift in community composition (with an increase in Firmicutes and γ-Proteobacteria, and a decrease in Bacteroidetes) (Tiao et al., 2012). These trends were not observed in the current experiment. Indeed the microbial community was consistent across all years and all treatments, with no statistically significant differences between treatments for the Proteobacteria, Actinobacteria, Acidobacteria or Bacteroidetes phyla. Community composition was also consistent across years and across treatments at finer levels of taxonomic resolution (the class, family, genus and species level), indicating that the broad phylum categorisation (which may encompass a range of very different OTUs and associated metabolisms) was not obscuring any underlying trends (Geyer et al., 2014).

In the seal movement study microbial diversity at the new site decreased relative to the original and control sites (Tiao et al., 2012). This is presumably due to the seal carcass generating a new set of environmental conditions which imposed new selection pressures on the underlying microbial community. The study assumed that the new environmental conditions selected for species that were better adapted to the new conditions, and that these species rapidly increased in abundance, outcompeting other, less well adapted species (Tiao et al., 2012). In the current experiment, however, average alpha diversity across the five years was actually greater in all of the tray treatments than in the control soil. However, alpha diversity varied significantly from one year to the next, and looking at each year individually, no common trends in alpha diversity could be confidently identified. This indicates that other factors (i.e. year-to-year variability in soil

properties such as pH, conductivity and moisture content) were more important in determining alpha diversity.

As anticipated, the black upright (BU), translucent upright (TU), and black overturned (BO) tray treatments stabilised January RH relative to the control. The BU and TU trays decreased the number of days (hourly recordings) above zero during the summer months, while the BO tray increased the number of days above zero (relative to the control). The translucent overturned (TO) treatment had a 'greenhouse' effect, raising the temperature and consequently lowering RH (since warmer air can hold more moisture) (Georgia State University, 2016) (Table 2.9). However, contrary to predictions average RH over the two year measuring period (from 2012-2013) was significantly lower for the tray treatments than for either of the controls.

Table 2.9. Summary of tray treatment effects

	Upright	**Overturned**
Black	Blocked UV exposure	Blocked UV exposure
Tray	Stabilised Jan temperature	Mildly stabilised Jan temperature
	Stabilised Jan RH	Stabilised Jan RH
	Fewer days above 0 ^{o}C in summer	More days above 0 ^{o}C in summer
Translucent	Light	Light
Tray	Stabilised Jan temperature	Increased Jan temperature
	Stabilised Jan RH	Reduced Jan RH
	Fewer days above 0 ^{o}C in summer	More days above 0 ^{o}C in summer

In the seal carcass experiment the soil underneath the carcass had stabilized and significantly increased RH relative to the control soil (82.8 ± 1.8% and 37.7 ± 13.1% respectively) (Tiao et al., 2012). In the tray experiment average RH values for 2013 were already significantly higher, ranging from 72.08 ± 23.09% in the 4B.TO treatment, to ~100% in the BU, TU, BO and control treatments. Based on this RH data it would appear that the tray experiment was set up in a flat, low lying area where moisture accumulated. This was supported by a visual inspection of the site, as it was noted that significant volumes of snow collected around the trays, while the upper slopes of the valley remained snow-free (Figure 2.18).

Adding to this, during the experimental period (from 2012-2016) there were a series of very wet years with unusual snow fall compared to the years of the seal movement experiment (C. Cary, personal communication, February 9, 2017). This snow accumulation is likely to have a significant effect on the microbial communities as it provides the soil with additional moisture during the seasonal melt period (Fountain et al., 1999).

Geographical factors (altitude, topography, and proximity to sources of water) have a major influence on the structure and diversity of microbial communities (Cary et al., 2010). However it could be possible that within the Dry Valleys these factors are operating on a far smaller scale than previously realised, with subtle terrain features influencing soil moisture content (and thus selection pressures) in a highly localised manner. This hypothesis is supported by other Dry Valley studies showing that spatial partitioning of invertebrate communities and biogeochemical factors occur over very small distances (<10 m), and that this partitioning is associated with landscape features (Barrett et al., 2004). Local-scale terrain features could potentially account for some of the heterogeneity in microbial communities between valleys, as environmental factors are presently only able to explain ~50% of the microbial community variation observed (Van Horn et al., 2013).

Given that the moisture content of the soil was so high, another possibility is that the tray experiment was deployed in the path of an invisible, subsurface water track. Water tracks are areas containing high levels of moisture that are involved in down-hill water transport (Levy et al., 2011). These water tracks are generally characterised by a lack of well-defined channels, and thus usually go unseen in the Dry Valley landscape. Water tracks are formed from the melting of buried ice or snow packs, are rich in dissolved minerals and salt, and ultimately drain into Dry Valley lakes. While often cryptic in nature, water tracks are a major feature of Dry Valley systems (Levy et al., 2011). Water tracks and seasonal melt water streams from Dry Valley glaciers are among the few sources of liquid water in the Dry Valleys during the summer months. The wet areas of soil extending from these streams, lakes, and tracks can be several metres wide and reach 70 cm in depth, and are commonly associated with Cyanobacteria (if they manifest at the surface and have access to available ambient light) (Gooseff, McKnight, et al., 2003;

Niederberger et al., 2015). With increased water availability these hyporheic soils (the water zone associated with the edge of lakes and streams, and possibly water tracks) are hotspots of biological activity during the summer months (Niederberger et al., 2015).

Since the soil moisture content was so high initially, a reduction in surface sublimation (caused by the overlying trays) would have been negligible in terms of increasing RH. In the seal experiment however, RH in the control soil was significantly lower and thus it is likely that a reduction in surface sublimation would have had a significant effect on RH (and consequently the edaphic microbial community) (Tiao et al., 2012).

Figure 2.18. Snow accumulation around the tray experiment

(Image by Professor Craig Cary)

Based on these findings, the issue with the current experiment appears to be the site on which it was deployed. In retrospect, prior to experimental set up soil moisture content should also have been assessed to ensure moisture levels were not already too high to test for the effect of elevated RH on microbial communities (or the microbial community should have been examined to identify whether it was associated with wet or dry soil). It would also have been advisable

to conduct a topographic survey to ensure that the experiment was not placed in a depression that might gather snow.

Although the tray experiment was not able to test the original hypothesis that increased, more stable RH and temperature was driving the microbial community changes seen in the seal movement experiment, it did unearth some unexpected, extremely interesting results. While the microbial community composition remained quite consistent across years and treatments (with no statistically significant differences in the relative abundance of Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria or Bacteroidetes between the trays), there were statistically significant differences in the relative abundances of Cyanobacteria/ Chloroplasts between the TO treatment and the BO and BU treatments. It should be noted that these translucent tray treatments had greater Cyanobacteria/ Chloroplast abundances than even the control samples, which also would have received full sunlight. This suggests that another factor in addition to the sunlight (such as increased temperature and/or RH beneath the tray), was promoting this observed increase. Of all the tray treatments and control samples, the black tray treatments had the lowest Cyanobacteria/ Chloroplast abundances. This is intuitive given that Cyanobacteria/ Chloroplasts are autotrophs and require sunlight to carry out photosynthesis (Woese, 1987).

The south-east positioned TO tray treatment (4A.TO) had a statistically significant difference in the abundance of an unknown species from the *Bacillariophyta* family; an unknown Cyanobacteria/ Chloroplast species; an unknown species of *Lysobacter* (γ-Proteobacteria); and an unknown species of *Actinomycetales* (Actinobacteria) (relative to the other tray/ control treatments). However it should be noted that there were also statistically significant differences between the 4A and 4B.TO replicates, indicating substantial within-treatment variability and limiting the extent to which these findings can be extrapolated.

It should also be noted that of all the samples, the TO treatment had the highest conductivity and the lowest pH values. Given this, it is difficult to confidently identify the factors responsible for the increased Cyanobacteria/ Chloroplast abundance. It is unclear whether the TO treatment itself was responsible for the

increase (i.e. by increasing temperature and allowing light to reach the underlying soil), or whether the increase was an artefact of tray positioning and small-scale differences in soil properties. Ideally more than two replicates would have been used in the experiment so that more statistical support could be provided. However, it should be noted that a composite soil sample (5 small samples combined together) was taken from beneath each of the trays (providing a better representation of the underlying soil), and that practical considerations (e.g. time, impact, and experimental size) come into play when deciding the number of replicates to include in an experiment (particularly when working in Antarctica where there are stringent controls around environmental manipulations) (Antarctic Treaty Consultative Parties, 1960).

In keeping with the findings of the current investigation, previous multi-year *in situ* studies from Antarctic and sub Antarctic regions (Alexander and Signy Island) have demonstrated that microalga colonisation is significantly increased following open-top chamber (OTC) manipulations. However, as in the current experiment it is not apparent which factors are driving these changes, as OTCs have a number of physical effects (elevating temperature, reducing UV exposure, providing wind protection, and stabilising moisture availability) (Wynn-Williams, 1996). However this is a line of research worth investigating, as Cyanobacteria and other photoautotrophs are fast growing, light dependent and temperature sensitive; and thus have the potential to be monitored as early biological indicators of climate change (Wynn-Williams, 1996).

Complications and unexpected findings are all part of the scientific process, and can often lead to important, interesting discoveries. Since the tray experiment appears to have been set up in a moist, low-lying area or a hidden water track, a new line of investigation was explored. The microbial community composition of the moist, low elevation tray experiment was compared with the community composition of the drier, higher elevation seal control soil; as well as the community composition of the new seal site. Given that the seal increased RH in the underlying soil, comparisons were made to see whether the microbial community composition at the seal site shifted towards that of the moist TO site. While all of the tray treatments were similar in microbial community composition,

it was the TO tray treatment that was selected for comparison as it was the treatment where the notable increase in Cyanobacteria/ Chloroplasts was seen.

The literature was also examined to determine whether the microbial community at the moist tray treatment site was similar to the microbial communities in other high-moisture associated areas. If so it could be possible to identify a microbial community structure associated with water tracks that could be used as a bio-indicator of these hidden features (as is currently done for oil prospecting, and environmental pollution detection) (Avidano et al., 2005; Rasheed et al., 2015). This is quite feasible given that a number of recent studies have identified spatial segregation in invertebrate communities based on soil moisture content (Barrett et al., 2004; Gooseff, Barrett, et al., 2003; Powers et al., 1998; Treonis et al., 1999). This bio-indicator could then potentially be used in routine soil surveys to help achieve a greater understanding of the Dry Valley systems (i.e. identify water sources), and aid in experimental design (i.e. where to set up experiments).

The microbial community composition seen in the tray experiment was quite different to that reported at the control and new seal site in the seal movement paper (Table 2.10). In the tray experiment Bacteroidetes were the most dominant phylum (at ~17-19% relative abundance); followed by Proteobacteria (~15-17%); Acidobacteria (~11-14%); Cyanobacteria/ chloroplasts (~3-8%); and Actinobacteria (~3%). In the new seal site only three phyla dominated the community: Firmicutes (48.4%), Proteobacteria (39%) and Actinobacteria (7.7%) (Tiao et al., 2012). At the control seal site Proteobacteria and Firmicutes were < 1% abundant; Actinobacteria had a relative abundance of 37.3%; Acidobacteria had a relative abundance of 3.7%; Bacteroidetes had a relative abundance of 12.6%; and Cyanobacteria were < 1% abundant (Tiao et al., 2012).

Table 2.10. Community composition at the control, new seal, and tray experiment sites

Phylum	Seal control soil	New seal site	TO tray treatment
Firmicutes	< 1%	48.4%	< 1%
Proteobacteria	< 1%	39.0%	16.6%
Actinobacteria	37.3%	7.7%	30.0%
Acidobacteria	3.7%	< 1%	11.5%
Bacteroidetes	12.6%	< 1%.	19.5%
Cyanobacteria/ Chloroplasts	< 1%	< 1%	8.3%

As expected, the moist, low elevation TO tray treatment soil had a greater abundance of Cyanobacteria/ Chloroplasts than the drier, higher elevation seal control soil, or the soil at the new seal site (since the experiment appears to have been set up on a water track; which are known to be associated with Cyanobacteria) (Niederberger et al., 2015). Bacteroidetes were also present at greater relative abundance in the TO treatment than in the seal control. These findings are consistent with several other studies which suggest that Bacteroidetes are often found in wet environments, particularly microbial mats and biofilms (Ley et al., 2006), and that they are commonly associated with Cyanobacteria (which release organic compounds that can be utilised by Bacteroidetes) (Stanish et al., 2013).

Notably, the moist TO tray treatment soil also had a greater relative abundance of Proteobacteria than the drier seal control site. This is interesting as it is consistent with the increase in Proteobacteria seen in the new seal site (Tiao et al., 2012), hinting that it may be a moisture associated increase. In keeping with these findings, another study investigating the effects of increased water and organic matter on microbial community composition within the Dry Valleys found that in low to moderate salinity sites the addition of water and organic matter was associated with a significant increase in the abundance of Proteobacteria (Van Horn et al., 2014).

There was also a greater relative abundance of Acidobacteria, and a lower abundance of Actinobacteria in the moister, low elevation TO site than in the drier, control seal site. The lower relative abundance of Actinobacteria in the moister, TO site was consistent with a study of microbial communities along a transect from dry (arid) to wet hyporheic soils within the McMurdo Dry Valleys (Niederberger et al., 2015). However, the greater relative abundance of Acidobacteria in the moister TO soil was not seen in the hyporheic soil study. Indeed, in arid Dry Valley soils, Acidobacteria and Actinobacteria are generally two of the most dominant phyla (Lee et al., 2012; Niederberger et al., 2008; Pointing et al., 2009; Smith et al., 2006). There are several possible explanations for the increased relative abundance of Acidobacteria seen in this study, such as local scale niche factors (i.e. species interactions) and local-level environmental selection pressures. Numerous studies conducted within the Dry Valleys have demonstrated that there is significant variation in taxonomy and microbial community structure between different valleys, and that this diversity is largely driven by environmental factors (i.e. water availability, pH, altitude and salinity) (Lee et al., 2012; Niederberger et al., 2008; Smith et al., 2006). Further research would need to be conducted to identify the factors driving the increased relative abundance of Acidobacteria seen in this study.

2.4 Conclusions

The central hypothesis of this study was that changes in relative humidity (RH) and temperature (induced by overlying objects) would cause changes in the local environment that, in turn, would drive changes in the underlying microbial community. This would provide strong evidence that contemporary environmental conditions are having a significant effect on microbial communities, and that these communities respond quickly to such changes. However, it would appear that due to the continuous high moisture content within the soil on which the experiment was deployed, the effect of the tray treatments on the local environment (i.e. RH and temperature) was negligible.

In contrast to the seal movement study, microbial community composition in the tray treatment experiment stayed consistent across all years and treatments. One point of interest, however, was the significantly greater abundance of Cyanobacteria/ Chloroplasts in the TO treatment than in the BU or BO treatments. This is intuitive given that Cyanobacteria/ Chloroplasts are autotrophs and require sunlight for photosynthesis. Notably, the moist TO tray treatment soil also had a greater relative abundance of Cyanobacteria/ Chloroplasts, Proteobacteria, Bacteroidetes and Acidobacteria than the drier seal control site, in keeping with the observations of other studies investigating microbial community composition within wet environments (Ley et al., 2006; Niederberger et al., 2015; Van Horn et al., 2014; Van Horn et al., 2013). Based on these observations it would appear that the tray experiment was set up either in a flat, low lying region where moisture accumulated, or in a subsurface water track.

Chapter 3

Conclusions and future directions

Due to Antarctica's hostile environmental conditions, most plants and animals cannot survive, and as a result the terrestrial ecosystem supports a simple trophic structure and is dominated by microorganisms (Chown et al., 2015; Hogg et al., 2006). Consequently, Antarctica is the ideal setting to study the impact of physicochemical factors on microbial communities. This foundational knowledge can then be used to help develop models that predict how Antarctica, and ultimately the rest of the world, may respond to the environmental changes forecast under current climate change predictions (as microbial communities lie at the base of all food chains) (Hogg et al., 2006; Terborgh & Estes, 2013).

Early, culture-based studies led researchers to believe that Antarctic microbial ecosystems had extremely low diversity and were homogenous across the Dry Valleys (Hopkins, Sparrow, Novis, et al., 2006; Johnson et al., 1978). These communities were thought to be controlled by legacy carbon inputs, and consequently, microbial community structure was considered incredibly stable (with changes occurring over thousands of years) (Barrett et al., 2006; Moorhead et al., 1999). However, with the development of molecular based techniques it has become apparent that Antarctic soil is just as temporally and spatially heterogeneous as more temperate systems (Bockheim, 2002; Lee et al., 2012), and that microbial diversity and abundance within this soil is far greater than initially anticipated (Cowan et al., 2002).

Climate change is predicted to increase temperature, water availability, and vegetation cover, and reduce the frequency and severity of freeze-thaw cycles in the decades to come (Hogg et al., 2006). This is particularly pertinent given that water availability in the Dry Valleys is thought to be one of the main factors driving microbial community structure and diversity (Barrett et al., 2007; Cary et al., 2010; Kennedy, 1993; Zeglin et al., 2009), and that recent *in situ* studies suggest that environmental changes may be having a more immediate impact on microbial communities than originally anticipated (Tiao et al., 2012; Yergeau et al., 2012).

In a milestone study, Tiao et al. (2012) conducted a unique, multi-year, *in situ* experiment in which a mummified seal carcass was transplanted from its original site in Miers Valley to a new geomorphically similar site in close proximity, and changes in the underlying microbial community were monitored via yearly sampling over a five year period. Astonishingly, within just three years of the transplantation the new seal site had increased microbial biomass, decreased biodiversity, and had experienced significant shifts in microbial community composition towards that of the original seal site (with a significant increase in Proteobacteria and Firmicutes, and a major decrease in Bacteroidetes) (Tiao et al., 2012).

Statistical analysis revealed that changes in the soil's physicochemical properties (nitrogen and organic carbon content, pH, and conductivity) could not satisfactorily explain the changes in microbial community structure observed, and instead it was hypothesised that the changes observed may have been driven by abiotic factors induced by the seal carcass (increased, more stable RH; reduced UV exposure; and more stable temperature) (Tiao et al., 2012). However, due to the un-replicated, observational nature of the study, this was only a proposal.

The current study was designed to shed light on these unexpected findings and verify whether changes in RH and temperature (caused by overlying objects) were indeed driving changes in microbial community structure. To do this, a controlled, *in situ* experiment was designed to replicate the abiotic effects of the seal carcass (stabilise temperature, reduce UV exposure, and increase humidity in the underlying soil). Plastic trays were set up on undisturbed areas of Antarctic Miers Valley soil; soil samples were taken every summer over a five-year period; and 16S rRNA gene Ion Torrent sequencing was used to assess changes in the microbial community. However, it would appear that due to the constant high moisture content within the soil on which the experiment was deployed, the actual effect of the tray treatments on the local environment (i.e. RH and temperature) was negligible.

While microbial community composition in the tray treatment experiment stayed consistent across all years and treatments, one point of interest was the significantly greater abundance of Cyanobacteria/ Chloroplasts in the translucent

overturned (‘greenhouse’) treatment than in the black upright or overturned treatments (an intuitive finding given that Cyanobacteria/ Chloroplasts are autotrophs and require sunlight for photosynthesis). Notably, the moist tray treatment soil also had a greater relative abundance of Cyanobacteria/ Chloroplasts, Proteobacteria, and Bacteroidetes than the drier seal control site (in keeping with the observations of other studies investigating microbial community composition within wet environments) (Ley et al., 2006; Niederberger et al., 2015; Van Horn et al., 2014; Van Horn et al., 2013).

