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Environmental gradients drive biogeographic patterns in soil microbial communities

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Abstract

One of the central goals of the field of microbial biogeography is to better understand spatial patterns of microbial community diversity and how communities respond to gradients in environmental conditions, be they natural or anthropogenic in origin. The main aim of this thesis was to investigate how gradients in environmental conditions (i.e., across a mountain elevational gradient and across different land-use types) affect soil microbial community structure, diversity and functional traits, and to assess how these communities respond to differing environmental variables, using next-generation sequencing technologies.

Elevation gradients are commonly used to explore impact climate impacts on biological communities since declines in temperature with increased elevation can generate substantial climate gradients over small spatial scales. However, inconsistent spatial patterns in soil bacterial community structure observed across elevation gradients imply that communities are affected by a variety of factors at different spatial scales. Here, I investigated the biogeography of soil bacteria across broad (i.e., a ~ 1500 m mountain elevation gradient) and fine sampling scales (i.e., both aspects of a mountain ridge) using 16S rRNA gene sequencing. Across equivalent distances, variation in bacterial community composition changed more with variation in site aspect than elevation. Bacterial community composition and richness were most strongly associated with soil pH, despite the large variability in multiple soil climate variables across the site. These findings highlight the need to incorporate knowledge of multiple factors, including site aspect and soil pH for the appropriate use of elevation gradients as a proxy to explore the impacts of climate change on microbial community composition.

Similar to bacterial communities, inconsistent elevational patterns in soil fungal community diversity suggest that these communities are driven by a complex underlying mechanism. Thus, to enhance understanding of whether distinct biogeographic patterns can be distinguished between different microorganisms and how such gradients influence the potential interactions among individual taxa, I assessed variation in the co-occurrence of different fungal taxa at different elevations along the aforementioned mountain ridge, using fungal internal transcribed spacer (ITS1) DNA sequencing. Fungal community composition changed significantly along the gradient, and their co-occurrences were less frequent with increasing elevation. Such changes with elevation were associated with soil nutrient concentrations, likely driven by the relative ability of different taxa to compete for nutrients at different environmental concentrations. Evidence of nutrient-driven shifts in fungal community diversity and function in soil will enhance our understanding of underground nutrient cycling and the likely impacts of climate change and agricultural disturbance on soil microbial communities.

To further explore gradients in the functional potential of soil bacterial communities along an elevation gradient, I devised a method to ‘infer’ metagenomics data from bacterial 16S rRNA gene sequences. I evaluated the applicability of my ‘inferred metagenomics’ approach, by comparing bacterial community composition derived from the original bacterial data to communities derived only from the 400 taxa for which genomic information is available. The results generated from these two datasets were highly similar, suggesting that the subset of ‘inferred’ community was largely reflective of that of the wider environmental community. Further analysis indicates that bacteria with larger genome size appear to prevail across the elevation gradient, suggesting that microorganisms might successfully cope with harsh or various environmental conditions by retaining a larger burden of potential genes and related functions. These findings highlight the potential for using inferred genomic information, based on bacterial 16S rRNA gene data, to generate a general functional trait-based picture of microbial biogeographical patterns.

Apart from studies on elevational patterns of soil microbial communities, many other environmental gradients impact distributions of bacterial communities, including gradients of anthropogenic disturbance. Therefore, I studied how pastoral land management practices affect soil bacteria, both in agricultural soils and adjacent forest fragments along 21 transects bisecting pasture-forest boundaries. Decreased compositional dispersion of bacterial communities in the grazed pasture soils resulting in a net loss of diversity caused by community homogenisation after forest-to-pasture conversion. Additionally, a greater richness of pasture-only taxa for sites with a fence on the boundary between the two land uses revealed that boundary fences play an important role in protecting the integrity of soil bacterial communities in forests surrounded by agricultural land via restricting livestock invasion. The observed variation in bacterial community richness and composition was most related to changes in soil physicochemical variables commonly associated with agricultural fertilisation. Overall, my findings demonstrate clear, and potentially detrimental, effects of agricultural disturbance on bacterial communities in forest soils adjacent to pastoral land.

This thesis reports the findings of a comprehensive evaluation of the impact of different environmental gradients on soil microbial community composition and functional potential, encompassing sample data collected across different spatial scales and land use types, as well as between different microbial phylogenetic groups. These results confirm that spatial patterns in both bacterial and fungal community structure are driven by various interacting environmental variables related with natural gradients or agricultural disturbances.

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Chapter 1

General Introduction

1.1 Microbial life on Earth

Enormous numbers of taxonomically, phylogenetically, and functionally diverse microorganisms exist in the soil. For example, a teaspoon of soil may contain approximately 1×10^9 bacteria and 1×10^6 fungi (Ingham, 2011). Microorganisms, including bacteria, fungi, archaea, viruses and protists, have a direct influence on a wide range of ecosystem processes, such as nitrogen fixation, carbon and nutrient cycling, decomposition and other processes (Fierer and Jackson, 2006; Prosser et al., 2007; Fuhrman, 2009; Bardgett and van der Putten, 2014). Indeed, due in part to their high diversity, the structure of microbial communities is important for the way in which ecosystems function. To better understand soil microbial processes and perhaps to predict Earth's response to future global change, such as climate change and ocean acidification, scientists need to have a better knowledge of the strength and forms of relationships between bacterial communities and the environments that they inhabit (Fuhrman, 2009).

The discipline of biogeography explores the distribution of organisms across space and time (Lomolino et al., 2010; Hanson et al., 2012). One of the central goals in this field is to understand spatial patterns of biological diversity and what these biogeographic patterns reveal about the processes that drive microbial community diversity and functioning (Lavorel and Garnier, 2002). Since at least the eighteenth century, biogeographers have investigated the distributions of macroorganisms, i.e., plants and animals (Linnaeus, 1781; Green et al., 2008). More recently, microbiologists have partially overcome the geographic distributions of microorganisms, including bacteria, fungi, archaea, viruses and other microbial eukaryotes (Martiny et al., 2006; Fierer, 2008). In recent decades, the development of genetic methodologies has alleviated some of the problems associated with historical culture and microscopy-based biases, which are well understood to miss a majority of bacterial diversity (Ward et al., 1990; Amann et al., 1995; Ferrari et al., 2005; Fuhrman, 2009). Advances in molecular methods allow a more comprehensive view of microbial diversity than could have been achieved just a few decades ago (Schloss and Handelsman, 2004; Venter et al., 2004; Pedros-Alio, 2006). Microbial geography stands to benefit tremendously from multiple advances in molecular tools, confirming that microorganisms display biogeographic

patterns, some of which are similar to those of macroorganisms (Green and Bohannan, 2006; Ramette and Tiedje, 2007; Lindström and Langenheder, 2012), while differing in other regards (Fierer and Jackson, 2006; Fierer et al., 2010; Meier et al., 2010; Meng et al., 2013).

1.2 Do microorganisms exhibit biogeography?

A long-held concept in microbial ecology “*everything is everywhere, [but] the environment selects*”, often referred to as the Baas-Becking hypothesis (Baas Becking, 1934), prompted the formulation of later theories in microbial biogeography, such as exploration of the relative roles of species sorting for microbial community composition (Leibold et al., 2004; Holyoak et al., 2005). The early work by Baas Becking (1934) prompted an increase in the frequency of studies on microbial biogeographic patterns that continues to this day, and is still often used as the question-raising starting point of studies (Garcia-Pichel et al., 1996; Zwart et al., 1998; Staley and Gosink, 1999; Cho and Tiedje, 2000; Fenchel, 2003; Richards et al., 2005; Telford et al., 2006; Chu et al., 2010; Wilkinson et al., 2012; Fondi et al., 2016). The rapid and ongoing development of high-throughput technologies has greatly facilitated microbial community research (Riesenfeld et al., 2004; Eisen, 2007; Wooley et al., 2010; Bailey et al., 2013; Bergkemper et al., 2016; Yang et al., 2017). As the traditional culture-based methods of bacteria in soil only can recover about 1% of the bacterial species present (Amann et al., 1995; Ferrari et al., 2005), the emergence of high-throughput technologies in biology have overcome historical culture-based biases in microbiology. With the development of these technologies, microbiologists now are able to survey a large portion of the microbial diversity on Earth and to quantify the microbial biogeographic patterns in a variety of environments. While Baas-Becking proposed that the remarkable dispersal potential of microbes leads to community distributions commonly shaped by environmental conditions rather than geographical distance, using modern molecular approaches, scientists can study microbial gene distributions at a broad scale, therefore extending the Baas-Becking hypothesis from the single organism to microbial genes. For example, using metagenomics sequencing data, Fondi et al. (2016) found that overlapping microbial gene pools were likely to be found in geographically disparate environments and these gene pools were affected by their

ecological niche. Likewise, microbial mercury methylation genes can be detected across a broad range of environments, encompassing thawing permafrost soils, coastal soils, sediments and extreme environments (Podar et al., 2015). Such findings confirm the global dispersal potential of microbes and their respective genetic functional traits into disparate habitats and environments.