Based on these observations it would appear that the tray experiment was deployed in either a flat, low lying area where moisture accumulated, or in a subsurface water track. Further investigations should be conducted in the area to identify which option holds true, and determine which factors were driving the increased relative abundance of Cyanobacteria/ Chloroplast observed in the translucent overturned tray treatment. If the first option is the case it is possible that low lying areas experience greater moisture accumulation than higher elevation, more sloped regions, and that these subtle differences in topography are responsible for significant heterogeneity in microbial community composition within the Dry Valleys.

Topography, altitude, latitude, and proximity to local water bodies are known to play a significant role in the structure of microbial communities (Cary et al., 2010), however it could be possible that these factors are operating on a far finer scale than originally anticipated. Subtle terrain features within a valley (dips and ridges) may be capable of influencing water availability (and hence selection pressures) in a highly localised manner. This is supported by studies showing that differences in biogeochemical factors and invertebrate biodiversity occur over very small scales, and are associated with landscape features (Barrett et al., 2004). If this hypothesis is proven correct, local-scale subtle terrain features could potentially help explain some of the inter-valley microbial community heterogeneity, as environmental factors are currently only able to account for ~50% of the microbial community variation observed (Van Horn et al., 2013).

Alternatively, if the experiment were set up in a subsurface water track, there is the potential to use the microbial community structure observed to identify a

microbial community "signature" that could act as a bio-indicator of hidden water tracks. Current knowledge of water movement in the Dry Valleys comes primarily from topographical surveys (Fountain et al., 1999), however recent studies have shown that below ground there is a network of cryptic erosional channels that dictate subsurface water movement and accumulation (Levy et al., 2011). These water tracks play a major role in salt and solute transport in the Dry Valleys, act as a chemical and ecological connection between Dry Valley lakes and soil, and provide the local edaphic ecosystems with a continuous source of moisture (producing conditions more suitable for microbial growth) (Levy et al., 2011). Microbial species/ communities are currently used as indicators of environmental pollution (Avidano et al., 2005); prospective regions for gas/ oil exploration (Microbial Prospecting) (Rasheed et al., 2015); as indicators of water quality (Griffin et al., 2001; Markert et al., 2003); and have been suggested as potential 'bio-indicators' of climatic warming (Oliverio et al., 2016). Thus it is conceivable that microbial community composition could also be used to identify hidden landscape features (water tracks) within the Dry Valleys, especially as recent studies have identified spatial-partitioning in invertebrate communities, based on soil moisture content and salinity (Barrett et al., 2004; Gooseff, Barrett, et al., 2003; Powers et al., 1998; Treonis et al., 1999).

Given these two exciting possibilities, soil/ topographic surveys should be conducted to identify subsurface water tracks, as well as low lying regions in which snow/ moisture would accumulate. Samples should be collected along transects of these areas (from the centre of the feature to dry soil), and Ion Torrent sequencing of 16S rRNA gene amplicons used to identify any changes in the microbial community composition. Given that dead cells and the inherent biases associated with PCR limit the reliability of DNA sequencing based analyses, it may be wise to team DNA sequencing based approaches with reverse transcription polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization (FISH) to better capture the active microbial diversity within Dry Valley soils (Niederberger et al., 2015). If proven correct, these findings would revolutionise our understanding of the factors controlling microbial communities within Dry Valley soil, and/ or provide an invaluable bio-indicator that can be implemented into routine Antarctic soil sampling practices to enhance our understanding of the Dry Valley system hydrology.

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Appendices

3 Supplementary Figures

Appendix 1

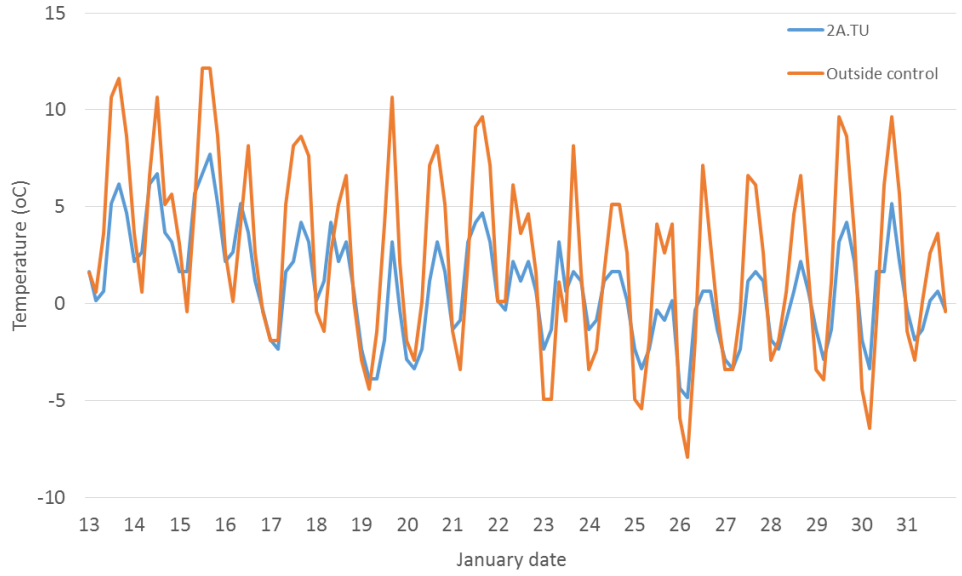


Figure 3.1. Temperature readings for the 2A.TU tray treatment (January 2012)

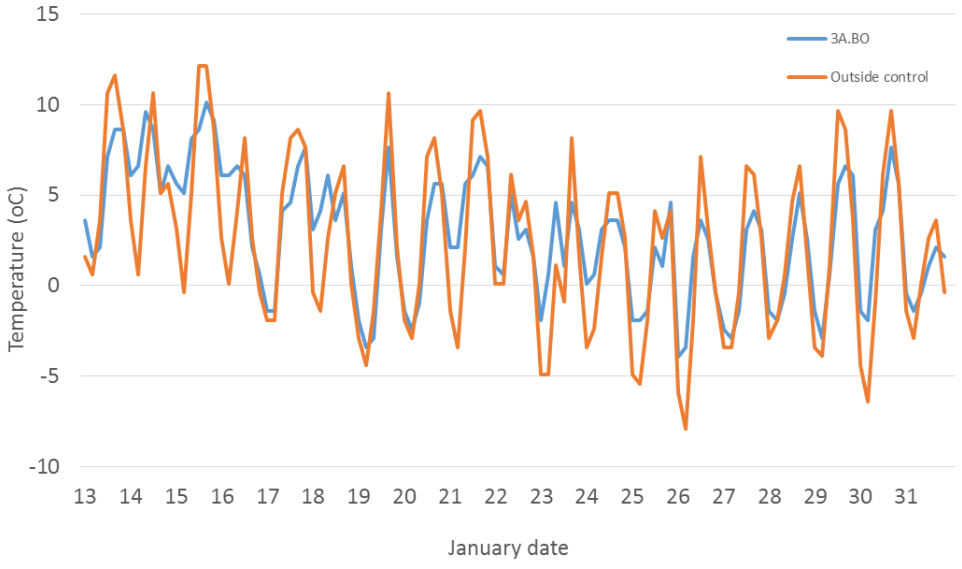


Figure 3.2. Temperature readings for the 3A.BO tray treatment (January 2012)

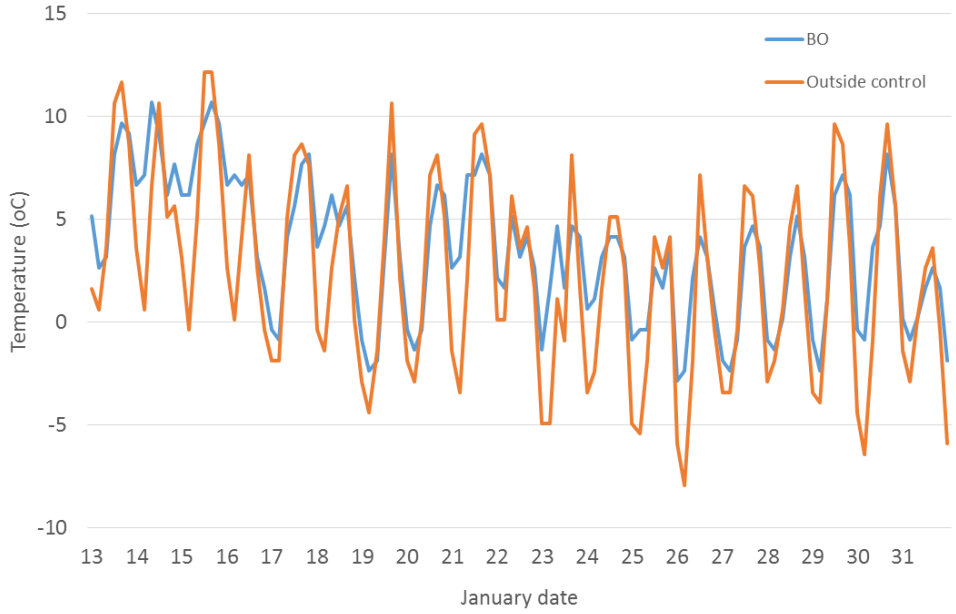


Figure 3.3. Temperature readings for the 3B.BO tray treatment (January 2012)

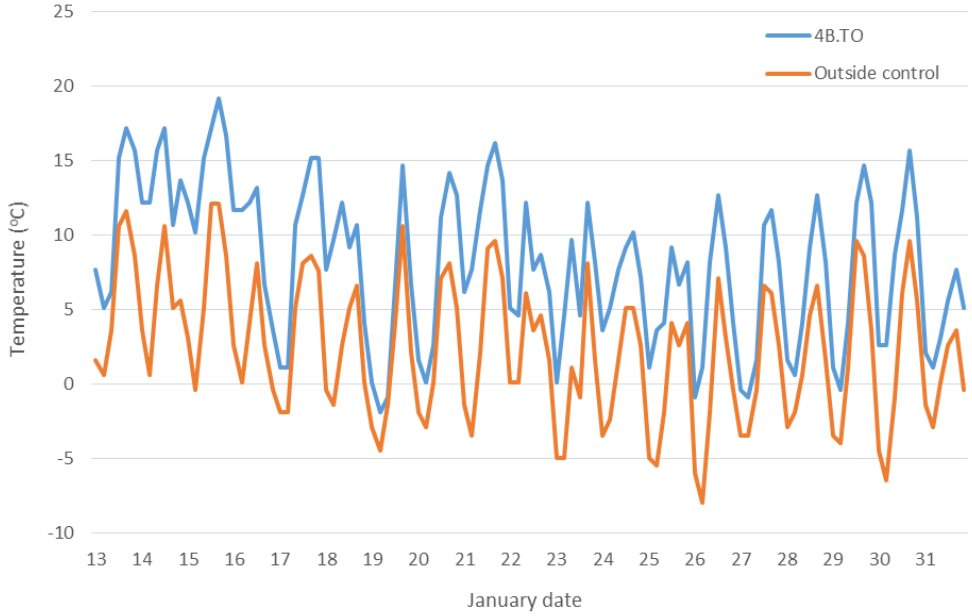


Figure 3.4. Temperature readings for the 4B.TO tray treatment (January 2012)

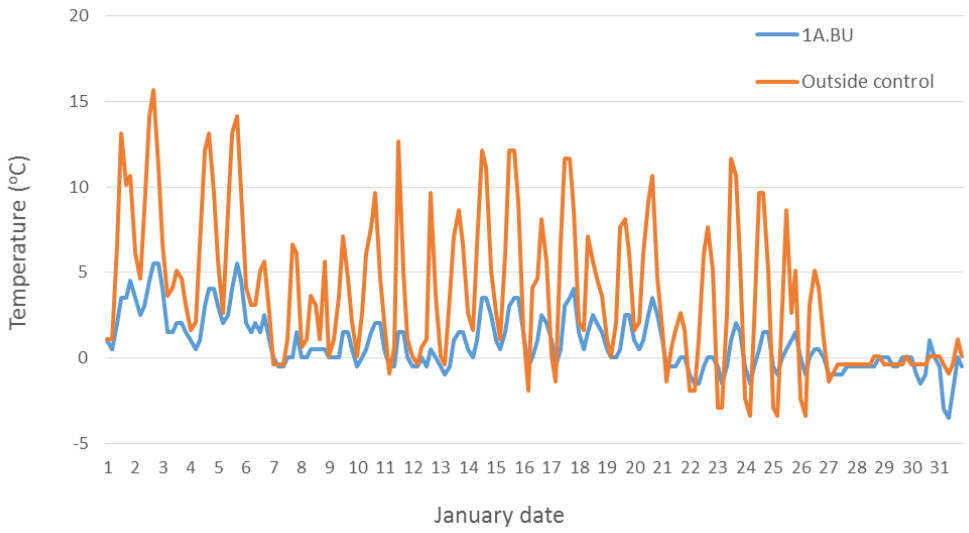


Figure 3.5. Temperature readings for the 1A.BU tray treatment (January 2013)

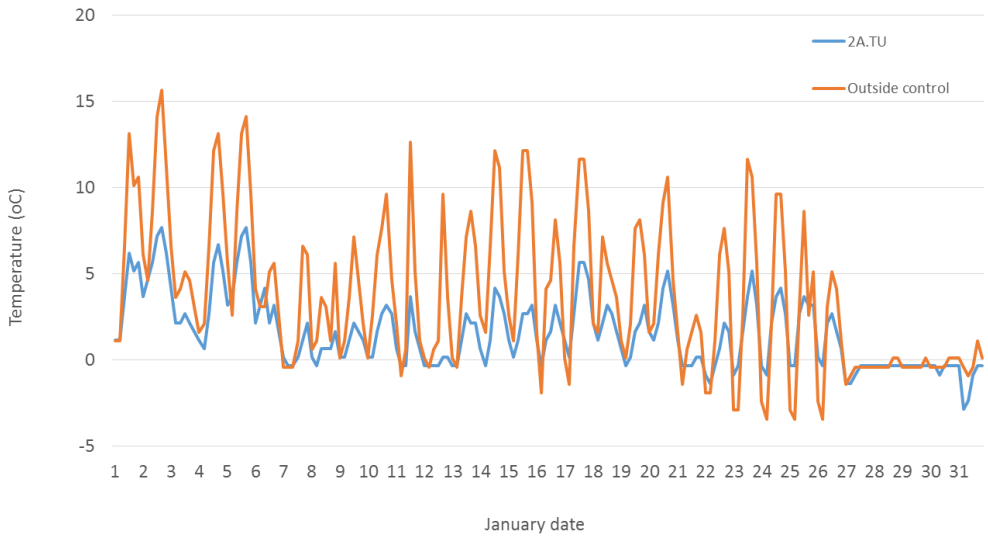


Figure 3.6. Temperature readings for the 2A.TU tray treatment (January 2013)

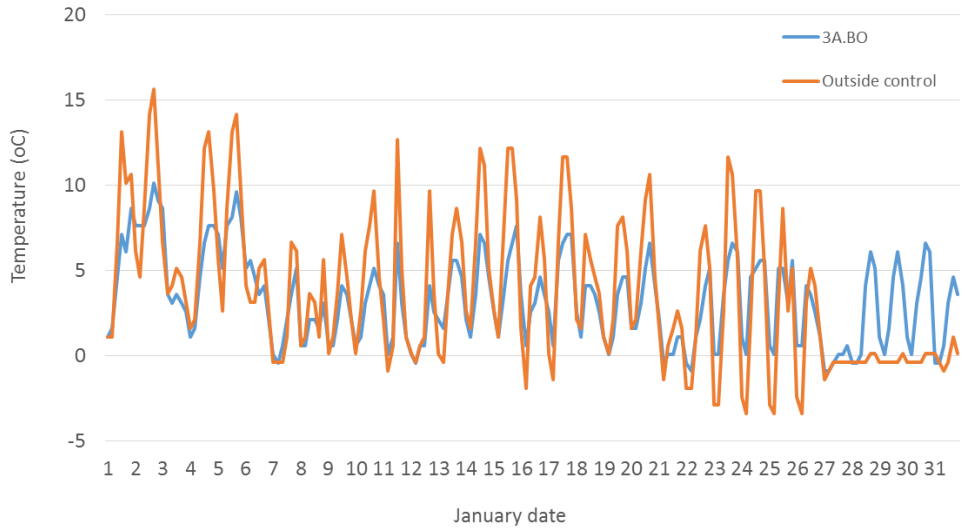


Figure 3.7. Temperature readings for the 3A.BO tray treatment (January 2013)

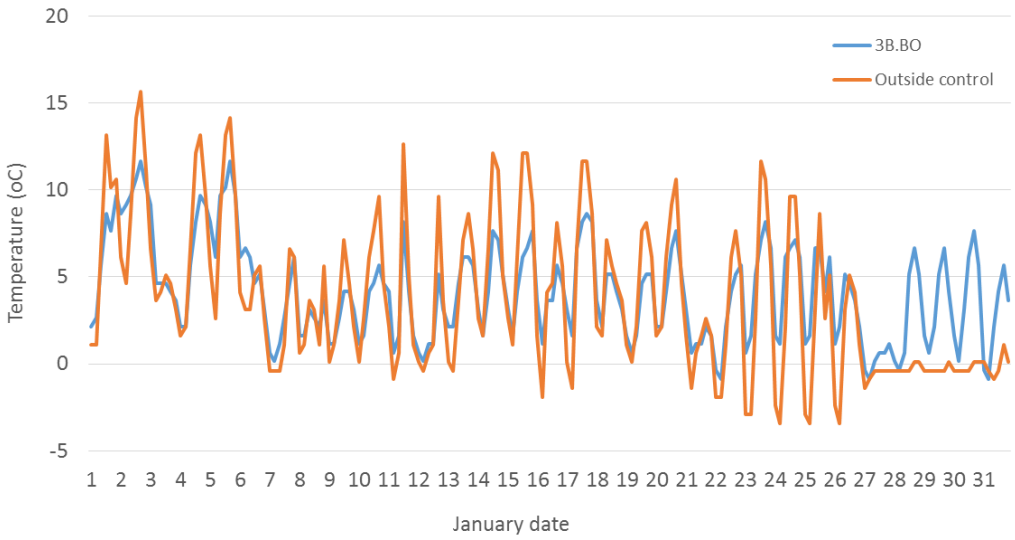


Figure 3.8. Temperature readings for the 3B.BO tray treatment (January 2013)