Although there was some debate about the existence of biogeographic patterns in microbial data, as can be found in earlier research (Fenchel et al., 1997; Staley, 1997; Finlay, 2002), a growing body of current microbial biogeography research provides overwhelming evidence that spatial patterns exist in soil microorganisms (Green et al., 2004; Horner-Devine et al., 2004; Green and Bohannan, 2006; Martiny et al., 2006; King et al., 2010; Lindström and Langenheder, 2012), aided by the aforementioned advances in DNA sequence analysis. The simplest evidence for microbial biogeography is that microbial community attributes across a landscape are non-random. Many studies have found spatial patterns in microbial community composition or richness across environmental or geographic gradients. Crucially, these patterns have been correlated with environmental parameters such as soil pH (Fierer and Jackson, 2006; Lauber et al., 2009; King et al., 2010; Shen et al., 2013; Hermans et al., 2017), salinity (Casamayor et al., 2002; Crump et al., 2004; Herlemann et al., 2011; Fortunato et al., 2012; Campbell and Kirchman, 2013), or with broad spatial attributes such as latitude as a proxy for climate/temperature change (Staddon et al., 1998; Schwalbach and Fuhrman, 2005; Pommier et al., 2007; Yergeau et al., 2007; Fuhrman et al., 2008; Lear et al., 2017b). More specifically, there are two dominant types of evidence that microorganisms display spatial patterns in their distribution. The first evidence is the existence of endemic microbial species, restricted to a particular location, region or habitat type (Hanson et al., 2012). As endemism is not evenly distributed on the Earth, the existence of free-living endemic species is perhaps the clearest demonstration of microbial biogeography. For example, some taxa are endemic to specific habitat types, such as those in habiting geothermal soils or hot springs (Whitaker et al., 2003; Stott et al., 2008; Takacs-Vesbach et al., 2008; Hug et al., 2014; Sharp et al., 2014). Another type of evidence is the exploration of patterns in microbial community similarity with geographic distance. Recent studies have found reductions in bacterial community similarity with increasing geographic distance (i.e., distance-decay relationships),

suggesting not only that the composition of microbial communities is different among locations, but that this variation is correlated with spatial distance (Green et al., 2004; Horner-Devine et al., 2004; Soininen et al., 2007). The distance-decay relationship has been widely observed for microorganisms in a wide range of habitats, and at a variety of taxonomic resolutions (Cho and Tiedje, 2000; Hewson et al., 2006; Casteleyn et al., 2010; Soininen et al., 2011).

Distance-decay relationships are further evidence for microbial biogeography, which reflects that the taxonomic composition of communities is often observed as becoming less similar with increasing geographic distances (Nekola and White, 1999; Green and Bohannan, 2006). This relationship received the early interest of Whittaker (1972) and Preston (1962) in their studies of plants, and became increasingly popular after Nekola and White (1999) formalised it. Distance-decay relations are mainly assumed to result from environmental changes occurring across distances as well as due to population dynamics (e.g., spatial variation in colonisation, extinction or speciation) (Hubbell, 2001; Zinger et al., 2014). Such relationships have been detected repeatedly across a wide range of macroorganisms, geographic gradients and environments (Condit et al., 2002; Tuomisto et al., 2003; Novotny et al., 2007; Qian and Ricklefs, 2007; Soininen et al., 2007). Now, microbiologists have reported similar patterns for microbial communities across both terrestrial and aquatic environments (Hillebrand et al., 2001; Reche et al., 2005; Bell, 2010; Astorga et al., 2012). Further, scientists also observed a significant decrease in bacterial compositional similarity with elevational distance, implying bacterial communities are spatially structured by elevation, rather than a random distribution (Bryant et al., 2008).

1.3 Ecological theories and microbial biogeography

1.3.1 Gradients with latitude and elevation

Ecological theories regarding the biogeography of macroorganisms have allowed generalized conclusions to be drawn from specific observations of organisms for decades, even centuries (Linnaeus, 1781; Merriam, 1890; Whittaker and Niering, 1965).

For example, one of the most fundamental and the oldest observed patterns in the biogeography of macroorganisms is the latitude gradient of diversity, whereby diversity increases at warmer, more equatorial latitudes (Pianka, 1966; Rosenzweig, 1995; Hillebrand, 2004).

A variety of plant and animal communities exhibit increases in diversity from the poles to the equator. Many competing hypotheses have been proposed to explain the pattern (Willig et al., 2003; Hillebrand, 2004; Lomolino et al., 2006). One of the most common hypotheses for explaining this broad-scale diversity gradient is the ‘water-energy dynamics hypothesis’, focusing on the direct or indirect (through influences on net primary productivity) constraints of water availability, solar energy, or water-energy balance for the maintenance of biodiversity (Hawkins et al., 2003; Kreft and Jetz, 2007). In other words, species richness at higher latitudes are limited by the availability of solar energy, and, in thermally suitable environments (i.e., at lower latitudes), by water availability (Wright, 1983; Stephenson, 1990; Allen et al., 2002). The theory of ‘biological relativity to water-energy dynamics’ proposed by O'Brien (2006) has been corroborated with numerous studies on the distributions of different macroorganisms, such as plants (Field et al., 2005; Vetaas and Ferrer-Castán, 2008), mammals (Diniz Filho et al., 2008; Hortal et al., 2008; Aragón et al., 2010) and birds (Hawkins et al., 2003; Li et al., 2013).

Alternative theories for latitudinal diversity gradients include Rapoport's Rule, in which diversity gradients are proposed to be driven by latitudinal gradients in environmental variation, and particularly temperature variation, associated with correlated gradients in geographical range size (Rapoport, 1982; Stevens, 1989; Smith and Gaines, 2003). Stevens (1989) associated Rapoport's Rule (Rapoport, 1975) with the observed pattern of decreasing species ranges closer to the equator. According to Steven's hypothesis, the latitudinal diversity gradient is caused by the principle of changing environmental tolerance of species with latitude, and consequently the latitudinal trend of individual's and species' potential to survive at various latitudes (Šizling et al., 2009).

Alternatively or additionally, Blackburn et al. (1999) identified Bergmann's rule as an explanation for large-scale latitudinal gradients in diversity. This rule describes the tendency for a positive relationship to exist between the body mass of species belonging to a monophyletic higher taxon and the latitude inhabited by these species. Scientists have demonstrated results consistent with Bergmann's rule for macroorganisms including birds and mammals (Rosenzweig, 1968; Zink and Remsen Jr, 1986; Blackburn et al., 1999; Ashton et al., 2000; Blackburn and Hawkins, 2004; Ramirez et al., 2008).

As climatic conditions, such as temperature, change with elevation as they do with latitude (Sunday et al., 2014), it is not surprising that elevational patterns in biodiversity can, to some extent, mirror latitudinal patterns (MacArthur, 1984; Brown, 2001), and consequently the aforementioned theories for latitudinal diversity gradients can be potentially adopted to elevational patterns. For example, scientists have applied Rapoport's rule to elevational patterns observed for distributions of macroorganisms, including, bats (Patterson et al., 1996), fishes (Bhatt et al., 2012), moths (Brehm et al., 2007), flies (Kubota et al., 2007; Rohner et al., 2015), ants (Sanders et al., 2003), spiders (Chatzaki et al., 2005) and butterflies (Sanchez-Rodriguez and Baz, 1995; Fleishman et al., 1998); coincident with the decline in species richness with increasing elevation is an increase in the latitudinal range of species (Stevens, 1992). Rapoport's Rule postulates that climates at higher elevations are more variable, so species at higher elevations can tolerate more variability and therefore have larger elevational ranges. Meanwhile, the 'low elevation' species cannot persist at higher elevations. As a result, species richness typically decreases monotonically with elevation (Sanders, 2002).