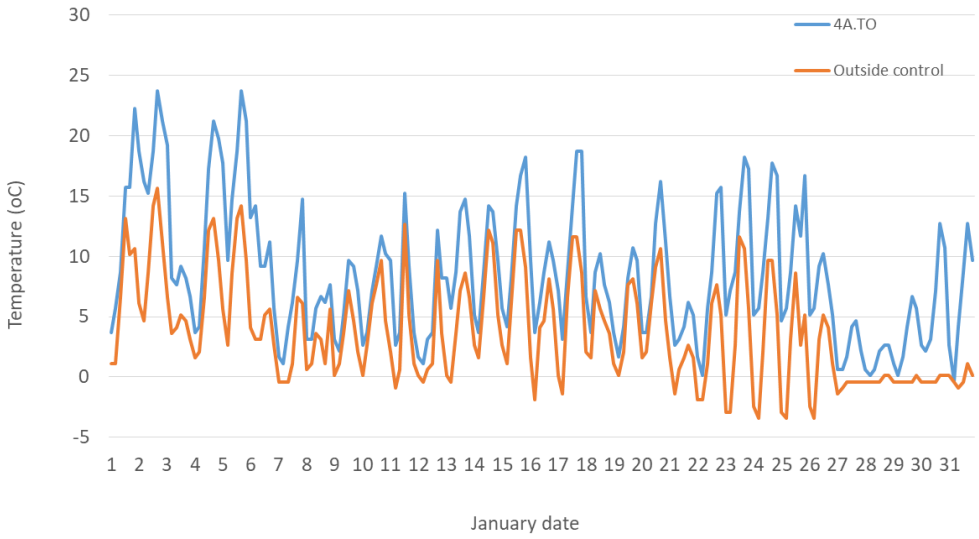


Figure 3.9. Temperature readings for the 4A.TO tray treatment (January 2013)

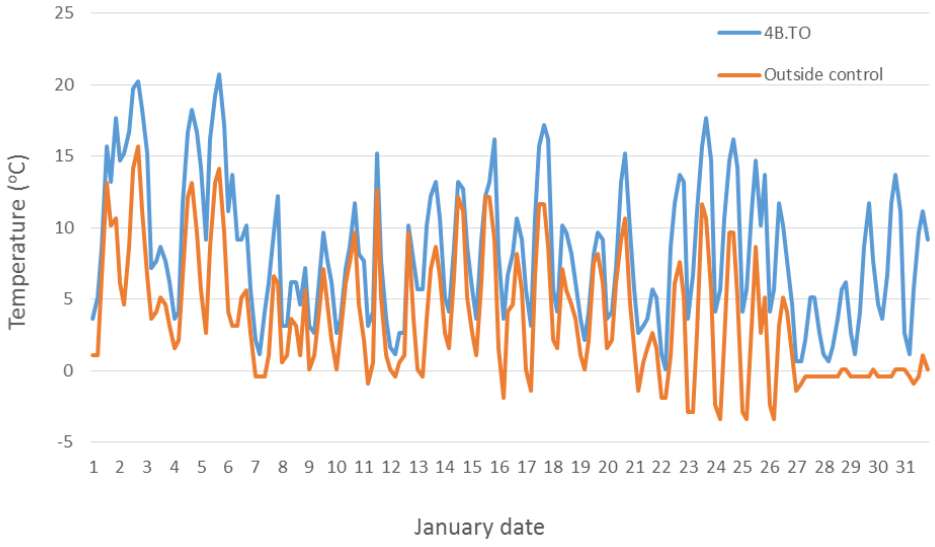


Figure 3.10. Temperature readings for the 4B.TO tray treatment (January 2013)

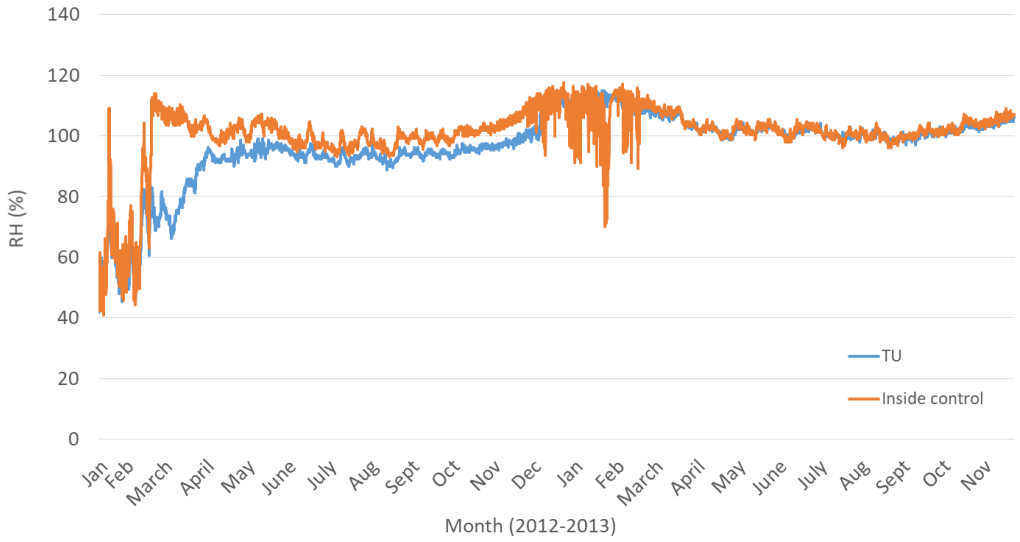


Figure 3.11. RH for the TU tray treatment (2012-2013)

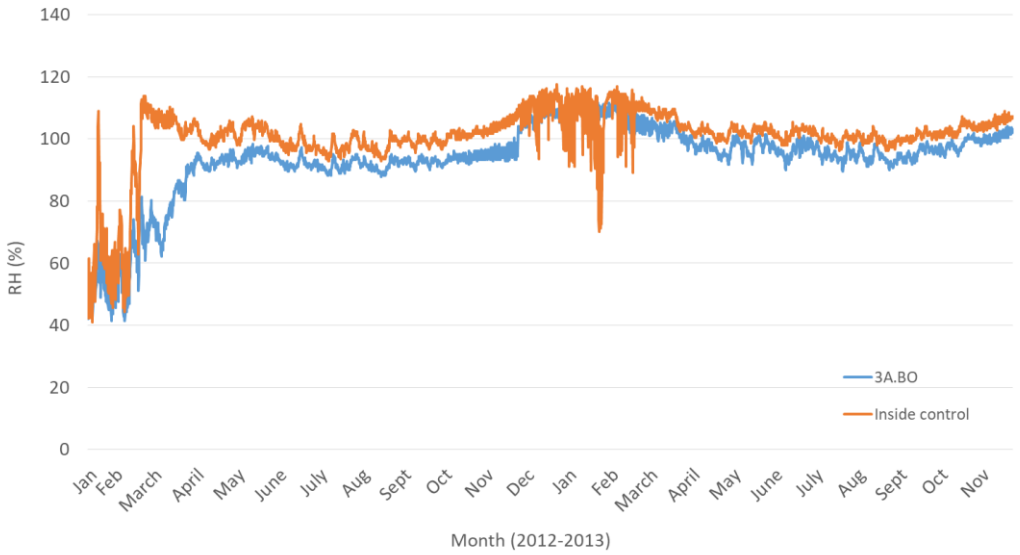


Figure 3.12. RH for the 3A.BO tray treatment (2012-2013)

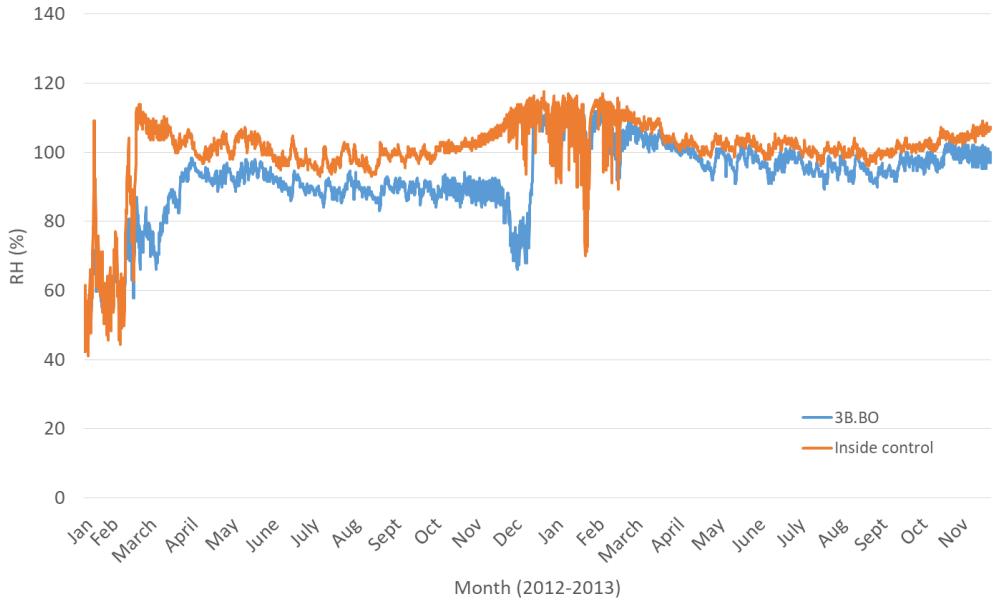


Figure 3.13. RH for the 3B.BO tray treatment (2012-2013)

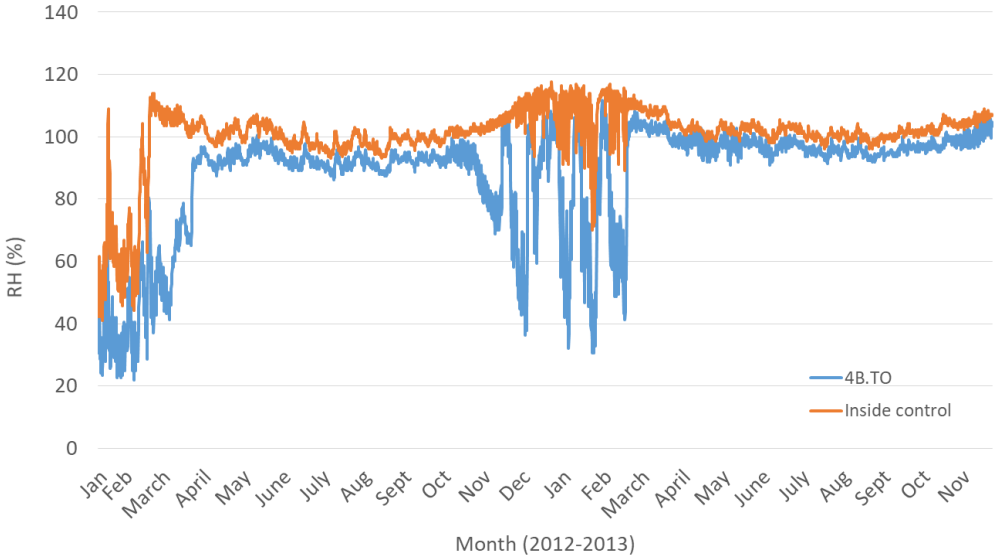


Figure 3.14. RH for the 4B.TO tray treatment (2012-2013)

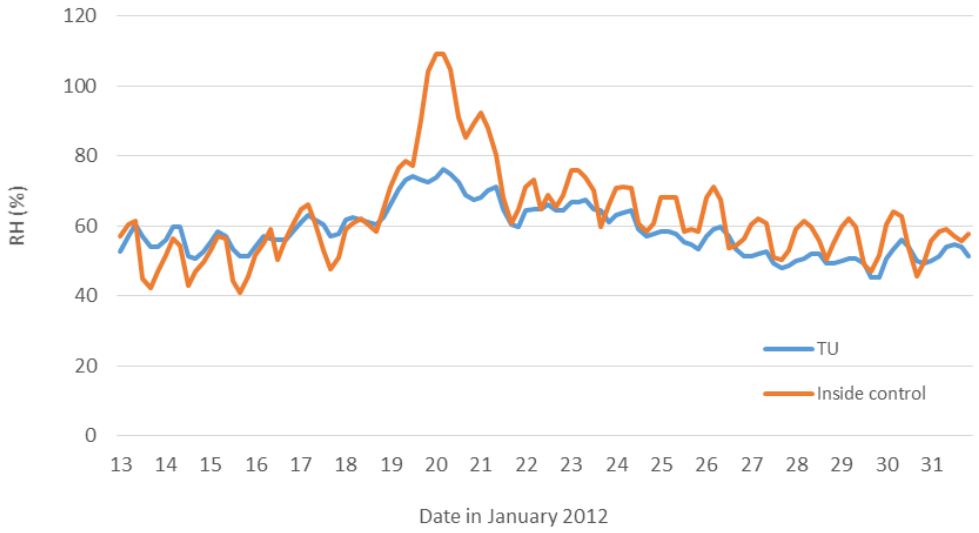


Figure 3.15. RH for the TU tray treatment in January 2012

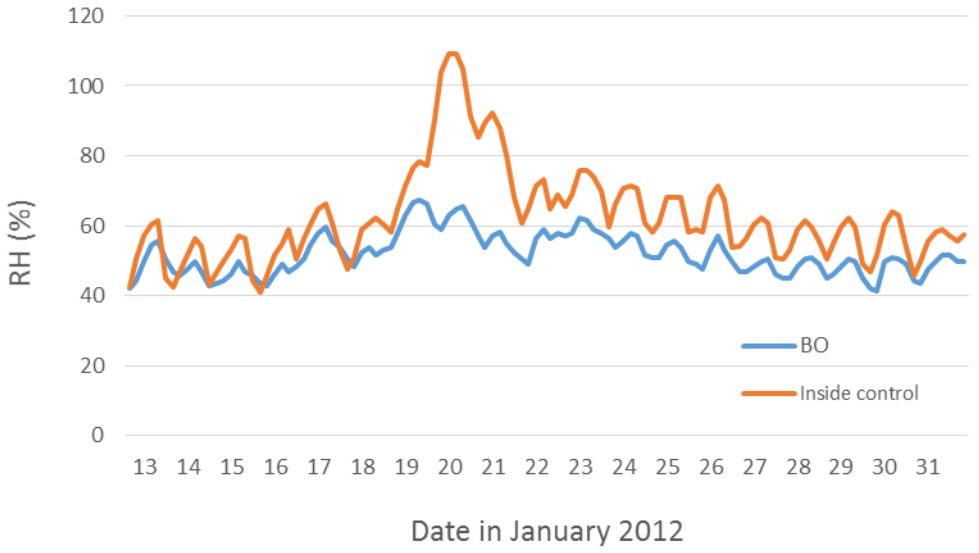


Figure 3.16. RH for the 3A.BO tray treatment in January 2012

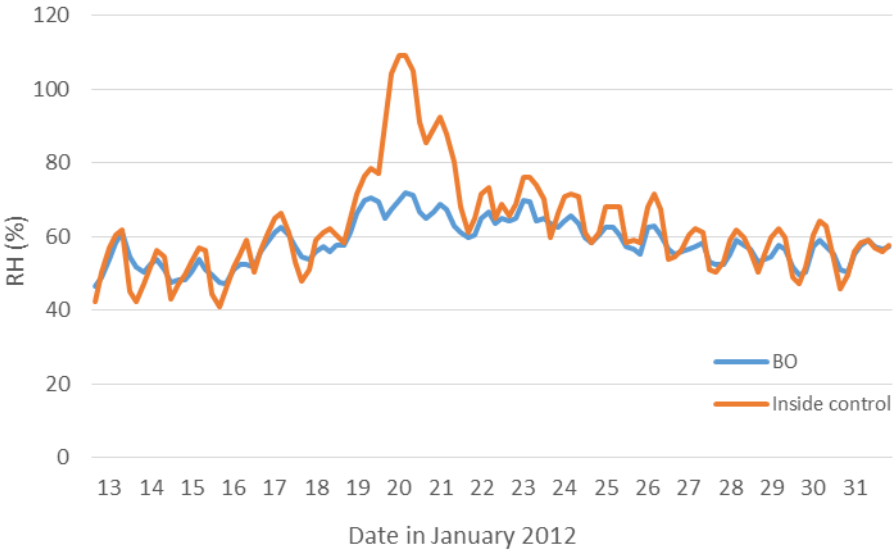


Figure 3.17. RH for the 3B.BO tray treatment in January 2012

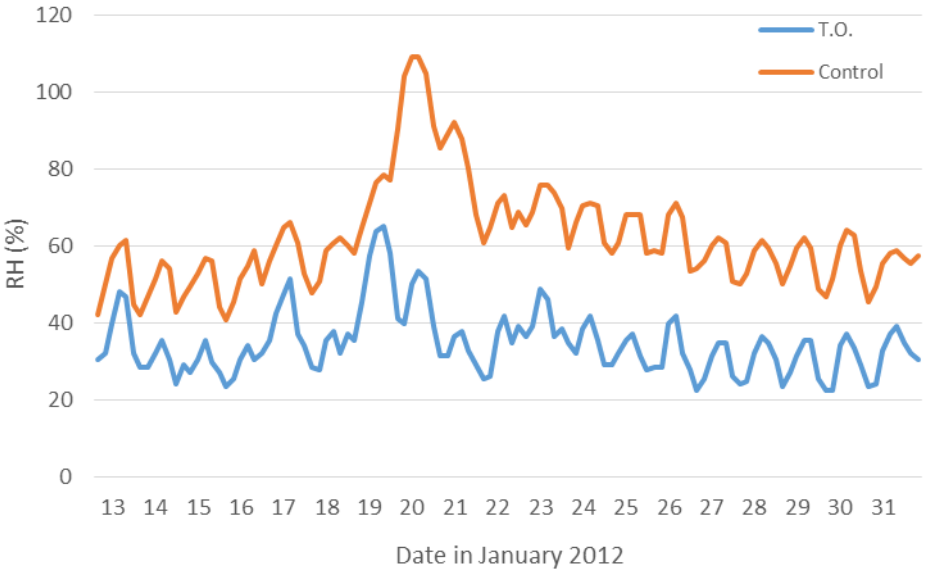


Figure 3.18. RH for the 4B.TO tray treatment in January 2012

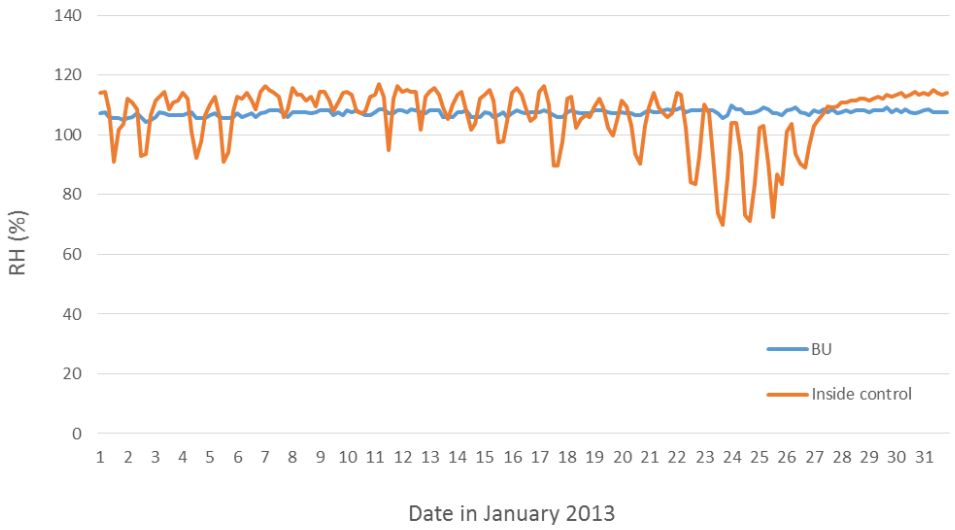


Figure 3.19. RH for the BU tray treatment in January 2013

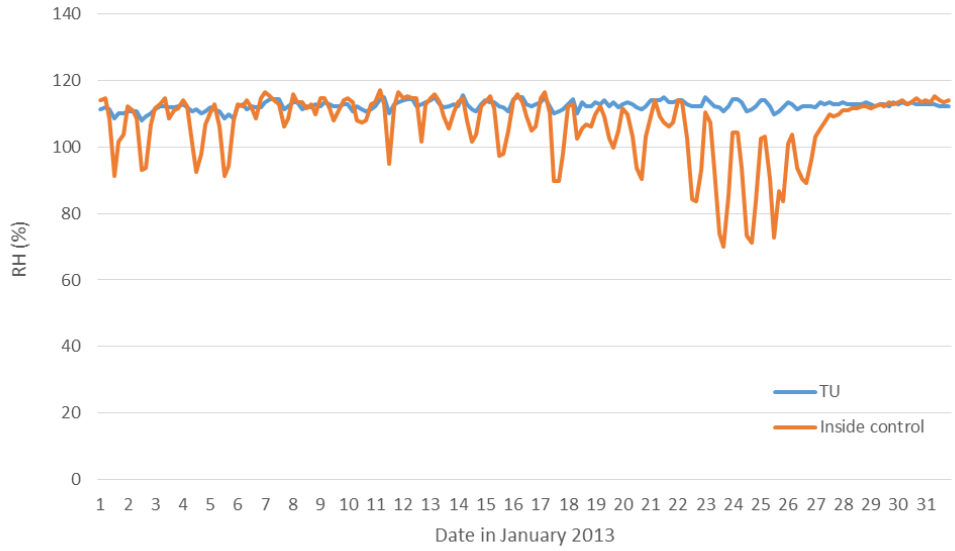


Figure 3.20. RH for the TU tray treatment in January 2013

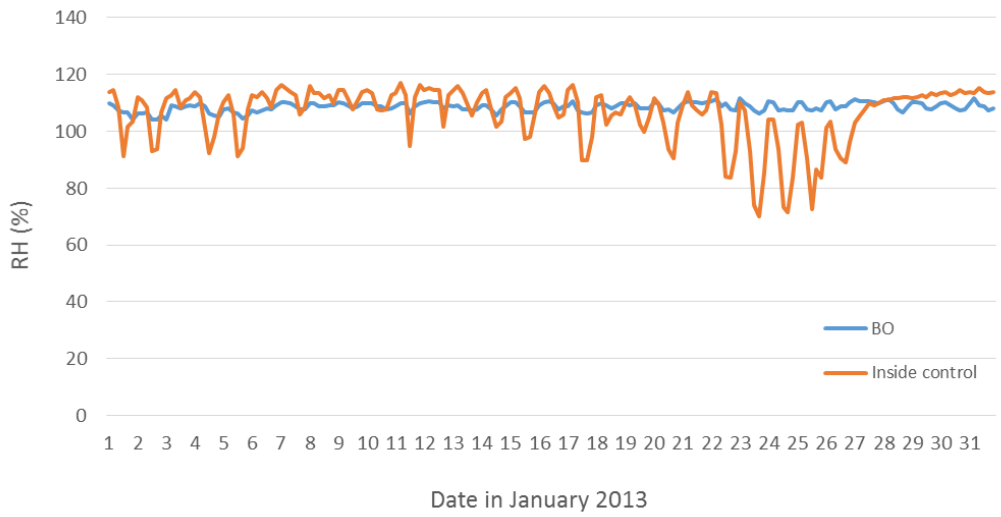


Figure 3.21. RH for the 3A.BO tray treatment in January 2013

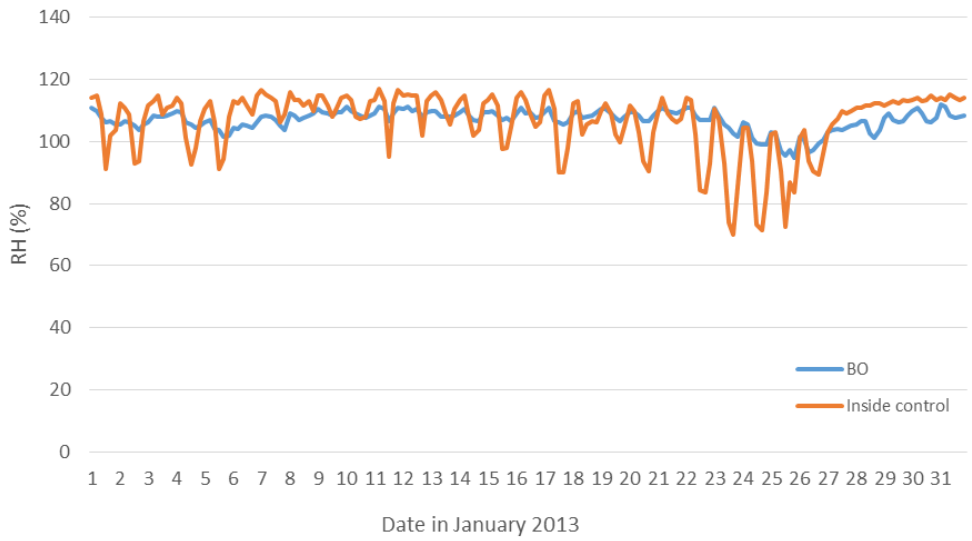


Figure 3.22. RH for the 3B.BO tray treatment in January 2013

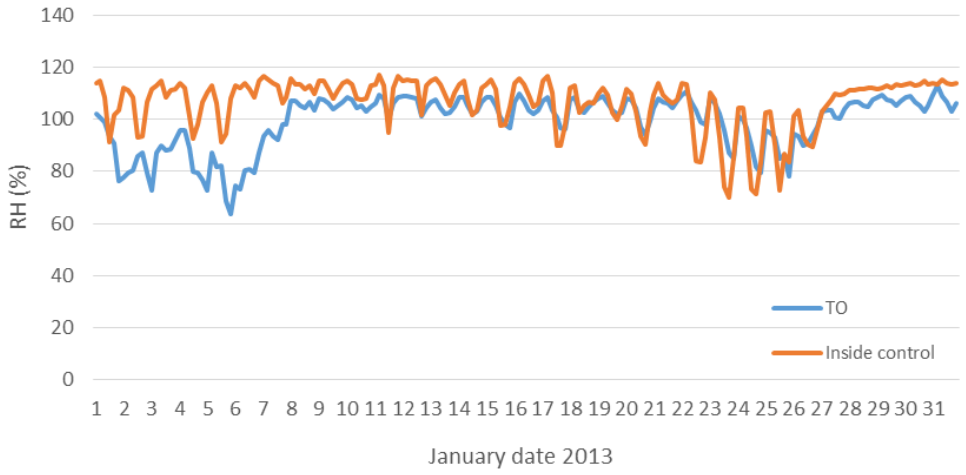


Figure 3.23. RH for the 4A.TO tray treatment in January 2013

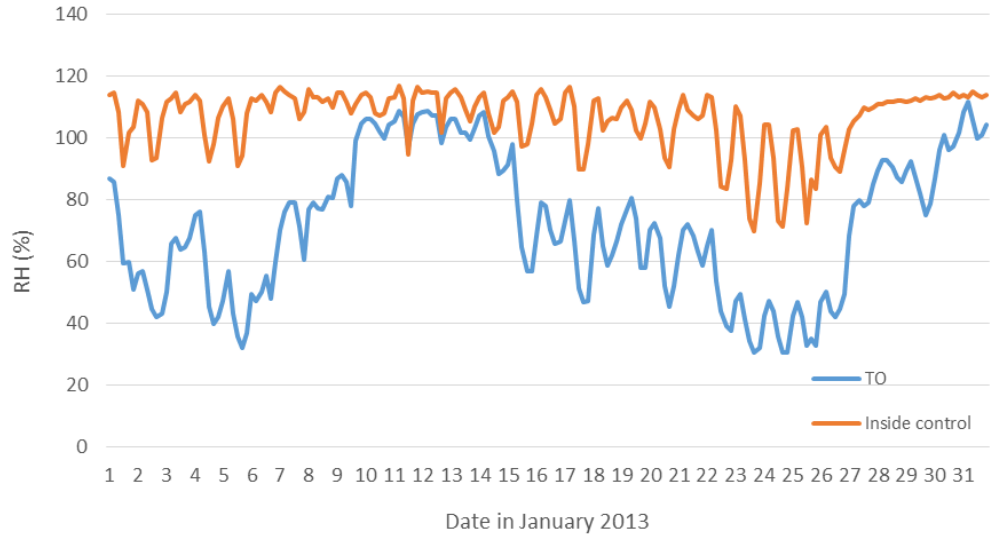


Figure 3.24. RH for the 4B.TO tray treatment in January 2013

Appendix 2

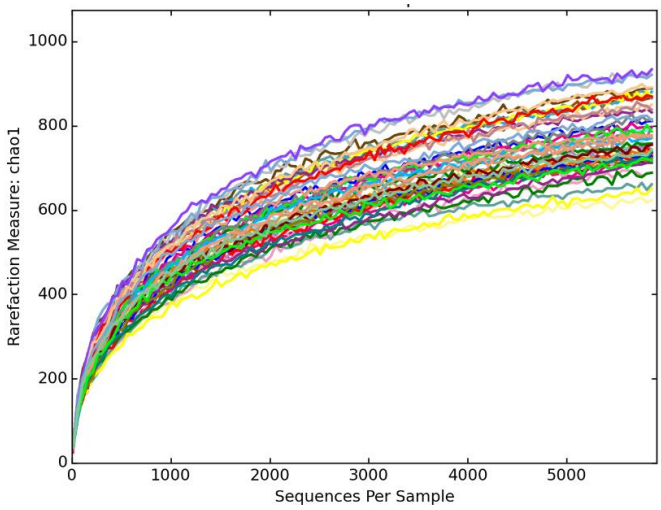


Figure 3.25. Rarefaction curves for each tray treatment (Chao1 index)

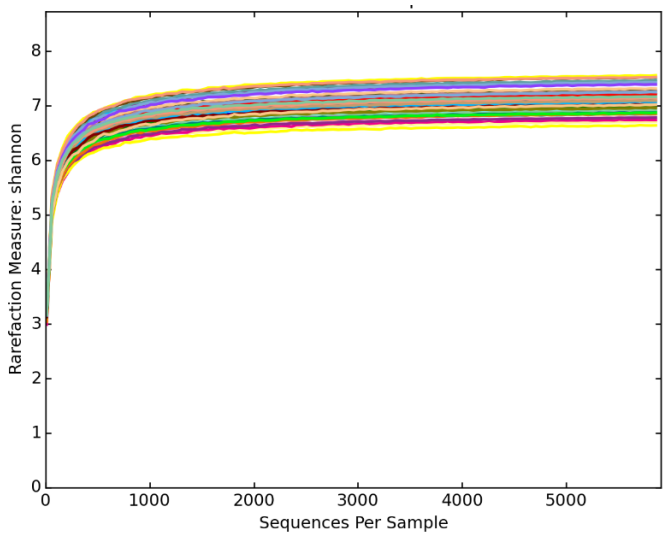


Figure 3.26. Rarefaction curves for each tray treatment (Shannon Index)