1.3.2 Taxa-area relationships

Another of the cornerstones in the field of modern biogeography is the equilibrium theory of island biogeography developed by MacArthur and Wilson (1967). The taxa-area relationship describes how the number of taxa tend to increase with increasing sample area (Martiny et al., 2006; Fuhrman, 2009). MacArthur and Wilson's theory provides a conceptual explanation for the species-area relationship, one of the most-

studied and best-documented patterns in plants and animal biogeography (Rosenzweig, 1995). This relationship is generally expressed with the equation:

$$S = cA^z$$

Where S is the richness of observed taxa; c is a fitted constant; A is the sampled area and z is the slope of the taxa-area relationship. Values of z are determined empirically, and for plant and animal taxa, z values generally range from 0.1 – 0.2 in contiguous habitats and 0.25 – 0.35 across discrete island habitats (Rosenzweig, 1995; Horner-Devine et al., 2004). Apart from sampling effects, the taxa-area relationship is mainly presumed to be caused by (1) the accumulation of habitats and taxa, when increasing the area considered, and (2) population dynamics, where higher probabilities of colonisation and speciation, but lower extinction rate, occur in larger areas (Connor and McCoy, 1979; Hubbell, 2001). Since current ecological studies increasingly address issues associated with habitat fragmentation, global environmental change and loss of biodiversity (Drakare et al., 2006), the taxa-area relationship remains a central theory for the prediction of species loss in response to global environmental change (Thomas et al., 2004), regional habitat loss (Ney-Nifle and Mangel, 2000) and the risk of future diversity loss because of reduced speciation (Rosenzweig, 2001).

An increasing number of studies have shown that microbes demonstrate a positive taxa-area relationship, with taxonomic richness increasing with the sample area (Green et al., 2004; Horner-Devine et al., 2004; Bell et al., 2005; Green and Bohannan, 2006; Peay et al., 2007; Prosser et al., 2007; Barreto et al., 2014; Terrat et al., 2015). This predictive pattern can also be applied to better understand variance in spatial microbial diversity along elevation gradients, as land area increases at lower elevation, and hence lower elevation sites consequently can harbour greater taxon richness.

1.3.3 Applying ecological theories to better understand patterns of microbial biogeography

To deepen our understanding of the ecology of microbes and also their role in ecosystem functioning, it makes sense to first explore if traditional ecological ideas and

theories derived from studies on plants and animals can be applied to microorganisms (Prosser et al., 2007; Ramette and Tiedje, 2007; Falkowski et al., 2008; Costello et al., 2012; Carbonero et al., 2014). However, to a certain extent, the application of traditional ecological theory is not driven by the assumption that microorganisms would simply follow the same patterns as observed for macroorganisms, but perhaps more simply by the fact that the biogeography of macroorganisms is fairly well-studied and scientists have adopted similar approaches for the investigation of microbial data (Martiny et al., 2006). We might lose sight of the fact that the biology of plants and animals are different from that of microorganisms, if microbiologists directly adopt traditional ecological theory without any adaptation (van der Gast, 2015). Indeed, there has been a growing number of studies investigating the biogeography of microorganisms in light of traditional ecological theory. For example, Carbonero et al. (2014), explored traditional ecological concepts of specialist and generalist species for microbial community data, but challenged their results by demonstrating that metabolic flexibility can be a major predictor of spatial distribution in microbial communities. On the other hand, Zinger et al. (2014) reported taxa-area relationships, one of the few universal principles in the ecology (an increase in species richness with increasing size of sampled area) (Rosenzweig, 1995; Lawton, 1999), for bacterial communities at magnitudes consistent with those observed for macroorganisms, while distance-decay relationships derived from the same dataset were much smaller than those derived from macroorganisms. Such studies, which provide contrasting results for microbial data, indicate that a major challenge in microbial biogeography is to determine the extent to which microorganisms show unique features or have patterns in common with macroorganisms (i.e., plants and animals).

1.4 Mountain elevation gradients and microbes

To explain large-scale patterns of species richness and composition and the mechanisms underlying these patterns are longstanding goals in ecology, as scientists used latitudinal and/or elevational gradients as proxies of climate change, particularly changes in temperature. Compared with studies conducted across latitudinal gradients, studies of patterns across elevational gradients provide a powerful ecological method for studying community richness and composition along a steep environmental

gradients but across relatively small spatial scales (Wang et al., 2012a). For example, a rapid decrease in temperature is commonly observed with increasing elevation ($\sim 6^{\circ}\text{C}$ per 1000 m), in contrast to a similar temperature decrease occurring over ~ 1000 km of latitude (i.e., 6.9°C) (Colwell et al., 2008; Jump et al., 2009). More than two centuries ago, Linnaeus (1743) documented a compressed and very orderly succession of climate, vegetative zones and animal communities across an elevational gradient. Later, Willdenow (1811) made the key observation that variation in plant diversity corresponded with variation in climate along elevational gradients. Since then, elevation gradients have continued to serve as a heuristic tool and natural laboratory for generations of scientists: from Von Humboldt (1849) in the Ecuadorian Andes, Darwin (1859) in the Chilean Andes, Merriam (1890) in the North American Rockies, to the study of Whittaker and Niering (1965) in North America, and the survey of Brehm et al. (2003) in southern Ecuador in the current century. Elevational gradients have shown their distinguished historical position in the testing and development of biogeography and ecological theory.

Community attributes and distributions are constrained by environmental factors which may form natural gradients that are inherently complex, combining variation in climate, soil resource factors and disturbance (Dubuis et al., 2013). Elevation gradients are frequently used as a proxy for identifying particularly climate-related factors that drive patterns of communities in the environment. Along a mountain ridge line, slopes encompass variation in climatic conditions over relatively short distances as well as variation in other soil physicochemical attributes and biology. Variation in climate attributes across mountain slopes are commonly used to explore their impacts on species distributions over short spatial distances. As a result of such studies, scientists have found that climatic conditions have emerged as a particularly important driver of species distribution through space (Hillebrand et al., 2010; Pellissier et al., 2013) with species' geographical ranges being driven by their climatic tolerance (Kerr et al., 2015).

While there is certainly evidence that climatic conditions, such as temperature alone can influence species diversity and turnover (Currie, 1991; Kluge et al., 2006; Zhou et al., 2016; Lewthwaite et al., 2017), other environmental factors likely play important

roles in controlling community diversity (Peay et al., 2017). For example, Fu et al. (2004) found that soil organic matter, positively related with elevation, had a close relationship with shrub richness and diversity, suggesting that elevation might exert indirect influences on species diversity via gradients in soil physicochemistry. Such observations might be caused by declines in nutrient availability, especially N and P, with increasing elevation, mediated by an increase in precipitation (Grubb, 1977). Slower rates of decomposition with increasing elevation might also cause an increase in soil C:N and declining pH (Bellingham and Sparrow, 2009).

1.4.1 Elevational patterns in macroorganism distributions

Studies of how individual macroorganisms and their community composition respond to elevation has led to a search for generalised elevational patterns of biodiversity (Brown, 2001; Lomolino, 2001; McCain, 2005). Studies have documented elevational patterns of biodiversity for a wide range of macroorganisms, including trees (Carpenter, 2005; Behera and Kushwaha, 2006; Acharya et al., 2011), mammals (Heaney, 2001; Rickart, 2001; Sánchez-Cordero, 2001), birds (Goodman and Rasolonandrasana, 2001; Kessler et al., 2001; Sekercioglu et al., 2008), reptiles (Rodríguez et al., 2005; Chettri et al., 2010), insects (Romero-Alcaraz and Ávila, 2000; Axmacher et al., 2004; Descombes et al., 2017) and amphibians (Stuart et al., 2004; Grenyer et al., 2006). Together, these results indicate that macroorganisms generally display either monotonic decreases or hump-shaped richness patterns (i.e., maximum richness at some intermediate point of the gradient) with elevation (Stevens, 1992; Rahbek, 2005). However, many elevational patterns are not sampled down to the lowest possible elevation, perhaps missing the detection of diversity ‘humps’ at lower elevations (Lomolino, 2001; Rahbek, 2005).

Changes in species richness along elevation gradients have been instrumental in developing a variety of theories about the general drivers of biodiversity. For example, one of the best known theories relate to the ‘species-area relationship’ (Rosenzweig, 1995), which assumes there is more chance for the maintenance of richness and speciation in larger areas. Therefore, an increase in the number of species is likely

observed at low elevation due to increasing patch size and a decreasing degree of isolation. Another explanation for the patterns observed relates to the ‘temperature hypothesis’, which correlates species richness with differing biochemical kinetics along these gradients, particularly the availability of thermodynamic energy (Stephenson, 1990; Rohde, 1992; Allen et al., 2002; Mittelbach et al., 2007). The ‘water availability hypothesis’ provides a similar explanation for elevational patterns of species richness, as at the highest elevations water availability decreases as precipitation decreases and evapotranspiration increases above the cloud layer; additionally, greater runoff may occur on the steeper slopes typically found at higher elevations sites and water resources water may be less accessible where stored as seasonal snow and ice (McCain, 2006). Therefore, this hypothesis mainly focuses on the direct or indirect constraints of water for the maintenance of biodiversity (Hawkins et al., 2003; Kreft and Jetz, 2007). Identifying theories that might drive large-scale patterns of biotic interaction is fundamental for understanding how communities respond to changing environmental conditions.