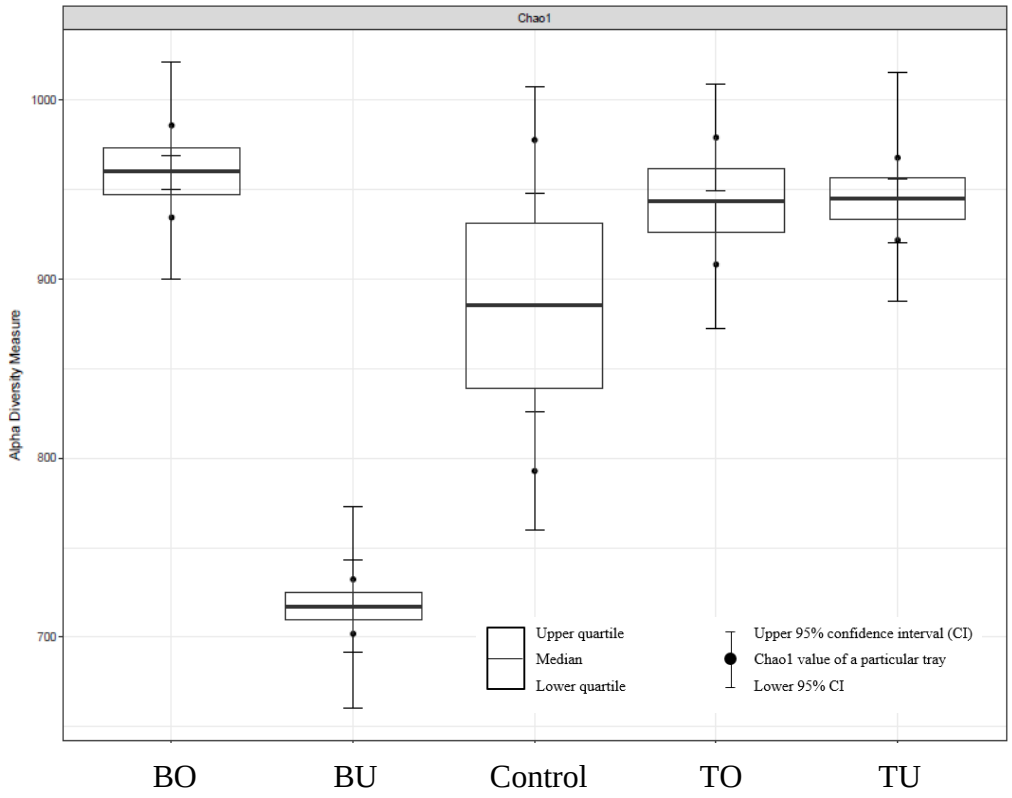


Figure 3.27. Alpha diversity (Chao1) across tray treatments in 2016

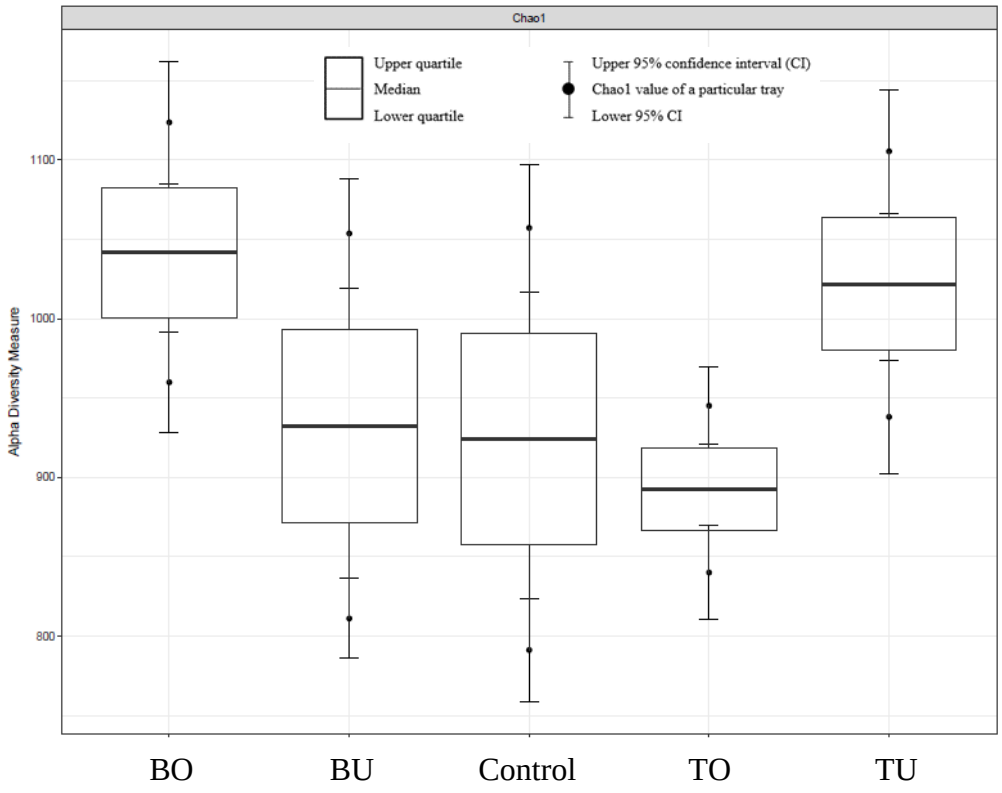


Figure 3.28. Alpha diversity (Chao1) across tray treatments in 2015

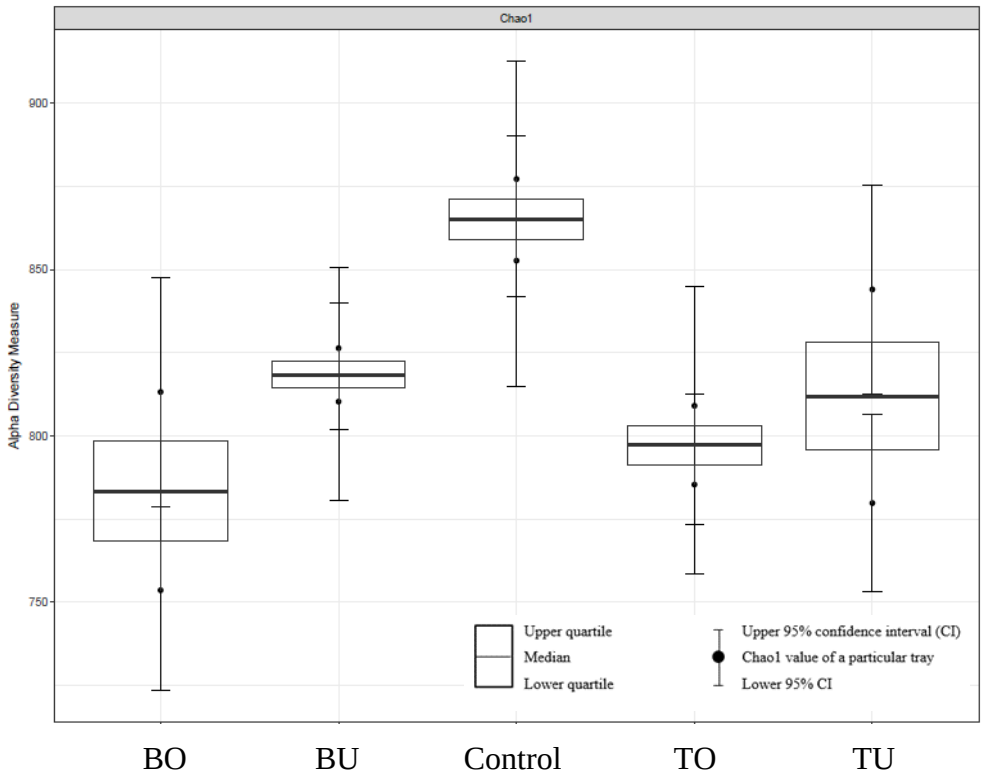


Figure 3.29. Alpha diversity (Chao1) across tray treatments in 2014

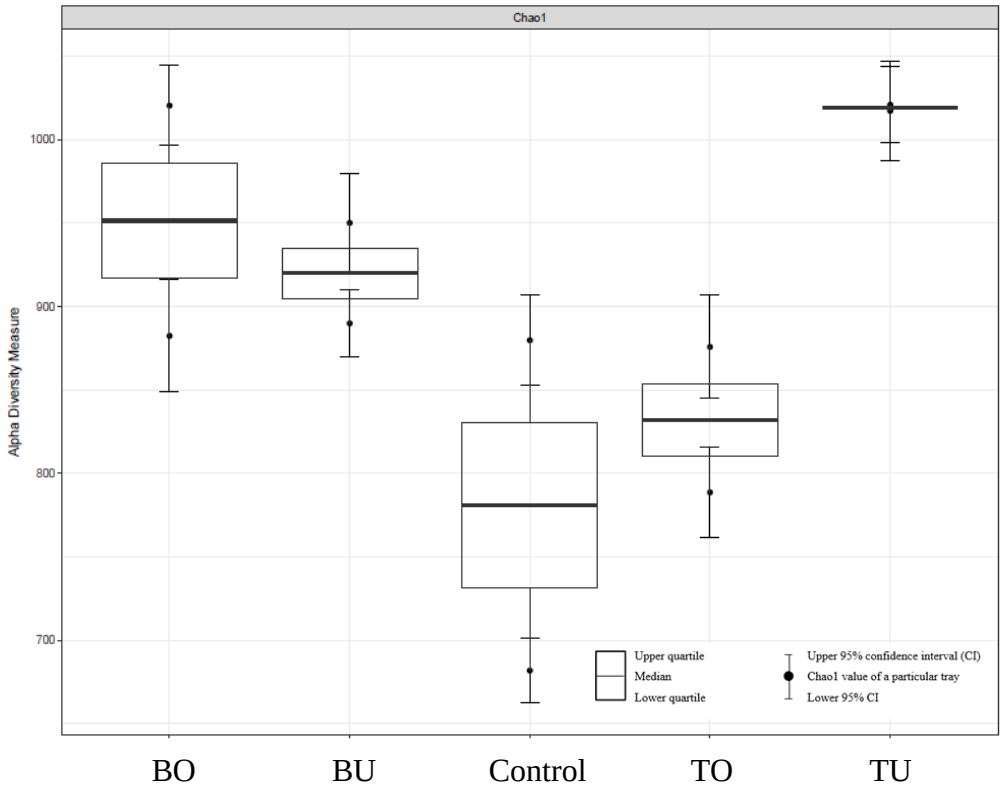


Figure 3.30. Alpha diversity (Chao1) across tray treatments in 2013

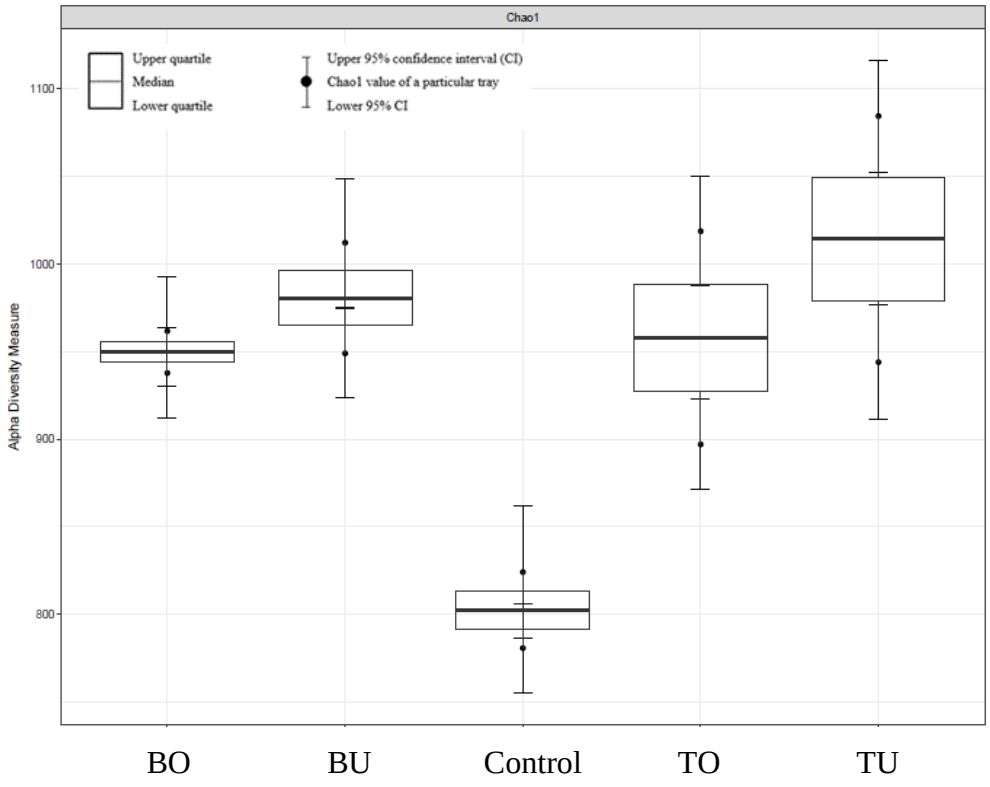


Figure 3.31. Alpha diversity (Chao1) across tray treatments in 2012

Appendix 3

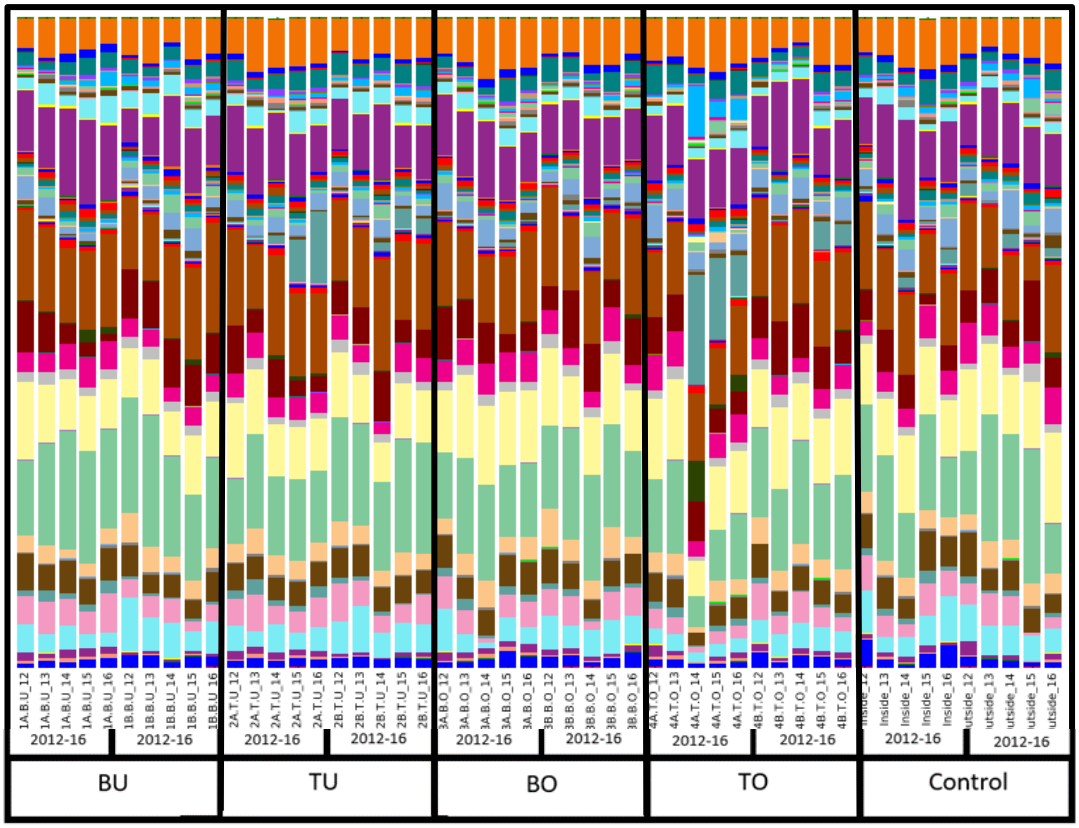
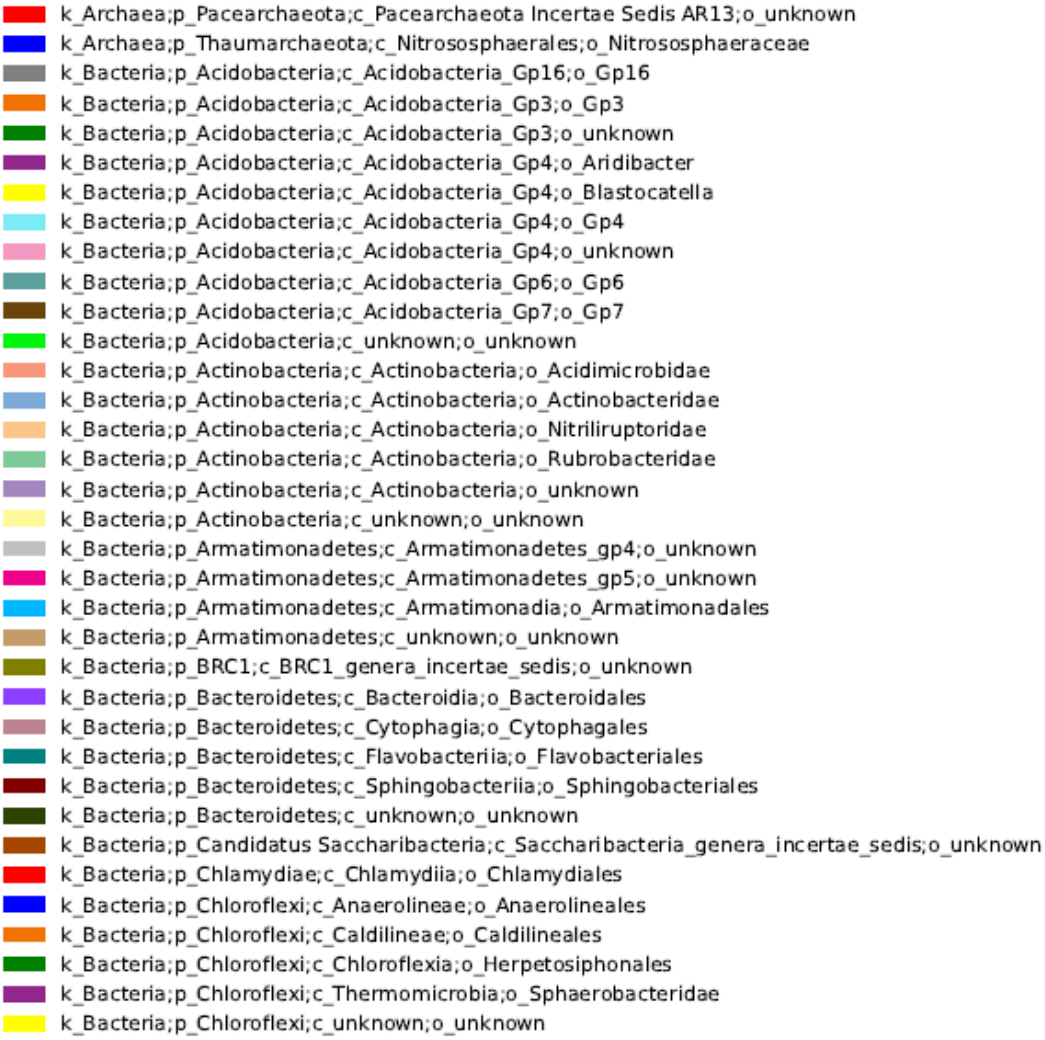


Figure 3.32. Relative abundance of different order for each tray treatment

Years 2012-2016 reading from left to right in each quadrant for each duplicate treatment.


k_Archaea;p_Pacearchaeota;c_Pacearchaeota Incertae Sedis AR13;o_unknown
k_Archaea;p_Thaumarchaeota;c_Nitrososphaerales;o_Nitrososphaeraceae
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp16;o_Gp16
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp3;o_Gp3
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp3;o_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp4;o_Aridibacter
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp4;o_Blastocatella
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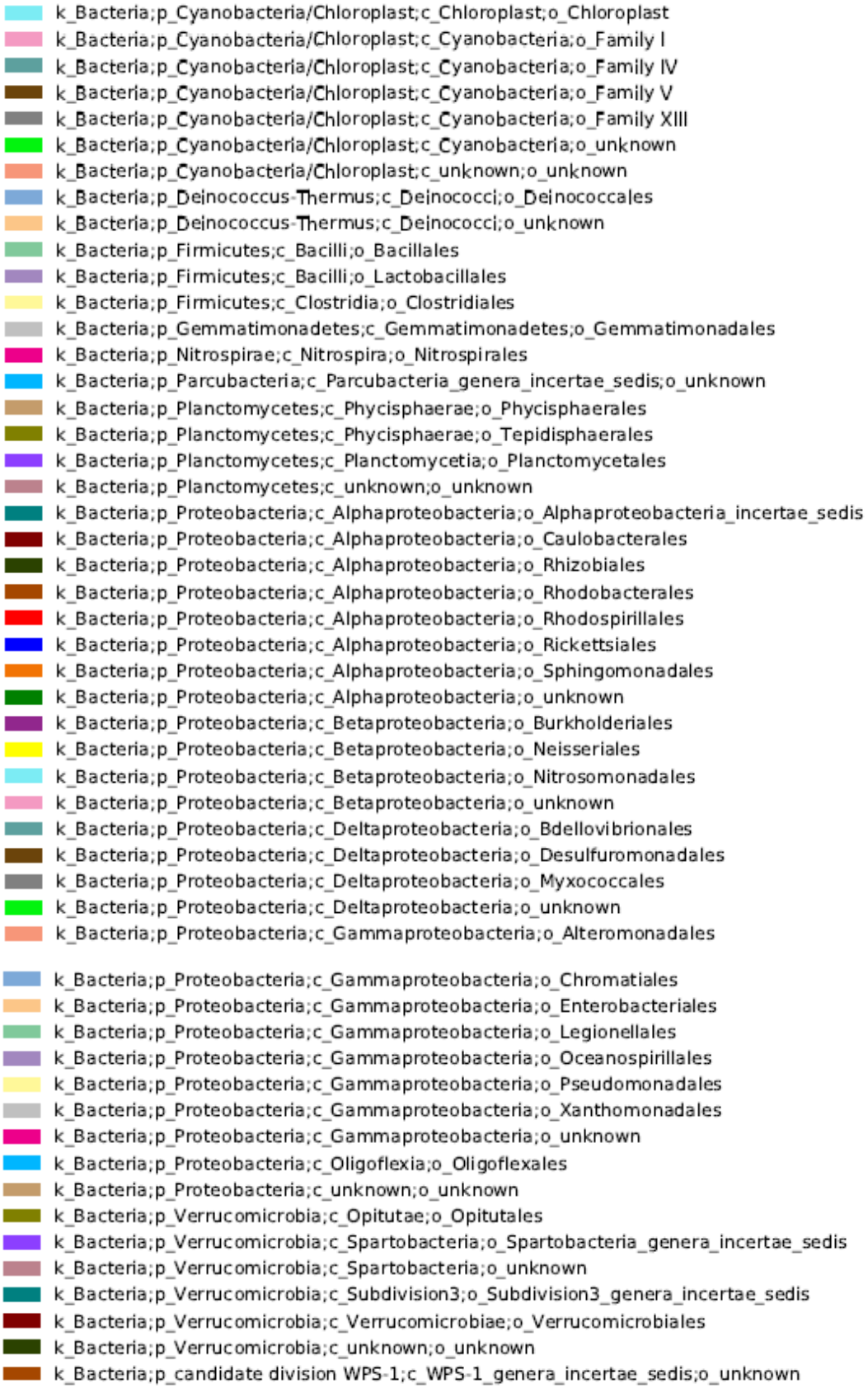
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k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales
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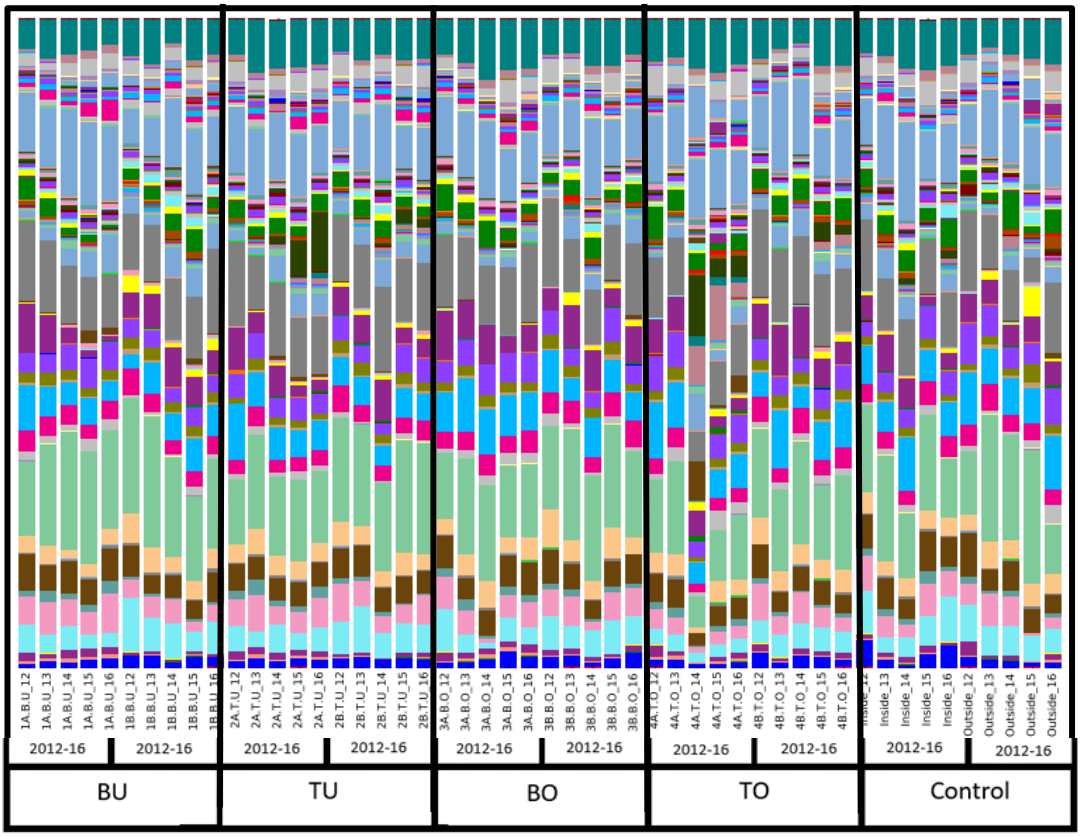
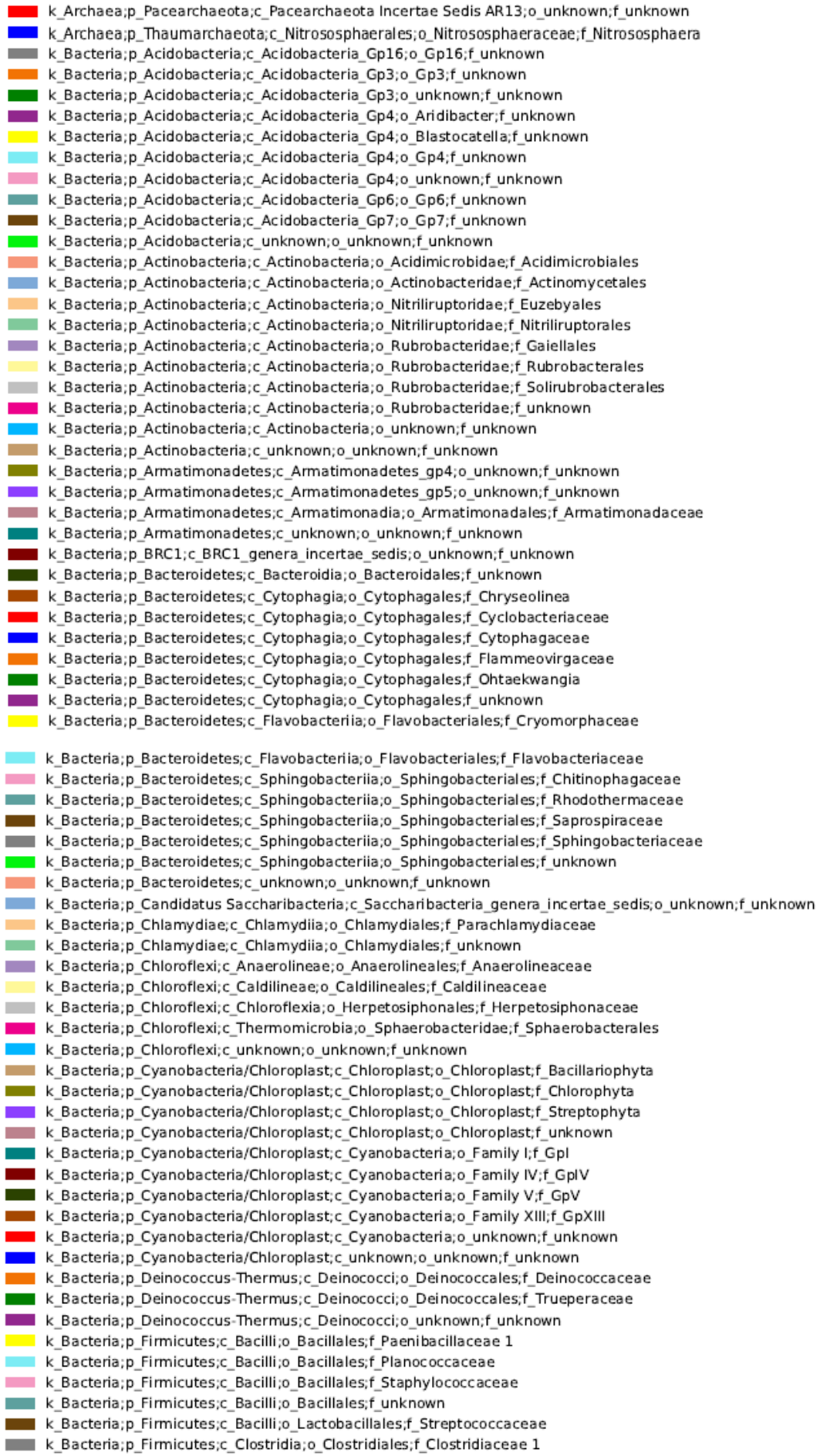