1.4.2 Elevational patterns in soil bacterial communities

As elevational patterns in macroorganism diversity along mountain gradients have been widely acknowledged, a growing number of microbiologists have started to study whether microorganisms show similar features or have patterns in common with macroorganisms along elevation gradients. In fact, it was as late as 2008 that the first high-profile study on the elevational pattern in bacterial diversity was published (Bryant et al., 2008). Bryant and colleagues (2008) found a monotonic decline in *Acidobacteria* richness with elevation. After this study, research on elevational patterns in bacterial community attributes began to attract more attention in the field (Fierer et al., 2010; Wang et al., 2011; Singh et al., 2012; Shen et al., 2013). For example, Fierer et al. (2010) observed no gradient in soil bacterial community diversity along elevational gradients, while, conversely, communities of macroorganisms, such as birds, bats and trees, collected at the same study site showed a clear decrease in richness with elevation. In the study of Wang et al. (2011), an increase in the richness of bacterial communities with increased elevation was observed, related to an increase in either carbon supply or temperature variability at high elevation. Further, Singh et al. (2012)

found a unimodal pattern in bacterial richness with elevation, which was not parallel to the diversity of plant communities. Other studies that have examined bacterial diversity along elevational gradients have shown variable results (Corneo et al., 2013; Looby et al., 2016; Hendershot et al., 2017; Wu et al., 2017). Together, all these findings indicate that elevational patterns in bacterial community attributes do not follow a universal rule, and fundamentally differ from the corresponding findings for macroorganisms. In addition, some scientists suggest that as elevation offers a strong gradient in climatic conditions (e.g., temperature), elevational gradients are a highly suitable proxy for the studies of microbial biogeography (Cordier et al., 2012; Soininen, 2012; Yang et al., 2014; Wu et al., 2017).

The inconsistency observed among spatial patterns in microbial communities with elevation is likely due to the different elevational ranges where samples were collected in different studies. For example, the topographic and climatic features are clearly distinct along a mountain incline on Changbai Mountain in China, encompassing a typical temperate forest below 1100 m, an evergreen coniferous forest from 1100 to 1700 m, a subalpine forest from 1700 to 2000 m, and a unique alpine tundra above 2000 m (He et al., 2005). Therefore, not surprisingly, researchers found distinct spatial patterns in bacterial community diversity across different elevational bands, including a linear decrease in taxon richness along 2000-2500 m elevations (Shen et al., 2015) as well as a lack of patterns from 500 m to 2200 m (Shen et al., 2013; Shen et al., 2014).

Climate conditions likely play a crucial role in generating and shifting bacterial diversity along elevation gradients, as bacterial taxa vary in their response to the gradient they inhabit (Vellend, 2010; Hanson et al., 2012; Whittaker and Rynearson, 2017). For example, climatic conditions (e.g., temperature and precipitation), which are highly correlated across elevation gradients (Rahbek, 1995; Gaston, 2000; Fierer et al., 2010), might significantly affect bacterial community structure and process, including respiration and enzyme activity (Fierer et al., 2003; Rinnan et al., 2009; Zeglin et al., 2013). Climatic conditions might further shape the functional abilities of bacterial communities (Strickland et al., 2015) although with adaptation taxa may begin to function outside of their normal 'climatic window' (Keiser and Bradford, 2017). For

example, environments with warm and moist conditions typically select for fast-growing competitive organisms. These conditions may therefore select for communities that generate higher rates of ecosystem processes than would be achieved by a stress-adapted communities (e.g., at low temperature and moisture content), when placed under similarly favourable environmental conditions (Vries et al., 2012; Crowther and Bradford, 2013). Climatic conditions might also impact soil bacterial community functional attributes through substrate-specific enzyme production whereby cold- or warm-adapted enzyme production is dependent on their habitat (Wallenstein et al., 2010). Therefore, the effect of climate gradients might be composed of the direct influence of temperature and moisture on the biotic activity of bacterial communities, reaction rates and substrate availability, and indirect effects mediated via the changing structure of these communities (Averill et al., 2016; Keiser and Bradford, 2017).

While there is certainly evidence that climate alone can influence bacterial diversity, the inconsistent responses to elevation in the microbial literature (Bryant et al., 2008; Peay et al., 2017) suggests that other environmental factors (e.g., soil pH) likely also play important roles in controlling the diversity of soil microbial communities. For example, Peay et al. (2017) found that although bacterial community richness strongly correlated with elevation, it was also correlated with other soil physicochemical factors across the study site (e.g., soil pH). Likewise, Bryant and colleagues (2008) found a strong influence of soil pH on bacterial community composition across elevation gradients; pH has previously been demonstrated as a dominant determinant or correlate of bacterial community composition (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011; Shen et al., 2013; Hermans et al., 2017). The strong correlation between soil pH and bacterial community structure could result from pH being an integral measure of a variety of soil physicochemical attributes (Fierer and Jackson, 2006). For example, elevational gradients in soil pH are commonly attributed to an increase in rates of mechanical weathering that emerges up to elevations of ~2000 m (Hales and Roering, 2005). An increase in weathering causes soil acidification, as soil pH and concentrations of exchangeable cations (e.g., calcium, sodium, phosphate and magnesium) are closely correlated (Berthrong et al., 2009). Therefore, besides the direct influence of climatic conditions, soil physicochemistry, such as pH, possibly affected

by different rates of weathering across the gradient, could be a correlate of elevational gradients in bacterial diversity and composition.

1.4.3 Elevational patterns in soil fungal communities

While the term ‘microorganism’ encompasses a broad range of taxa (i.e., bacteria, fungi, archaea, viruses, and protists) that are phylogenetically, morphologically, and physiologically distinct, whether distinct biogeographic patterns can be distinguished between the different microorganisms has received little attention (Logue et al., 2015). Do fungi, for example, respond similarly to environmental variables as bacteria? It is perhaps unlikely that these taxonomic groups share similar patterns, as the intrinsic properties and functional attributes of fungal communities are different from those of bacterial communities. Current studies focusing on bacteria and fungi show differences in their sensitivity to drought (Berlemont et al., 2014), fertilisation (Koyama et al., 2014; Liang et al., 2015), and warming (DeAngelis et al., 2015; Liang et al., 2015), which in turn impact measured functional processes or traits (Berlemont et al., 2014; Liang et al., 2015). As fungi are widely distributed in all terrestrial ecosystems, often governing soil carbon cycling, plant nutrition, and pathology (Tedersoo et al., 2014), it is important to investigate their distribution (Fierer et al., 2009; Serna-Chavez et al., 2013; Xu et al., 2013).

Similar to the inconsistent elevational patterns in bacterial community attributes as already mentioned, mixed results have been found for fungal communities, with evidence for decreased richness at lower elevation (Pellissier et al., 2014) or increased richness at lower elevation (Logue et al., 2015), greatest richness at mid-elevations due to range overlap (Bahram et al., 2012; Counce et al., 2014; Miyamoto et al., 2014), or a lack of pattern with elevation (Zimmerman and Vitousek, 2012; Jarvis et al., 2015). These inconsistent elevational patterns imply that soil fungal community richness and diversity attributes might not be simply driven by a single rule, but instead by more complex ecological mechanisms. Previous studies have investigated how fungal distribution and diversity differ when assessed at the level of fungal biomass (Zhang et al., 2013), at functional group levels (Bahram et al., 2012), and at different taxonomic

levels such as by phylum (Looby et al., 2016) across elevational gradients. These variations become more apparent as lower taxonomic levels and/or ecological trait differences are considered.

1.4.4 The impact of mountain aspect and microclimate on spatial patterns in soil microbial communities

Apart from variation in climatic conditions along elevation gradients, mountain aspect, slope, shading and related microclimate factors are also relevant to climatic variation across mountain environments (Titshall et al., 2000). It is indeed a well-established fact that the microclimate of a site is largely determined by its geographical orientation (Xu et al., 2002), which causes sites separated by distances of only a few meters to experience very different microclimate conditions. Slope aspect is an important topographic factor affecting microclimate, mainly because it determines the amount of solar radiation received, that in turn influences soil temperature (Weiss and Weiss, 1998; Thomas et al., 2001; Davies et al., 2006), soil moisture (Carter and Ciolkosz, 1991; Schaetzel and Anderson, 2005), evaporative demand (Bennie et al., 2008) and biological activity (Nielsen et al., 2001; Selvakumar et al., 2009). To summarise, the aspect of a mountain which receives greater solar radiation, is typically hotter, dryer and subject to more rapid seasonal and diurnal changes in microclimate (Sariyildiz et al., 2005; Carletti et al., 2009). Particularly, at higher altitudes, soil microclimate varies remarkably, largely due to difference in the number of daily insolation hours received at different locations (Zumsteg et al., 2013). Although research on the relationships between microclimate or aspect differences and soil microbial communities remain low, there have been a few studies which demonstrate the impact of mountain aspect or microclimate on changes in microbial community attributes. For example, some microbial taxa, such as *Pseudomonas*, were more abundant on the cooler aspect of a mountain (Sikorski et al., 2008; Selvakumar et al., 2009), suggesting that aspect induced microclimatic conditions selecting particular microbial genotypes that are highly adapted to the prevailing cooler conditions. The relevance of aspect induced microclimate differences is also confirmed by Zumsteg et al. (2013), showing that the microbial activities were higher on the colder and moister side of a mountain, likely due to greater substrate availability at lower temperatures and higher moisture levels.

Moreover, Wu et al. (2017) found that variation in bacterial community composition was more closely associated with the aspect of the study site than its elevation. Overall, it is important to note that variations in microbial community attributes are influenced not only by the whole elevation gradient at a large-scale, but also by microclimate features occurring at fine-scales. Therefore, more studies on spatial patterns in soil microbial community attributes along a mountain incline encompassing both broad as well as fine-scale variation are desirable, especially when little work has currently been done regarding the impact of microclimate or mountain aspect on soil microbes.

1.5 The impact of different land use on soil microbial communities

Soils are typically physically, chemically, and biologically heterogeneous in nature, thereby providing a wide range of gradients in soil characteristics to explore the distributional patterns in microbial communities and the factors driving these patterns. There is no shortage of evidence that soil heterogeneity can, to some extent, directly affect spatial distributions of microbial communities. For example, across a 180-m distance of the Hoosfield acid strip (that is, a pH gradient ranging from 4.0 to 8.3 within 200 m that resulted from a one-time uneven application of chalk in the mid 19th century), Rousk et al. (2010) found that the relative abundance and diversity of bacterial communities were positively associated with soil pH from pH 4 to 8. This finding has confirmed observations from other studies conducted from continental (Fierer and Jackson, 2006; Lauber et al., 2009) to submeter scales (Baker et al., 2009), showing that the influences of soil properties on soil microbial communities are robust across different spatial scales.

The effects of changes in land use on the physical and chemical properties of soils in agricultural land and even adjacent natural habitats have been well studied (Post and Mann, 1990; Murty et al., 2002; Kuramae et al., 2012; Didham et al., 2015), while a growing body of evidence documents how changes in land use also alters soil microbial community attributes and the biogeochemical processes they carry out (Jangid et al., 2008; Wu et al., 2008; Ramirez et al., 2010; Rodrigues et al., 2013; Paula et al., 2014).

For example, both Rodrigues et al. (2013) and Gossner et al. (2016) similarly report increases in taxon richness and decreases in compositional dispersion of soil microbial communities after forest to pasture conversion. Additionally, fertiliser application frequently causes a decrease in soil pH (Fox and Hoffman, 1981; Liu et al., 2012), consequently reducing nutrient availability and microbial biomass in agricultural soils (Bardgett, 2005). The influences of land-use change on microbial community attributes arise from diverse management practices across different land-use types, for example fertilisation effects on soil (Garbeva et al., 2004; Wakelin et al., 2008), including soil pH (Fierer and Jackson, 2006; Griffiths et al., 2011; Wu et al., 2017), nitrogen (Di et al., 2009; Campbell et al., 2010; Fierer et al., 2012a) and phosphorus concentrations (Griffiths et al., 2011; Hermans et al., 2017). Interestingly, some scientists found that differences in microbial communities were not necessarily related to distinct land-use types, but rather to be directly associated with the impacts of that land-use change on underlying soil properties (Lauber et al., 2008; Kuramae et al., 2012).

Numerous studies have demonstrated that changes in land use have a considerable influence on both bacterial and fungal communities (Bossio et al., 1998; Steenwerth et al., 2002; Johnson et al., 2003; Rodrigues et al., 2013; Gossner et al., 2016). Such influences on microbial community structure are caused by the variability in specific soil characteristics associated with different land use types, rather than land use itself (Lauber et al., 2008). Additionally, although hydraulic activity and livestock invasion have been previously reported to transport microorganisms and agricultural nutrients further into the forests surrounded by agricultural land (Lim and Flint, 1989; Champagne et al., 2000; Maule, 2000; Wolf et al., 2010), we have a poor understanding of the spatial extent of the impact of different land uses on soil microbial community structure in natural forest fragments affected by their proximity to adjacent agricultural land.

1.6 Approaches for studying the structural and functional traits of microbial communities

1.6.1 Next-generation DNA sequencing technologies

Environmental samples collected from nature typically harbour complex microbial communities, which are highly diverse in both the composition and abundance (Degnan and Ochman, 2012). Advances in molecular biological technologies have revolutionised our ability to fully describe soil microbial communities with regard to their diversity, composition and biogeography (Pedros-Alio, 2006). Since 2005, the development of “next-generation” (or “high-throughput”) sequencing technologies has greatly facilitated bacterial community research (Sogin et al., 2006; Eisen, 2007; Bailey et al., 2013; Bergkemper et al., 2016; Yang et al., 2017). Such technologies have helped overcome historical culture-based biases, which often underestimated microbial community diversity and only provided limited information related to the taxonomic identity (Amann et al., 1995; Ferrari et al., 2005). Next-generation sequencing platforms, such as Roche 454 pyrosequencing (Margulies et al., 2005), Ion Torrent PGM (Rothberg et al., 2011) or Illumina (Gloor et al., 2010), can yield great numbers of sequencing reads at low cost, enabling high-through microbial analysis to explore microbial biogeography. Although some technical issues of next-generation sequencing exist, for example PCR primer biases (Caporaso et al., 2012), surveys of microbial communities from environmental samples across continental-or even global-scales have become the norm nowadays (Fierer and Jackson, 2006; Lauber et al., 2009; Tedersoo et al., 2014; Ma et al., 2016; Zhou et al., 2016). By studying these patterns, scientists may be able to develop or examine fundamental biogeographical theories and hypotheses that might be common to all of life, beyond merely documenting the existence of patterns. Using next-generation sequencing technologies, microbiologists can reveal microbial community responses to diverse environmental gradients and changes for archaea, bacteria, and fungi, as well as a vast range of habitats, and varying spatial and temporal scales. Therefore, we can widely investigate if microbial biodiversity is fundamentally different from that of macroorganisms and which underlying attributes and processes contribute to these differences, which would greatly further our understanding of the process regulating the diversity and distribution of life on Earth.

1.6.2 The distribution of microbial functional traits

A key research topic in ecology is how community composition impacts ecosystem functioning (Loreau et al., 2002). For microorganisms, there is a growing body of interest in the biogeography of functional traits that are linked with its fitness or performance (McGill et al., 2006). The observed elevational patterns in the distribution of functional traits can deepen our understanding of the complex mechanisms determining why organisms live where they do, how many taxa can co-exist in a habitat, and how they will respond to environmental fluctuation. However, compared with our understanding for plants and animals, we know little whether changes in microbial community composition lead to shifts in metabolic functions or whether shifts in these functions require associated changes in taxonomic composition (Green et al., 2008; Logue et al., 2015). Previous studies have indicated the potential of exploring functional traits to deepen our understanding of microbial biogeography (Martiny et al., 2015; Ruiz-González et al., 2015), and that the distribution of specific functional traits may govern the type of microbial response to environmental change (Shade and Handelsman, 2012). Yang et al. (2014) found greater abundances of bacterial cold shock genes encoding for adaptation to cold conditions at higher elevation. Their results also indicated that the abundance of gene *gdh*, converting ammonium into urea, and gene *ureC*, converting urea into ammonium contents, was consistent with soil ammonium contents. In addition, changes have been reported at the cellular level, whereby heterotrophic bacteria featuring a range of homeostatic regulation mechanisms from strong homeostasis to highly flexible biomass stoichiometry were able to accommodate changes in carbon and phosphorus concentrations (Godwin and Cotner, 2015). Overall, studying variation in microbial community functional traits is becoming particularly valuable to microbiologists, as new patterns of variation in functional traits can be discovered across environmental gradients, even within taxonomic groups once thought to be relatively homogeneous (Green et al., 2008). As such patterns in the distribution of microbial functional traits could be applied to understand complex phenomena, current advances in environmental molecular biology, such as metagenomics, co-occurrence network analysis and other trait-based approaches, would recast the argument of functional redundancy among taxa (Achtman and Wagner, 2008) to better

understand the importance of specific groups of microbial functional traits in the environment.