Figure 3.33. Relative abundance of different family for each tray treatment

Years 2012-2016 reading from left to right in each quadrant for each duplicate treatment.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k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Nitriliruptoridae;f_Euzebyales
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Nitriliruptoridae;f_Nitriliruptorales
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_Gaiellales
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k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Planococcaceae
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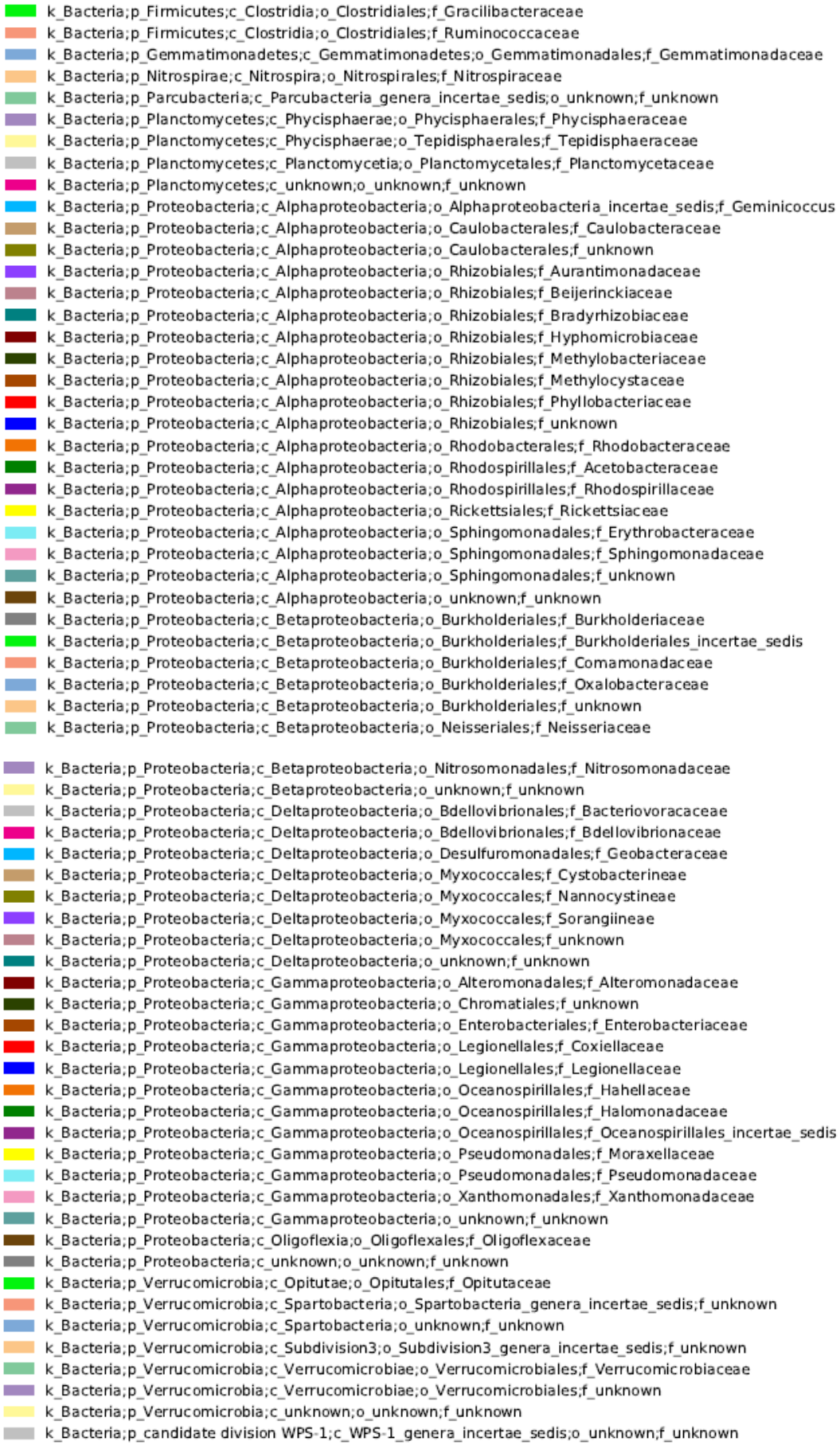
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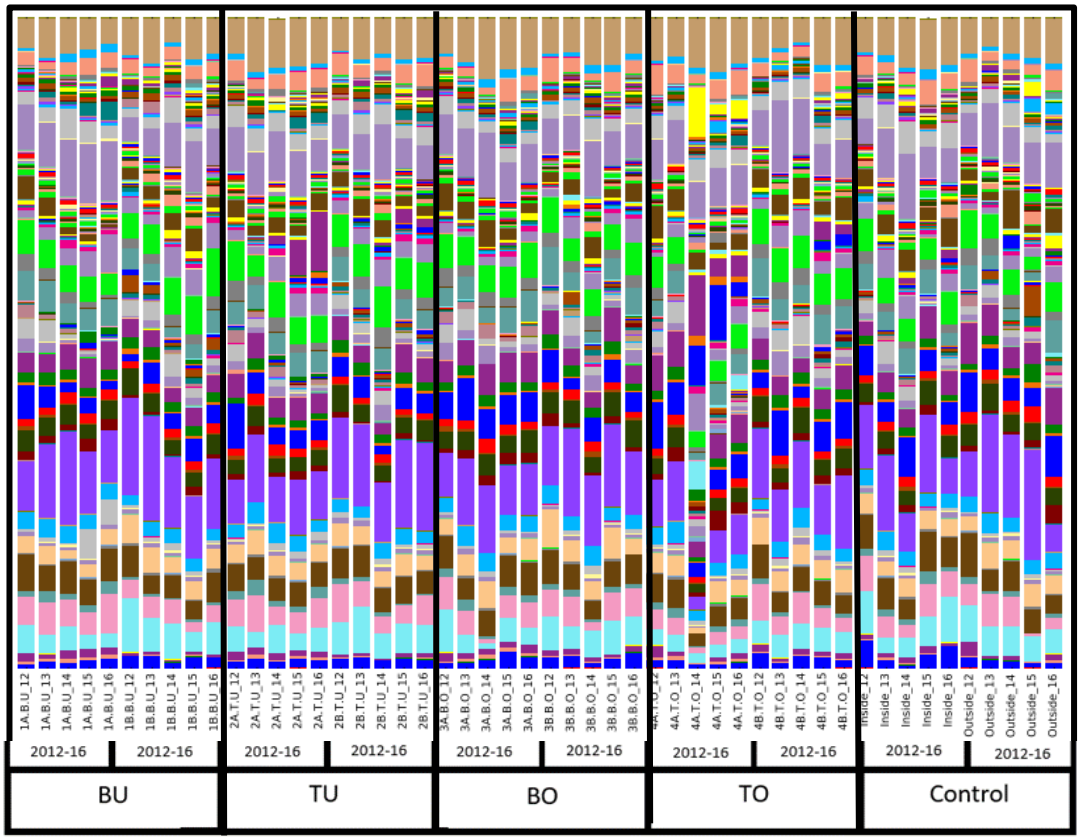
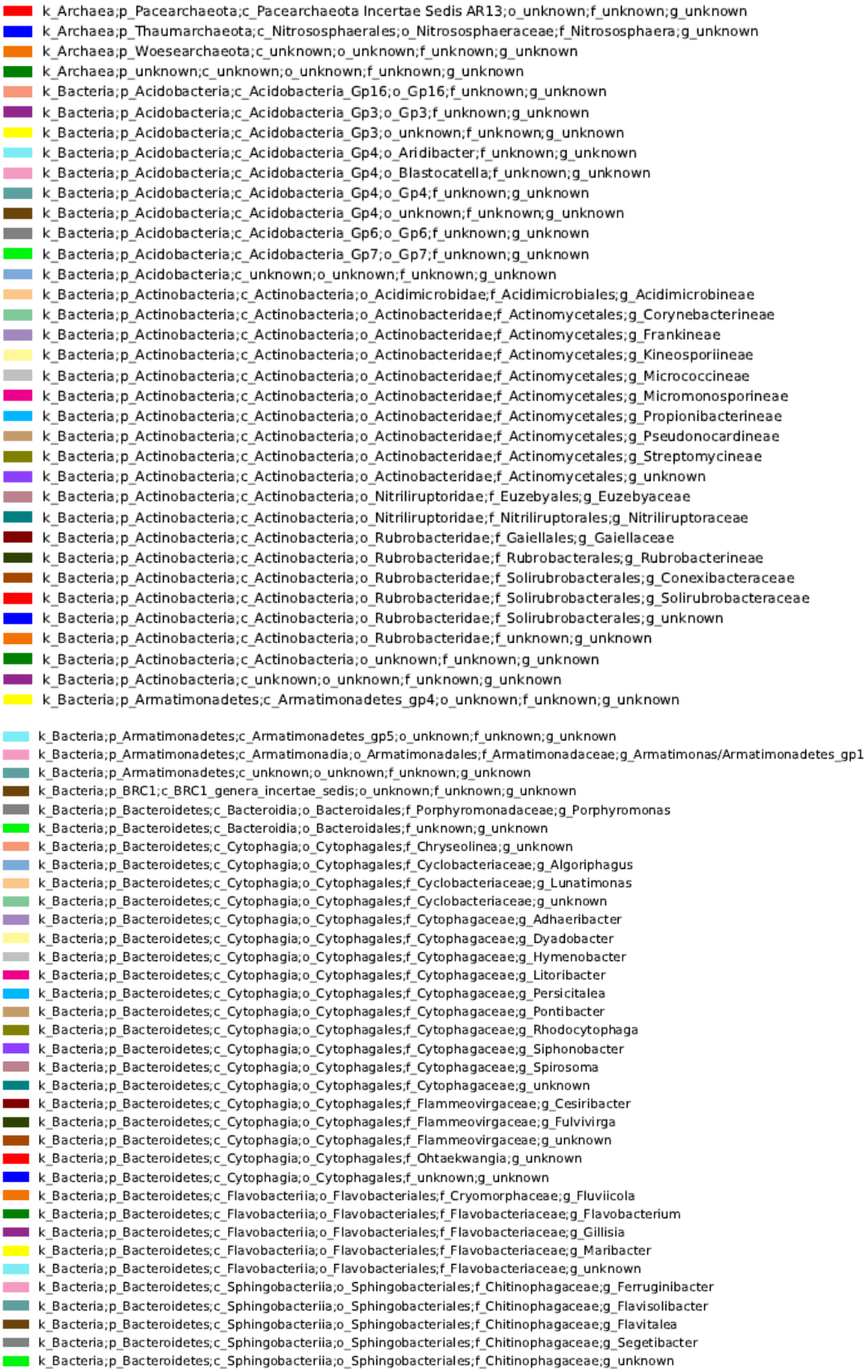