1.6.3 Metagenomics

Microbial communities, as part of natural ecosystems, are inherently complex. One of the biggest challenges facing microbiologists interested in functional traits is how to identify them. The traditional tools of microbiology, such as the analysis of pure cultures, only provide a reductionist view, studying each organism in isolation (Fuhrman, 2009). One approach to studying microbial functional traits without relying on culture is to use the DNA sequences from environmental genomics studies, which can provides a comprehensive and integrated approach to decipher microbial function and physiology. The emergence of metagenomics-based approaches in microbiology have helped overcome historical culture-based biases, which previously limited our ability to address the functional attributes of microorganisms and mechanisms underlying their interactions (Ferrari et al., 2005; Thomas et al., 2012). This approach can be used to annotate the sequences into open reading frames and then predict the encoded proteins and infer putative functions using annotation databases, such as the Kyoto Encyclopedia of Genes and Genomes, Clusters of Orthologous Groups, Functional Ontology Assignments for Metagenomes and SEED subsystems (Kanehisa and Goto, 2000; Koonin, 2002; Overbeek et al., 2004; Prestat et al., 2014). The rapid development and extraordinary cost reductions of next-generation sequencing technologies has greatly increased the number of available metagenomics datasets, and, consequently, our knowledge of the functioning of complex microbial communities (van Nimwegen et al., 2016). We are now capable of predicting the growth requirements (Pope et al., 2011; Walker et al., 2014) and stress tolerances (Mongodin et al., 2006; Yuan et al., 2012; Stuart et al., 2013; Trivedi et al., 2013) even of microorganisms that have never been grown in a laboratory environment. These advances are deepening our understanding of how soil bacterial diversity varies across natural environmental gradients and in response to treatment applications. The new era of high throughput metagenomics methods now provides microbial researchers the ability to catalogue distributions of multiple genes relevant to metabolic pathways,

energetics and regulatory circuits to directly address changing microbial functional potential, across time and space (Yang et al., 2014).

1.6.4 Ecological co-occurrence networks

Topology-based analysis of co-occurrence networks measure correlations between taxon abundances to reveal how taxa sometimes or never co-occur together in niches and how these relationships change under different environmental conditions (Gotelli and Graves, 1996; Gotelli and McCabe, 2002). A reductionist approach, such as a culture-based approach or a genetic study, is not well suited for learning about interactions and emergent properties of communities. In contrast, a more holistic approach, such as co-occurrence network analysis, can yield complementary data directly derived from natural habitats to help deduce the interactions among microorganisms and their variation with changes in environmental conditions (Ruan et al., 2006; Fuhrman and Steele, 2008; Fuhrman, 2009; Chaffron et al., 2010). These patterns reveal how communities are structured by co-association, and how particular community ‘structures’ and can be represented as mathematical interaction diagrams or networks.

Co-occurrence network analysis identifies the mathematical, statistical and structural properties of a set of items (nodes) and the connections between them (edge) within a specific community (Newman, 2003). Network analysis has been commonly used by biologists to explore feeding interactions between species in a food web (Krause et al., 2003). This analysis has been also used to describe host-parasitoid systems, focusing on distinct guilds of terrestrial hosts and tracing the links from host to the parasitoids, and mutualistic webs (e.g., pollination or frugivore networks) (Ings et al., 2009). Descriptions of different co-occurrence patterns help to describe the underlying structure of ecological communities, deepening our understanding of the relationship between community complexity and ecological stability (Melián and Bascompte, 2002; Ives and Cardinale, 2004; Bascompte and Jordano, 2007).

Co-occurrence network analysis offers new insight into the structure of complex microbial communities, insight that complements and expands on the information provided by the standard suite of analytical approaches, such as community richness and composition. Using culture-independent technologies (e.g., the 16S rRNA gene and shotgun metagenomics), large microbial datasets can be generated that take full advantage of network analyses, applying the method to explore co-occurrence patterns in complex ecosystems, such as in soils (Caporaso et al., 2011; Barberán et al., 2012; Shokralla et al., 2012). Current studies of microbial networks show co-occurrence network construction using correlation coefficients or other association metrics, although these networks do not reflect direct evidence of interaction between taxa (Ruan et al., 2006; Barberán et al., 2012; Eiler et al., 2012; Schwab et al., 2014). For example, in the study of Barberán et al. (2012), the diversity of network structures among habitats (e.g., aquatic vs soil) was observed, which may reflect different ecological rules guiding microbial community composition in different environments. Eiler and colleagues (2012) also found complex interdependencies within microbial communities and contrasting links to environmental conditions. Although co-occurrence network analysis is increasingly used to infer microbial interactions in soils (Barberán et al., 2012), oceans (Steele et al., 2011), lakes (Eiler et al., 2012) and even in genomic surveys at a global scale (Freilich et al., 2010; Fondi et al., 2016), the elevational patterns in microbial interactions have remained poorly studied until recently. For example, Mandakovic et al. (2018) found that, apart from large variation in environmental conditions with elevation, a proportion of co-occurrence patterns identified in the bacterial networks were resilient, likely due to the existence of persistent OTUs with similar association patterns across the gradient. In contrast, network analysis showed that soil bacterial networks were less resistant to environmental change at lower elevation than that at higher elevation (Siles and Margesin, 2017). These conflicting findings suggest the need for further studies regarding elevational patterns in microbial co-occurrences. Overall, co-occurrence network analysis represents an approach for identifying patterns in large and complex datasets, which may be more difficult to detect using the standard approaches widely used in microbial biogeography (Proulx et al., 2005).

1.7 Thesis objectives

Using a mountain elevation gradient as a proxy for climate change and using land use changes as shifts in soil properties, the main aim of my research was to determine the influence of environmental gradients on belowground bacterial and fungal community structure and their functional attributes across local (i.e., along a c.1500 m elevation gradient) and micro-scales (e.g., among samples located just a few meters apart) as well as across different land use types (i.e., along a transect line from the interior of forest fragments into adjacent grazed pasture, < 100 m). My PhD thesis has four main objectives:

- 1. To evaluate the relative influences of non-resource (e.g., soil temperature, moisture and pH) and resource conditions (e.g., soil carbon, nitrogen and phosphorus) on the biogeography of soil bacterial communities across broad (i.e., across a whole elevational gradient) and fine sampling scales (i.e., across two aspects of a mountain ridge) along a ~1,500 m mountain elevation gradient.**

Variation in soil bacterial community composition was analysed using 16S rRNA gene data with the aim of examining the influence of environmental variables at different spatial scales. First, I hypothesise that variation in bacterial community composition would correlate with elevation and that bacterial taxonomic richness would decline with increased elevation, since such changes are generally observed for macroorganisms (Rahbek, 2004; Aubry et al., 2005; Carneiro et al., 2013). Then, as environmental conditions, such as temperature and soil moisture are strongly affected by elevation as well as aspect differences, I hypothesise significant differences in bacterial community composition, and a decline in bacterial taxonomic richness, on shadier compared to sunnier aspects of a mountain ridge. Finally, I hypothesise that non-resource environmental conditions, such as soil temperature and moisture, would be a more important determinant of bacterial community attributes than environmental resource factors, such as concentrations of soil C, N and P. Evidence of a significant and dominant impact of climatic conditions on shaping bacterial community structure would provide evidence that the biogeographic pattern in microbial communities is fundamentally similar to that in macroorganisms.

2. To move beyond simple explorations of richness and composition patterns across a mountain elevation gradient to assess microbial co-occurrence patterns.

As fungi are widely distributed in soils and govern soil carbon cycling, plant nutrition, and pathology, it is important to know whether fungal communities respond in the same way to the environmental variables as bacterial communities along the same elevational gradient. The richness and composition of soil fungal communities collected across the same mountain gradient as in Chapter 2 is analysed to detect their elevation patterns using fungal ITS1 region sequencing. Likewise, I first hypothesise that fungal community richness would decline with increasing elevation. Thereby, using analysis of co-occurrence networks, I further hypothesise that the number of overall interspecific co-occurrences within fungal communities would increase with decreasing elevation, as environmental conditions, such as temperature, might be considered to be more optimal for life at lower elevation due to increased energy-nutrient availability (Hawkins et al., 2003). Finally, I predict that soil physicochemical variables, e.g., soil nitrogen and ammonium would be stronger correlates of fungal community network associations than climatic variables, i.e., temperature and soil moisture, since fungi are recognised as major contributors and mediators of soil nutrient cycles.

3. To determine microbial functional biogeography across a mountain elevation gradient using an ‘inferred metagenomics’ approach

Although metagenomics can be used to produce a large amount of data and these sequences can be used to assign functional traits of microbial communities across space, such methods are rarely used to study microbial biogeography, due to the high cost of DNA sequencing and the large number of samples typically required for analysis. Here, I explore the potential for using ‘inferred metagenomics methods’ based on the analysis of bacterial 16S rRNA gene sequence data (collected as described in Chapter 2), to assess the reliability of this ‘inferred metagenomics’ and to investigate patterns in the presumed

abundance of genomes and functional traits across a mountain elevation gradient. I hypothesise that: (1) bacteria with larger genome size would be prevalent across the elevation gradient, as in prior studies, bacterial communities requiring resilience to more fluctuating environmental conditions are found to have larger average genome sizes (Matz and Jürgens, 2005; Bentkowski et al., 2015; Cobo-Simon and Tamames, 2017); (2) the numbers of genes encoding for ‘cellular responses to stress’ would increase at higher elevation, as decreasing temperatures and increasing soil acidity with elevation across the study site might increase the environmental stress experienced by the bacterial communities.

4. To determine the variation in soil bacterial community richness and composition in forest fragments associated with land use conversion of adjacent land to grazed pasture

Apart from elevational patterns in the microbial community structure and their functional attributes across a mountain ridgeline observed in previous chapters, in this chapter I mainly focus on how pastoral land-use practices affected the spatial patterns in richness and composition of bacterial communities in adjacent forest soils. First, I hypothesise that the richness of bacterial communities would be lower in individual samples collected from within grazed pasture, relative to those in adjacent forest soils, as a decrease in plant and animal diversity is repeatedly reported after forest conversion to agricultural land (Bierregaard, 2001; Soares et al., 2006). Secondly, I investigate if the absence of a fence between pasture and forest systems increases the extent of biotic homogenisation between these land uses. This might occur directly because soil and microorganisms were transported across the study sites by stock animals, but also due to the indirect effects of the stock animals on the forest soil (e.g., soil compaction and nutrient additions). Thus, I hypothesise that the presence of a fence would increase the proportion of taxa occurring uniquely in the pasture of fenced farms, showing that fencing prevents migration of some pasture-associated taxa from grazed grasslands into adjacent forest soils. Finally, as how microbial communities in the adjacent forest soils respond to different

pastoral land use intensities remains poorly studied, I hypothesise that the richness of bacterial communities in a forest would be lower and their community dissimilarities would increase (compared to communities in the pasture soil) in response to existing gradients in adjacent agricultural land use intensity.

In general, my thesis seeks to explore the factors driving variation in diversity, structure and functional roles of soil bacterial and fungal communities across both large-and fine-scales in response to gradients in natural environmental conditions and anthropogenic activities. By providing an insight into how gradients in environmental factors affect soil microorganisms, my study improves our understanding of the likely responses of microbial communities to natural environmental gradients, as well as to climate change and anthropogenic disturbance.

Chapter 2

Aspect has a greater impact on alpine soil bacterial community structure than elevation

This chapter is a modified version of: Wu, J., Anderson, B.J., Buckley, H.L., Lewis, G., and Lear, G. (2017). Aspect has a greater impact on alpine soil bacterial community structure than elevation. *FEMS Microbiology Ecology* 93(3). doi: 10.1093/femsec/fiw253.

2.1 Introduction

Variation in climatic factors, such as temperature and precipitation, impacts the composition and diversity of a wide variety of natural biological communities, often in predictable ways (Bertrand *et al.* 2011; Knapp *et al.* 2002). Climatic gradients in conditions that occur along mountain inclines are commonly used to explore the impact of climate on community attributes, since substantial variation can occur across short geographic distances. Temperature declines by approximately 0.6 °C for each 100 m increase in elevation (McCain and Grytnes 2010); precipitation and related ecological variables, such as soil moisture, can also vary considerably over similar spatial scales (Brown *et al.* 2012). To date, biogeographic studies along elevation gradients have concentrated almost exclusively on the community response of macroorganisms, in part because of the relative ease that larger taxa can be visually identified. These studies frequently report significant elevational gradients in community composition and richness that are suggested to be directly driven by climatic variables and particularly by the availability of thermodynamic energy, water, or combined energy-water balance (Allen *et al.* 2002; Stephenson 1990). Alternative explanations for observed diversity patterns also include the impact of decreasing land area proportional to elevation increases (Rahbek 1997) and decreased range sizes of taxa at lower elevation, according to Rapoport's Rule (Stevens 1992). Although microorganisms may be considered to be the 'unseen majority' in soil ecosystems, where they dominate numerically and comprise a large portion of the genetic diversity, the relationship between microbial communities and fine-scale variability in abiotic conditions remains poorly resolved (Grundmann 2004; Morris 1999; Vos *et al.* 2013). A better understanding of the impacts of abiotic conditions on microbial communities is necessary since they provide vital ecosystem services, including soil formation, carbon and nutrient cycling and acquisition, and are a major determinant of plant productivity and diversity (van der Heijden *et al.* 2008). Here, we explore the relationship between abiotic micro-habitat conditions and the biogeography of soil bacterial communities across a single alpine elevation gradient. Improved knowledge of micro-habitat variability in soil bacterial community composition and diversity across elevation gradients will aid in developing and testing hypotheses regarding the response of these vital communities to global climate change.

If the factors driving the biogeographic distribution of microbial taxa along mountain elevation gradients are fundamentally similar to those affecting communities of macroorganisms, then equivalent patterns should be observed, such as declines in species richness at increased elevation. However, the inconsistent nature of the patterns so far observed across mountain elevation gradients for microbial taxa, imply that related variation in bacterial communities can be complex and follow no single rule; authors describe decreases in diversity/richness (Bryant *et al.* 2008), no trend (Fierer *et al.* 2010), or unimodal patterns (Singh *et al.* 2012) in bacterial community composition with increased elevation. Similarly, if elevational gradients in community diversity and richness are universal, then we would expect the same environmental drivers (i.e., temperature and availability of water) to be key predictors of these fundamental bacterial community attributes.

Temperature and precipitation significantly affect bacterial community structure and processes, including respiration and enzyme activity (Fierer *et al.* 2003; Rinnan *et al.* 2009; Zeglin *et al.* 2013). Therefore, where patterns in soil bacterial community attributes are not tightly related to gradients in climatic conditions across elevation gradients, we expect that alternative factors such as variation in soil type and management are likely to be the key drivers of community assembly (Fierer and Jackson 2006). However, the relative impact of edaphic factors on alpine bacterial communities remains poorly understood, particularly within single mountain elevation gradients where we would expect the uniform geology and land use attributes to allow variation in natural climatic and environmental factors, rather than anthropogenic factors, to be a stronger determinant of microbial community composition (Singh *et al.* 2014). It is important to note that even where land management and edaphic variables are controlled or otherwise accounted for, additional site attributes can contribute to observed environmental patterns in bacterial community composition and diversity. For example, climatic variation across mountain environments is related not only to site elevation, but also to aspect, slope and shading (Titshall *et al.* 2000), which can vary considerably over distances of only a few metres. Many studies have noted the effect of slope and aspect in determining soil temperature (Davies *et al.* 2006; Thomas *et al.*

2001; Weiss and Weiss 1998), evaporative demand (Bennie *et al.* 2008), soil moisture (Carter and Ciolkosz 1991; Schaetzl and Anderson 2005), soil chemistry (Hunckler and Schaetzl 1997; Miller *et al.* 2004; Thompson and Kolka 2005) and nutrient cycling (Gilliam *et al.* 2015; Sariyildiz *et al.* 2005; Sidari *et al.* 2008) principally via modifying the amount of solar radiation received. Although the relationships between fine-scale variation in site slope and aspect have been poorly investigated for microbial communities, Sidari *et al.* (2008) observed significant correlations between soil microbiological activity and aspect-induced microclimatic differences in the content and composition of soil organic matter. For these reasons, we chose to quantify variation in soil bacterial community attributes along a c.1500 m mountain incline encompassing a broad microhabitat gradient, as well as examining local-scale variation related to site aspect.

The availability of growth-limiting resources, especially nitrogen and carbon (Mason *et al.* 2014; Zinger *et al.* 2009) impact the abundance, diversity and composition of many communities, including plants (Edgar 2013; Kalra and Maynard 1991; Rahbek 2004), bacteria (Calleja-Cervantes *et al.* 2015; Zhang *et al.* 2015) and fungi (Lauber *et al.* 2008). For example, Shen *et al.* (2015) observed a significant relationship between soil carbon, nitrogen, and variation in bacterial community composition across a climatic gradient in alpine tundra. Variation in carbon and nitrogen concentrations and fluxes are frequently correlated with variability in temperature and precipitation (Altschul *et al.* 1990; Huber *et al.* 2007; Knapp *et al.* 2002; Weltzin *et al.* 2003) and related to both increasing microbial metabolism and decreasing energy use efficiency in warmer conditions, providing water availability is not limiting. Hence, while climatic factors such as temperature are predicted to be strong independent determinants of bacterial community composition and metabolism (Fierer *et al.* 2003; Kessler 2000), resource availability is expected to have additive and interacting impacts on bacterial communities across broad altitudinal ranges. For instance, Wang *et al.* (2011) observed decreasing bacterial community richness with increasing elevation, which they correlated with an increase in carbon availability at higher altitudes. Here, we quantify how fine- and coarse-scale gradients in resource and non-resource factors (e.g., temperature, soil pH) are related to soil bacterial community composition and richness across a range of sample site elevations and aspects across a single mountain ridge.