Figure 3.34. Relative abundance of different genus for each tray treatment

Years 2012-2016 reading from left to right in each quadrant for each duplicate treatment.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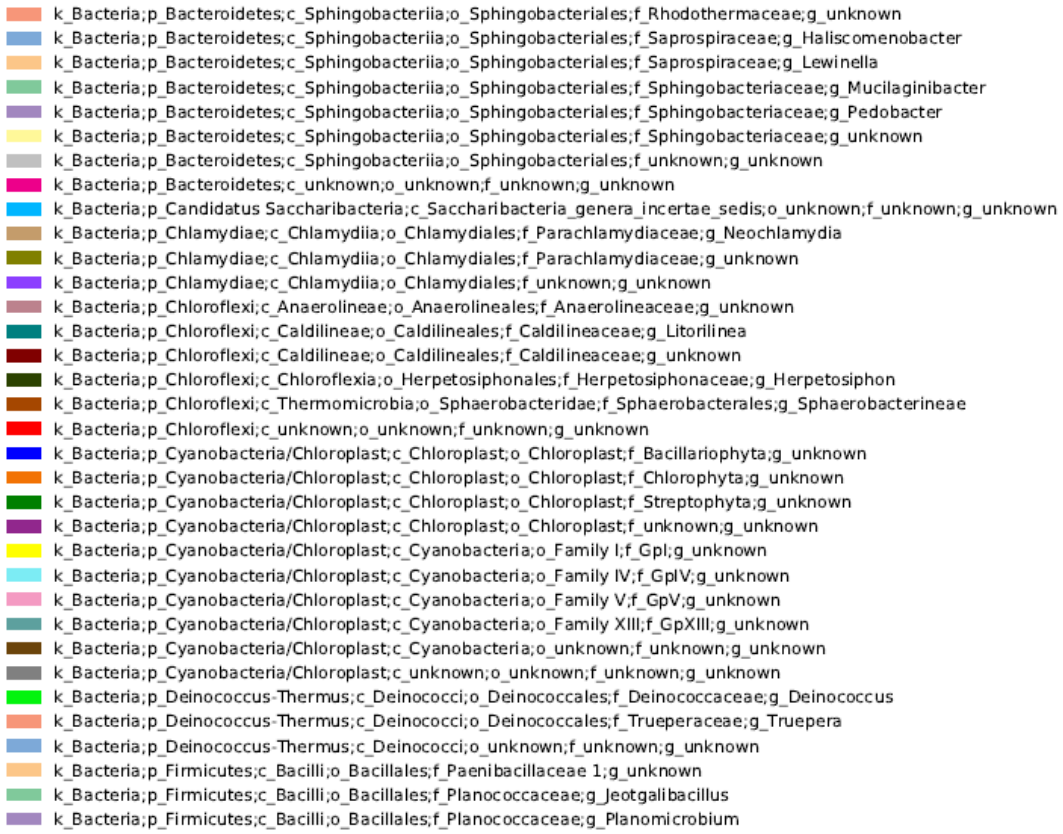
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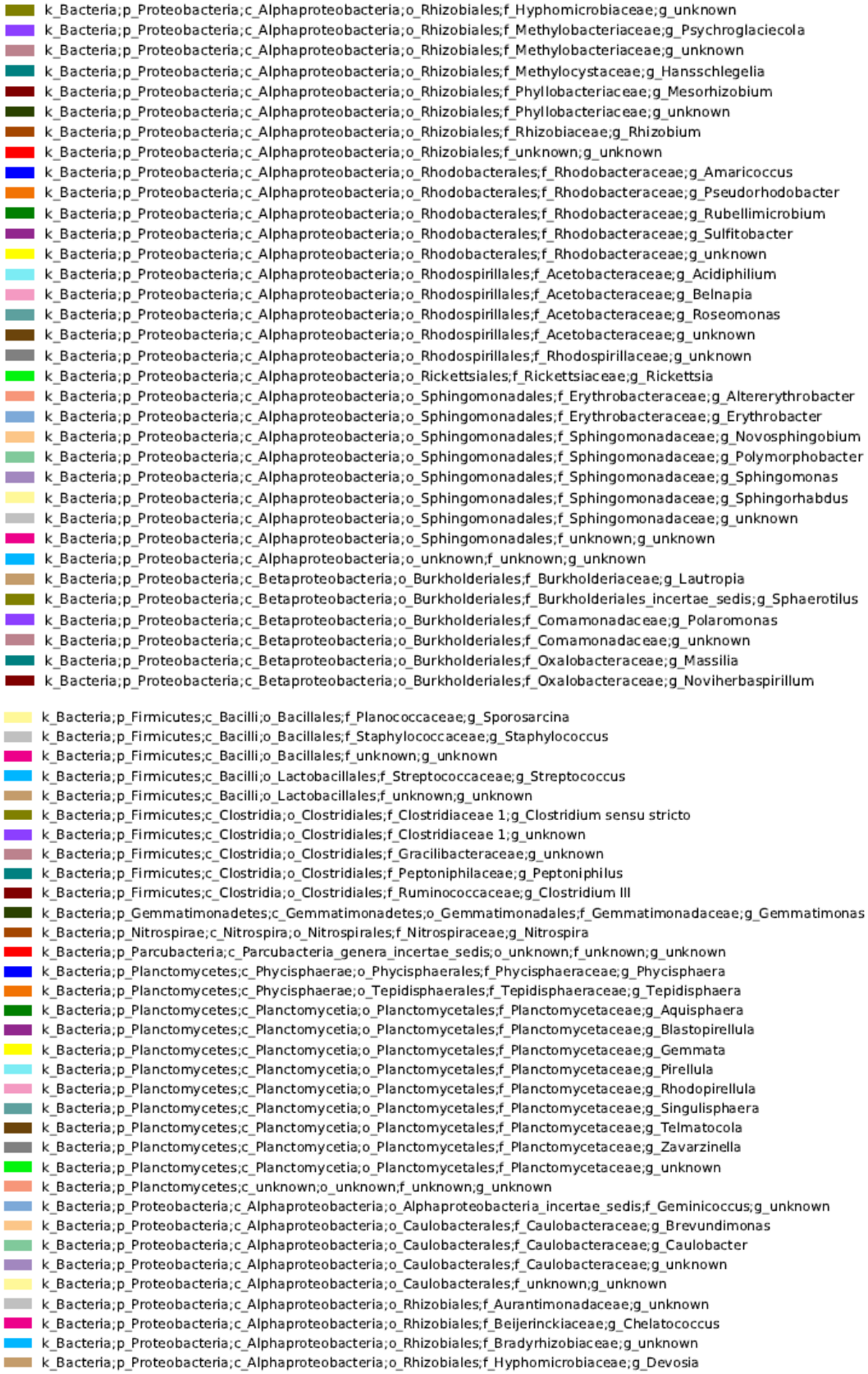
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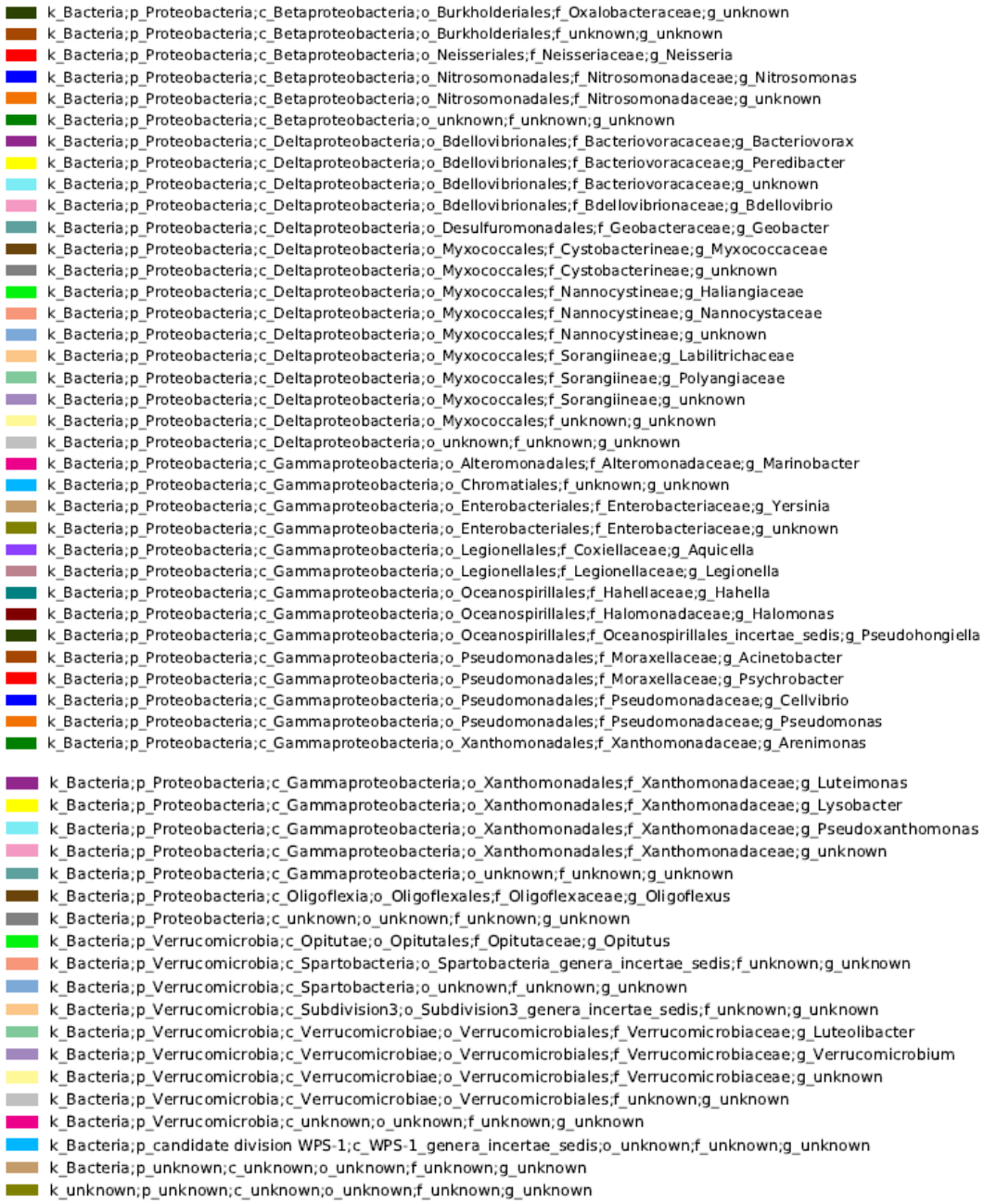
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k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Psychrobacter
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Cellvibrio
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Arenimonas
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Luteimonas
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Lysobacter
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Pseudoxanthomonas
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_unknown
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_unknown;f_unknown;g_unknown
k_Bacteria;p_Proteobacteria;c_Oligoflexia;o_Oligoflexales;f_Oligoflexaceae;g_Oligoflexus
k_Bacteria;p_Proteobacteria;c_unknown;o_unknown;f_unknown;g_unknown
k_Bacteria;p_Verrucomicrobia;c_Opitutae;o_Opitutales;f_Opitutaceae;g_Opitutus
k_Bacteria;p_Verrucomicrobia;c_Spartobacteria;o_Spartobacteria_genera_incertae_sedis;f_unknown;g_unknown
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k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;g_unknown
k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_unknown;g_unknown
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k_unknown;p_unknown;c_unknown;o_unknown;f_unknown;g_unknown

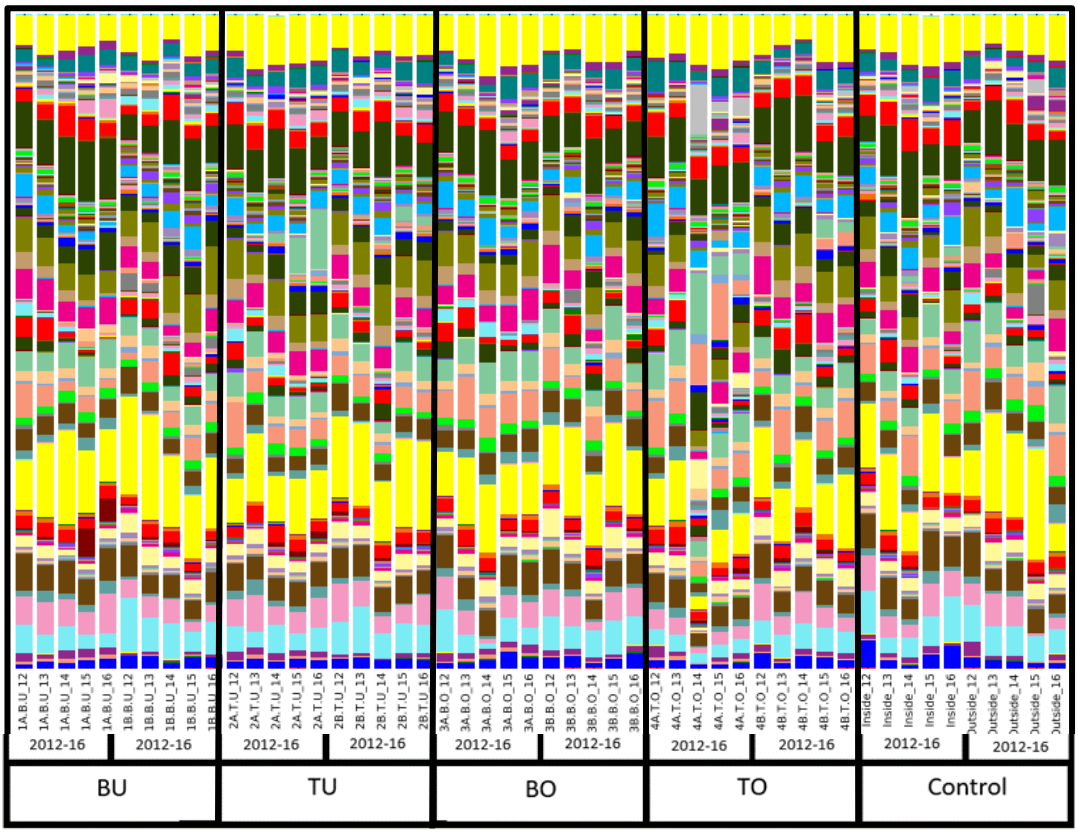

Figure 3.35. Relative abundance of different species for each treatment

Years 2012-2016 reading from left to right in each quadrant for each duplicate treatment.

k_Archaea;p_Pacearchaeota;c_Pacearchaeota Incertae Sedis AR13;o_unknown;f_unknown;g_unknown;s_unknown
k_Archaea;p_Thaumarchaeota;c_Nitrososphaerales;o_Nitrososphaeraceae;f_Nitrososphaera;g_unknown;s_unknown
k_Archaea;p_Woesearchaeota;c_unknown;o_unknown;f_unknown;g_unknown;s_unknown
k_Archaea;p_unknown;c_unknown;o_unknown;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp16;o_Gp16;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp3;o_Gp3;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp3;o_unknown;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp4;o_Aridibacter;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp4;o_Blastocatella;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp4;o_Gp4;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp4;o_unknown;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp6;o_Gp6;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_Acidobacteria_Gp7;o_Gp7;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Acidobacteria;c_unknown;o_unknown;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Acidimicrobidae;f_Acidimicrobiales;g_Acidimicrobineae;s_Acidimicrobiaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Acidimicrobidae;f_Acidimicrobiales;g_Acidimicrobineae;s_Acidimicrobineae_incertae_sedis
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Acidimicrobidae;f_Acidimicrobiales;g_Acidimicrobineae;s_Iamiaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Acidimicrobidae;f_Acidimicrobiales;g_Acidimicrobineae;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Corynebacterineae;s_Corynebacteriaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Frankineae;s_Geodermatophilaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Frankineae;s_Nakamurellaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Frankineae;s_Sporichthyaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Frankineae;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Kineosporiineae;s_Kineosporiaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Micrococcineae;s_Intrasporangiaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Micrococcineae;s_Microbacteriaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Micrococcineae;s_Micrococcaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Micrococcineae;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Micromonosporineae;s_Micromonosporaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Propionibacterineae;s_Nocardioidaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Propionibacterineae;s_Propionibacteriaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Propionibacterineae;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Pseudonocardineae;s_Pseudonocardiaceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinobacteridae;f_Actinomycetales;g_Streptomycineae;s_Streptomycetaceae
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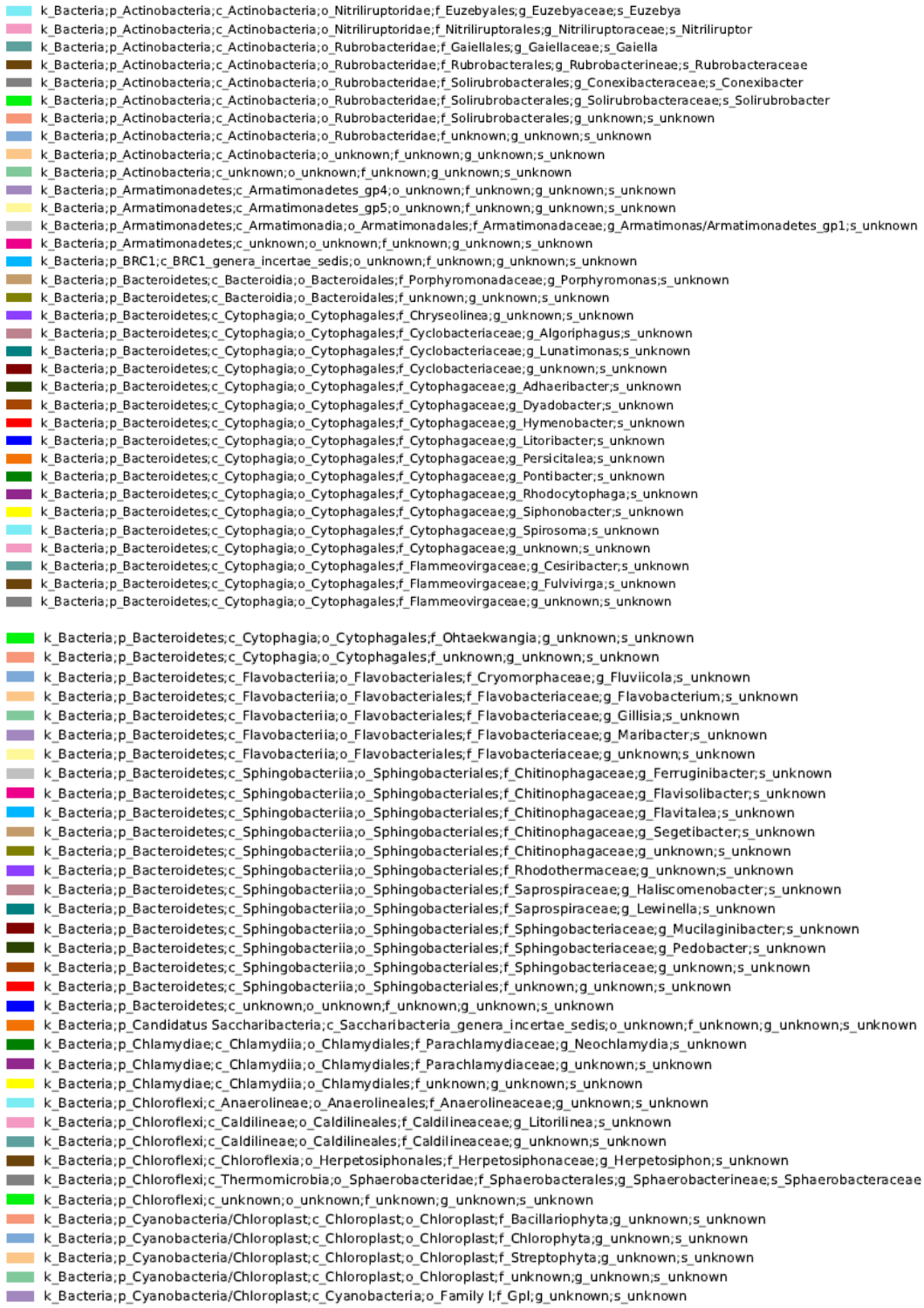
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k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_Gaiellales;g_Gaiellaceae;s_Gaiella
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_Rubrobacterales;g_Rubrobacterineae;s_Rubrobacteraceae
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_Solirubrobacterales;g_Conexibacteraceae;s_Conexibacter
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_Solirubrobacterales;g_Solirubrobacteraceae;s_Solirubrobacter
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_Solirubrobacterales;g_unknown;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Rubrobacteridae;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_unknown;f_unknown;g_unknown;s_unknown
k_Bacteria;p_Actinobacteria;c_unknown;o_unknown;f_unknown;g_unknown;s_unknown
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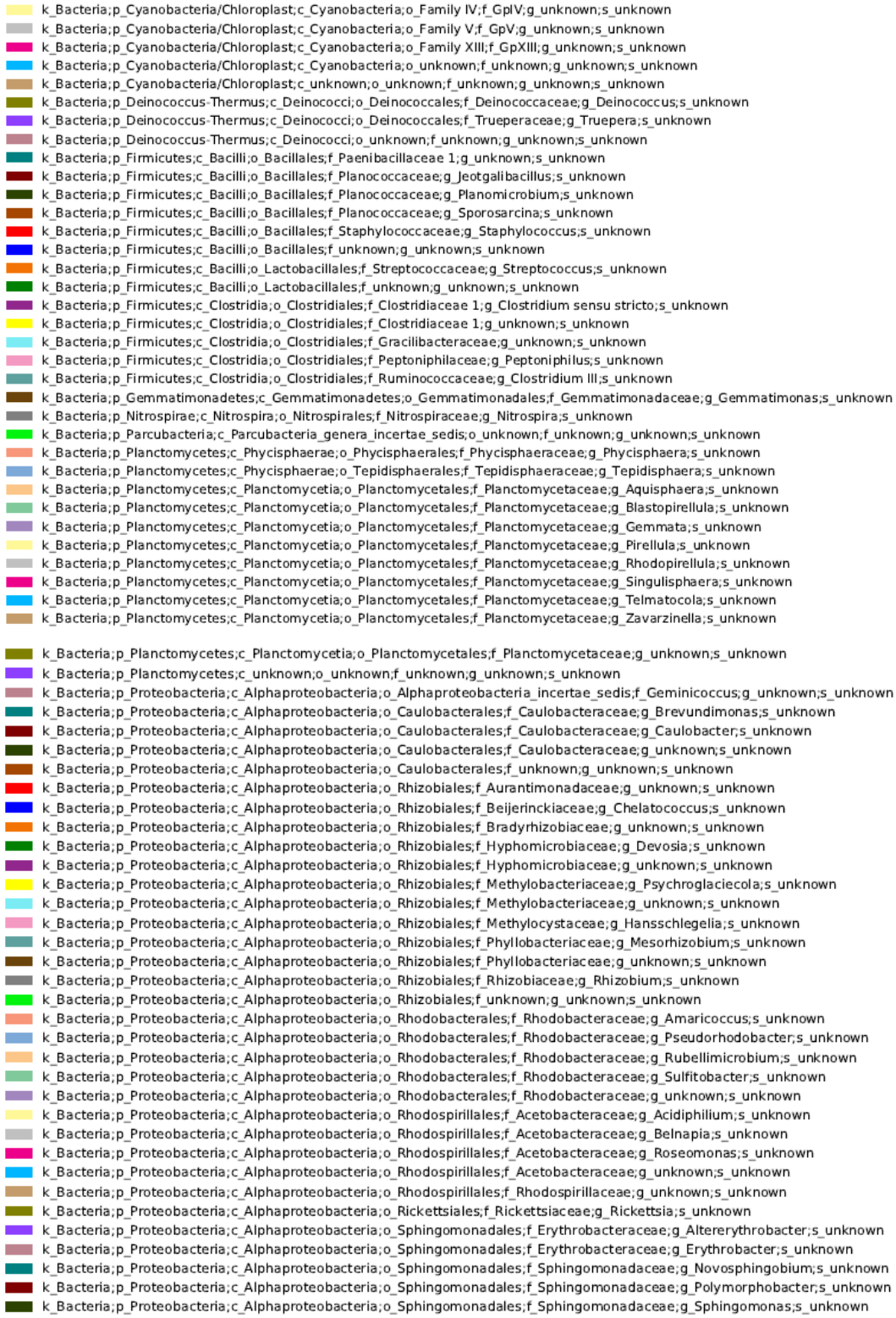
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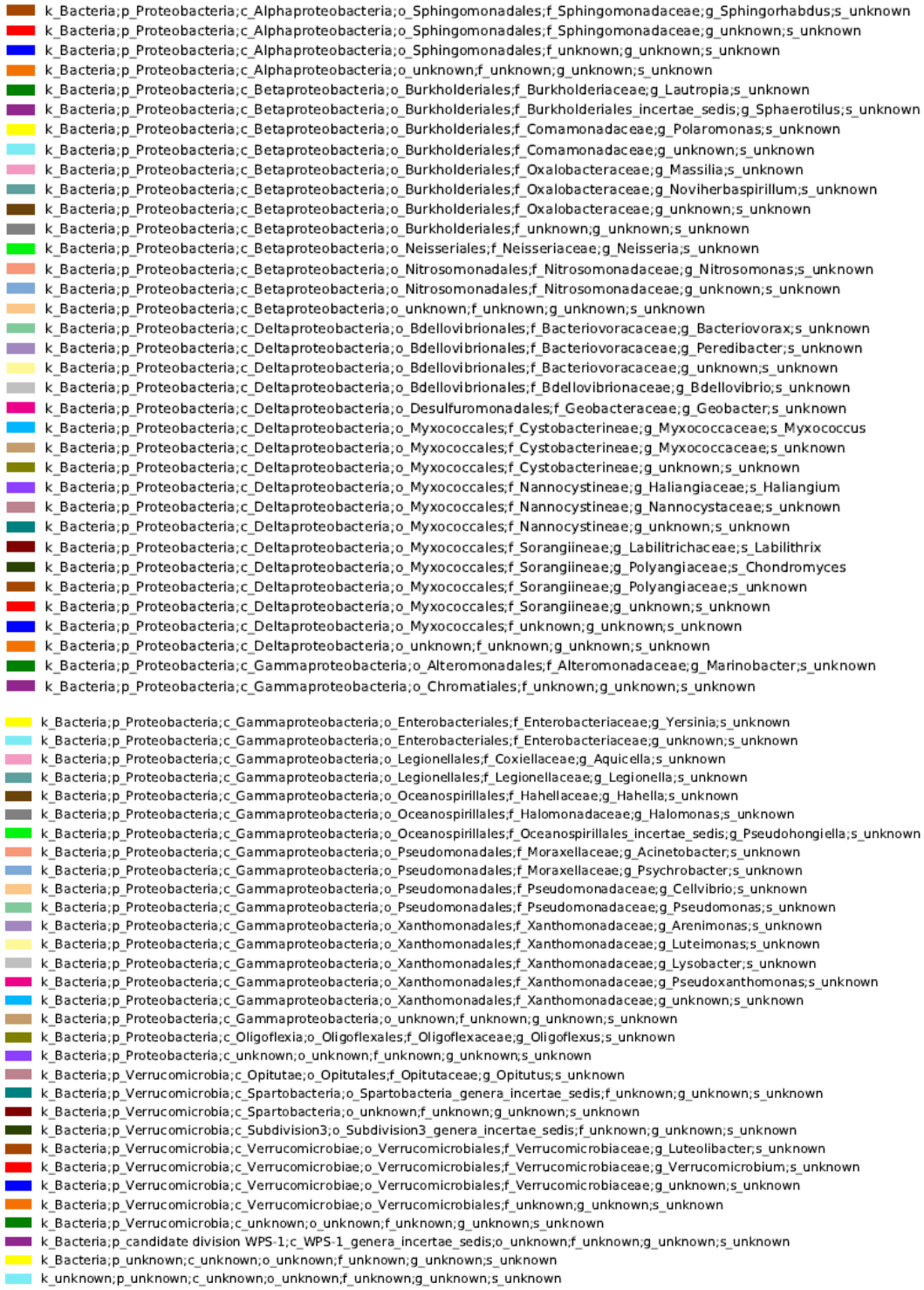
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Appendix 4

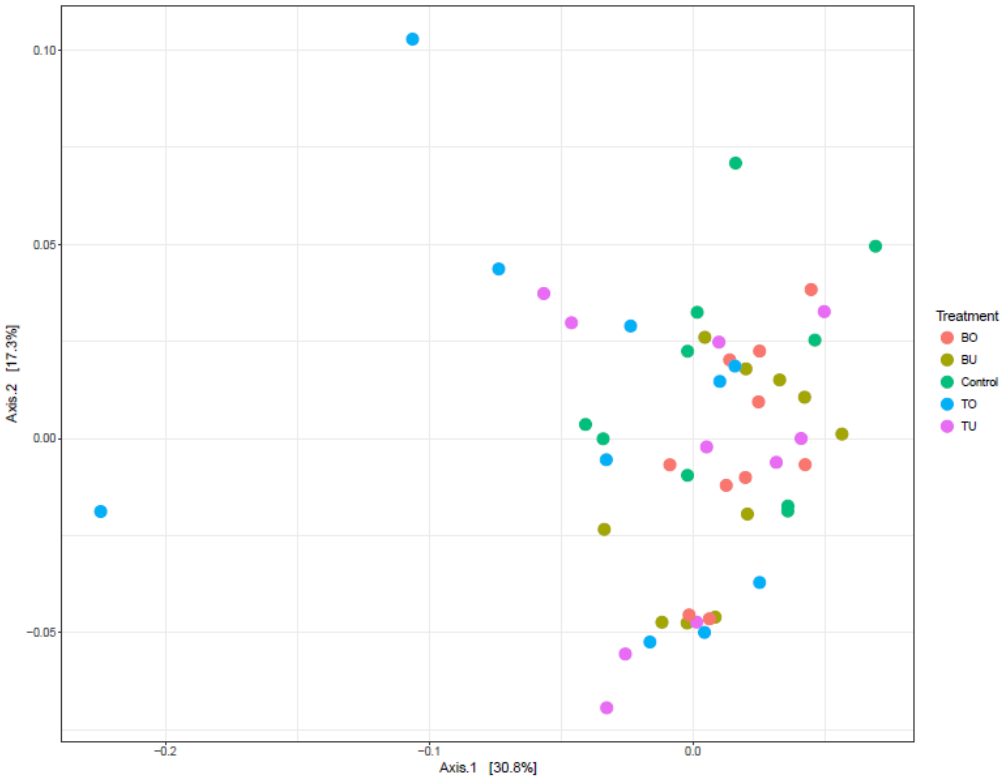

Figure 3.36. PCoA plot of tray treatments (2012-2016)

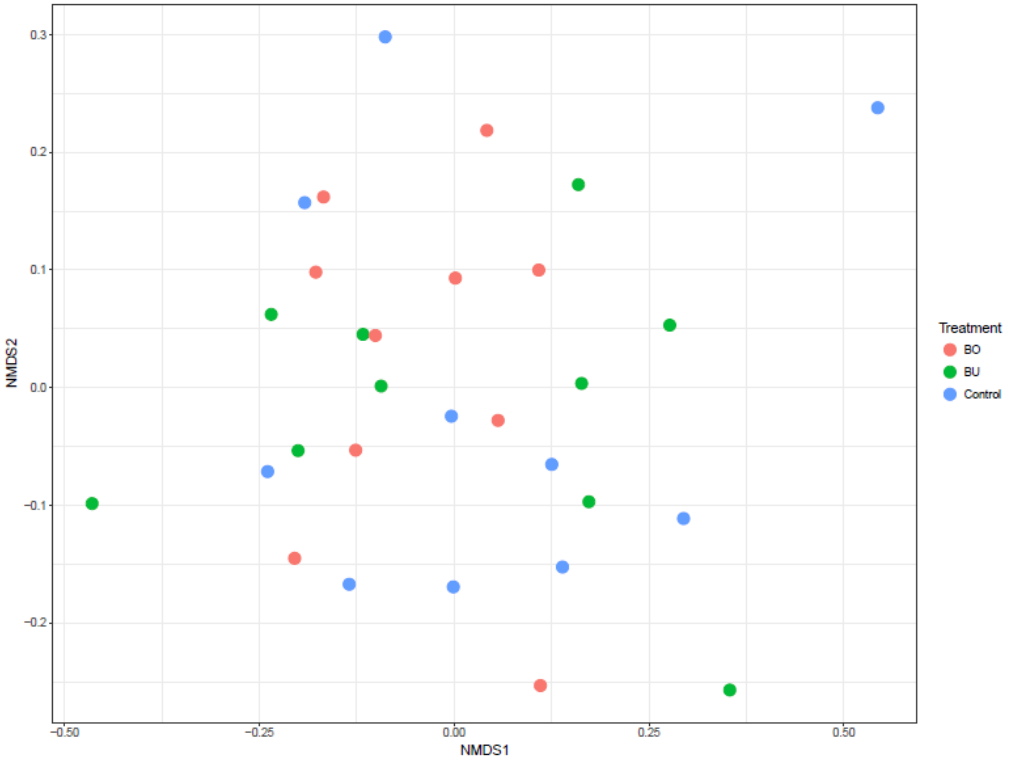

Figure 3.37. NMDS plot of BO and BU tray treatments (2012-2016)

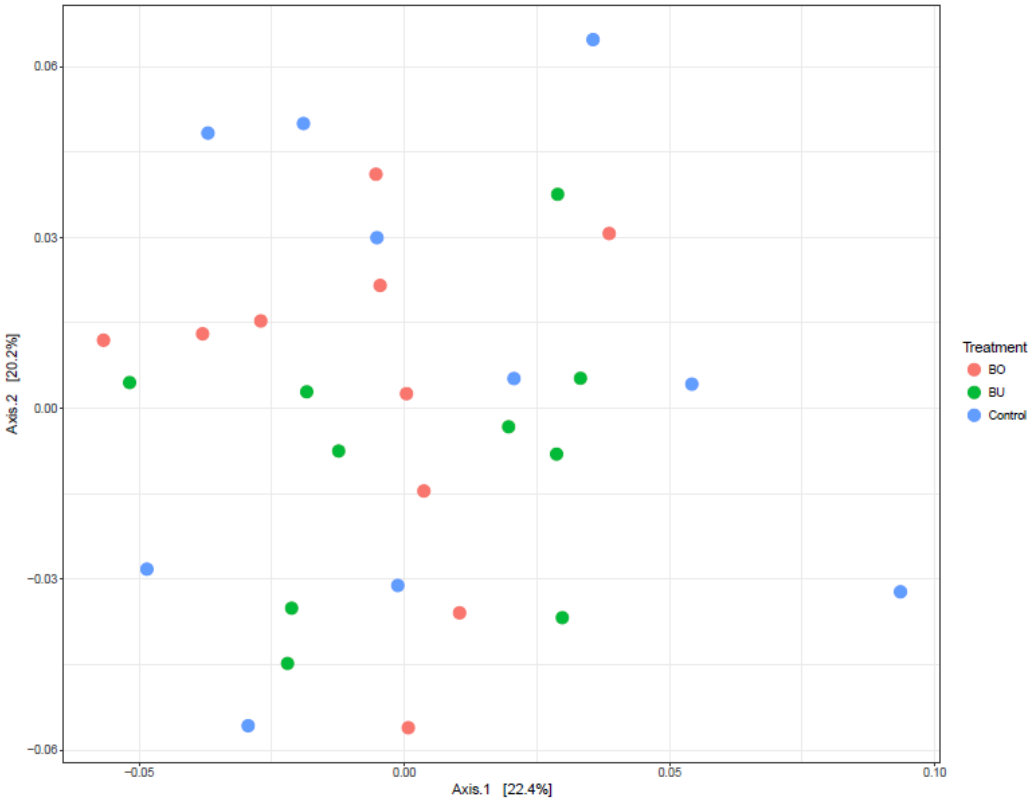

Figure 3.38. PCoA of BO and BU tray treatments (2012-2016)

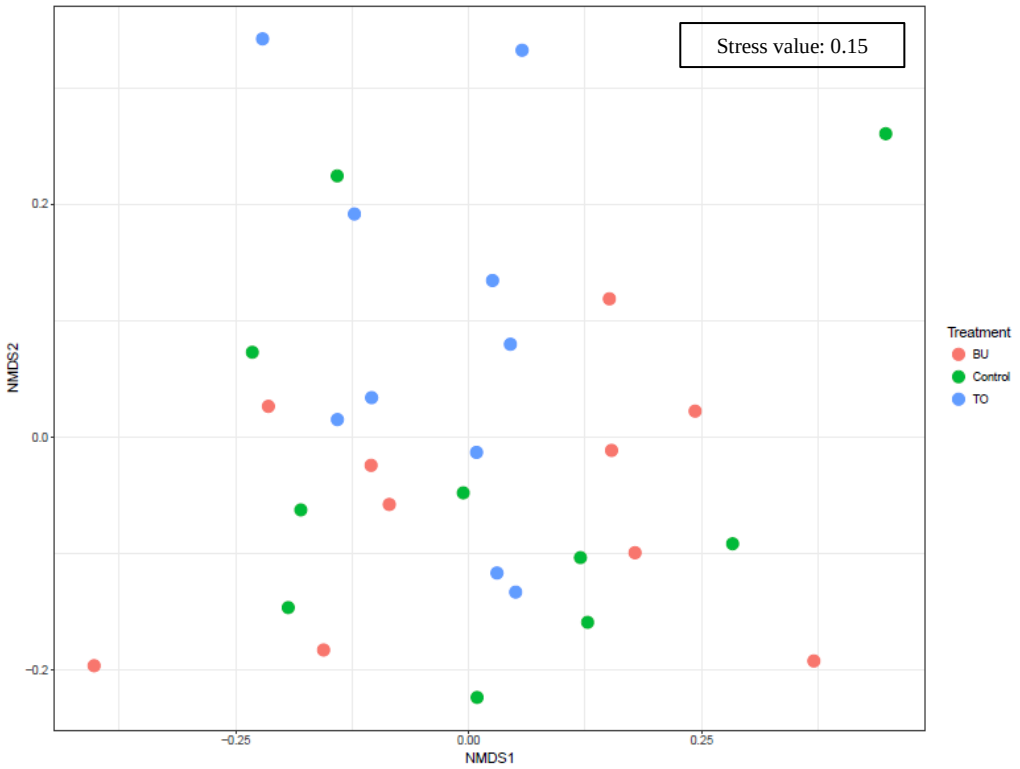

Figure 3.39. NMDS of BU and TO tray treatments (2012-2016)

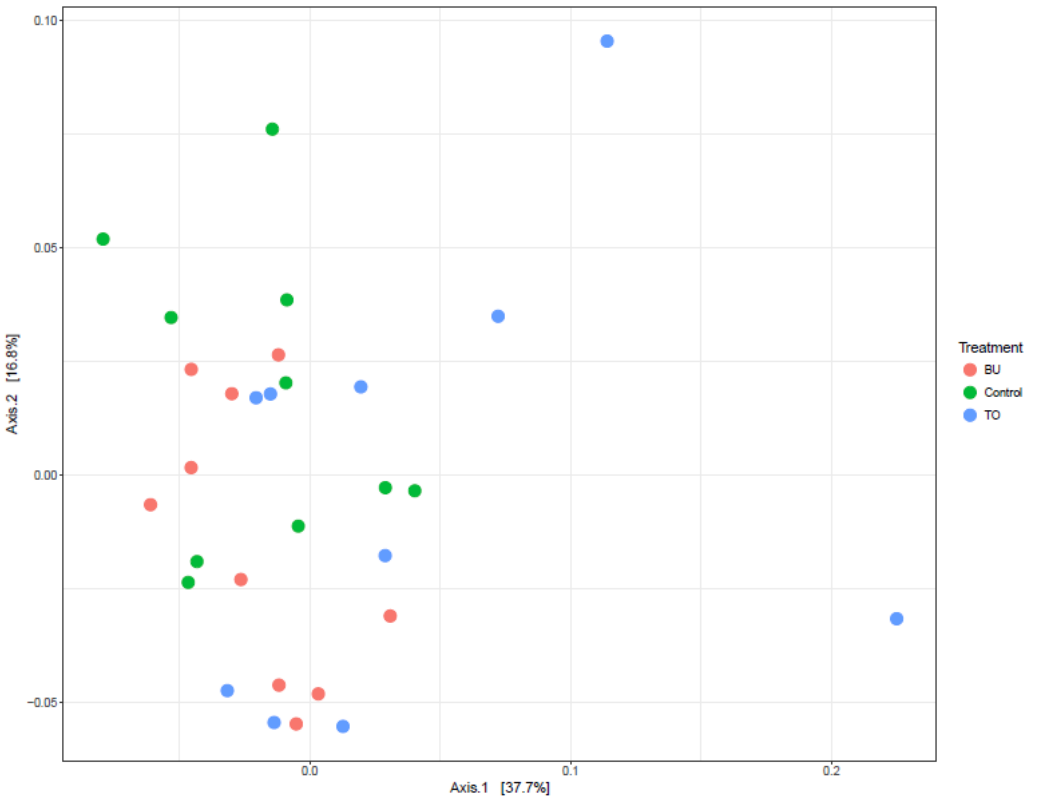

Figure 3.40. PCoA of BU and TO tray treatments (2012-2016)

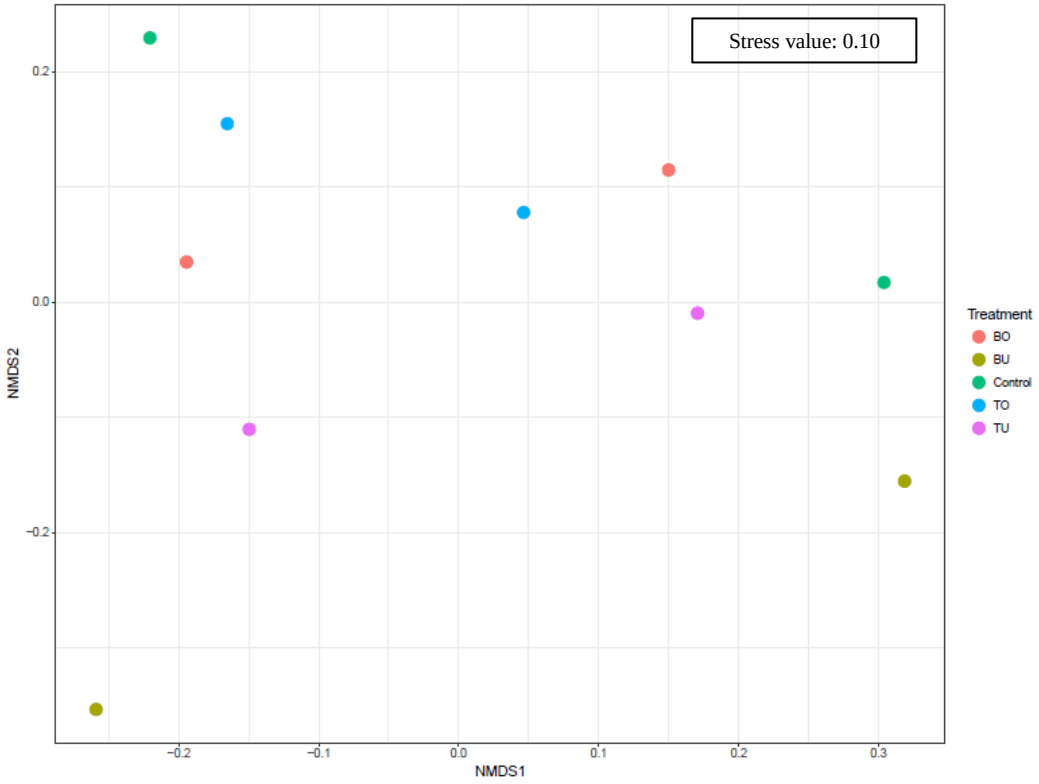

Figure 3.41. NMDS plot of 2016 tray treatment samples