We sought to test three hypotheses. First, we hypothesised that variation in bacterial community composition would correlate with elevation and that bacterial taxonomic richness would decline with increased elevation (Elevation H₁), as is frequently reported for macroorganisms (Aubry *et al.* 2005; Carneiro *et al.* 2013; Rahbek 2004). Second, since environmental conditions, particularly temperature, are strongly impacted by aspect, we hypothesised significant differences in bacterial community composition, and a decline in bacterial taxonomic richness, on shadier compared to sunnier aspects of the mountain ridge (Aspect H₂). Finally, we hypothesised (Environment H₃) that non-resource environmental conditions, such as soil temperature and moisture, would be a more important determinant of bacterial community attributes than environmental resource factors, such as concentrations of soil C, N and P. Evidence of a significant role for non-resource, and particularly climatic factors, in determining bacterial community composition and richness would imply that the biogeography of bacterial communities is fundamentally similar to that of macroorganisms.

2.2 Materials and methods

We conducted a survey of soil bacterial 16S rRNA gene data collected across a continuous ridge leading to the peak of Mount Cardrona, New Zealand. We compared communities across both sunny and shady sides of the mountain ridge and ridgeline, at 100 m elevation intervals across an elevation gradient of almost 1,500 m to test the influence of aspect relative to elevation on bacterial community richness and composition (i.e., Elevation H₁ & Aspect H₂). Our final hypothesis (Environment H₃) was tested in combination with the collection of both soil resource (e.g., concentrations of soil C, N, P, and the average biomass of aboveground plants) and non-resource environmental data (e.g., soil pH, temperature and moisture).

2.2.1 Site description and sample collection

To assess the impact of microclimate variability on soil bacterial community attributes, we collected a total of 405 soil samples from a continuous ridge on the north-eastern side of Mt. Cardrona, New Zealand (44.85° S, 168.95° E; Figure 2.1). We identified 15 elevation bands (Ebands) located at 100 m elevation intervals from 500 m to 1900 m along the ridge. Since the slope of the ridge at 1300 m is very shallow, this Eband was separated into two (i.e. one at the upper edge of the ridge called E1301 and another at the lower edge called E1300). Hence, samples were collected from a total of 16 Eband locations.

In addition to elevation, microclimate conditions are impacted by variability in site aspect. For this reason, each Eband encompassed five clusters of soil samples. One of the five clusters (R0) at each Eband was located on the centre of the leading ridge line, two (SU1, SU2) to the north (warmer/sunnier side), and two (SH1, SH2) to the south (cooler/shadier side). All of these clusters were geographically equidistant (i.e., collected at 25 m intervals). A single sample cluster was collected at the summit of the mountain at 1936 m. Within each sampling cluster, five individual soil samples were collected across a 1.5 m transect using a soil corer to 10 cm depth allowing us to also quantify the fine-scale variability in bacterial community composition and relative richness at each sample location. Hence, there are 405 samples in total (16 Ebands x 5 sample clusters x 5 site samples + 5 summit samples = 405). All soil samples were contained in labelled ziplock bags to avoid contamination, and stored at -20 °C within 24 h of collection.

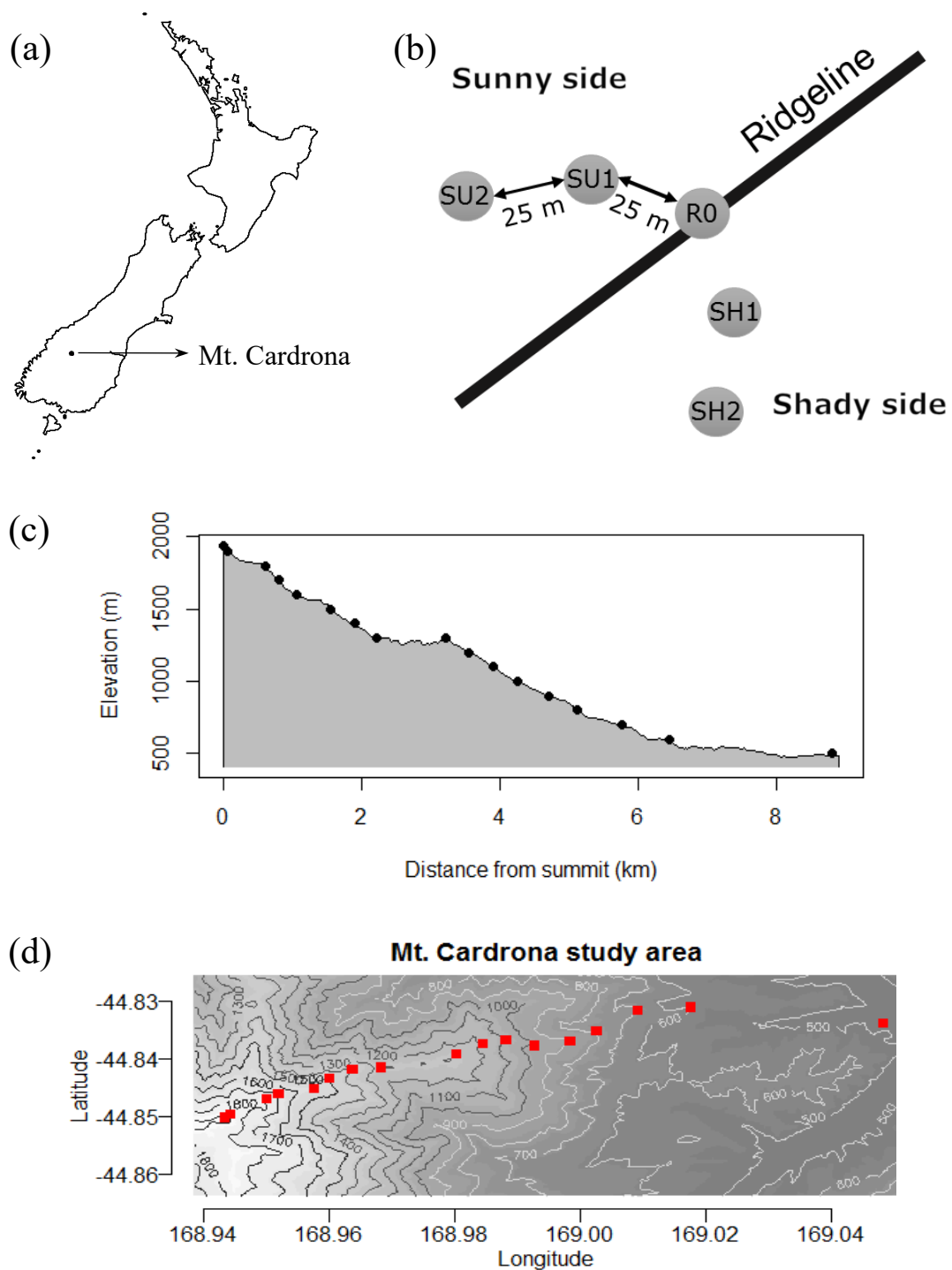


Figure 2.1. Sample locations on Mt Cardrona. (a) Mt. Cardrona is located in Otago (44.85° S, 168.95° E), New Zealand; (b) at each elevation, samples were collected on both sides, and on the main ridge of mountain at intervals of 25 m (geographic distance); (c) mountain profile showing the distance from the summit of each sampling location along the elevation gradient; (d) samples were collected at elevation intervals of 100 m along either side of the main ridge on north-eastern side of Mt Cardrona.

2.2.2 Environmental conditions

We used ibutton temperature loggers (Maxim Profile, USA) to collect real-time temperature data (below ground temperature at 10 cm depth) from each sampling cluster (n=81) at Mt. Cardrona in the summer (from February to March in 2014). Soil moisture was measured gravimetrically (i.e., soil samples were weighed before and after being placed in an oven at 105 °C for 96 hours.) (Rayment and Lyons 2011). Soil pH was measured by the Landcare Research Environmental Chemistry Laboratory using method 106i. Briefly, a 1:2.5 suspension of soil:water was stirred vigorously then left to stand overnight before measurement with a pH electrode. Full descriptions of each method are available from <http://www.landcareresearch.co.nz/resources/laboratories/environmental-chemistry-laboratory>.

2.2.3 Resource availability

Soil physiochemical properties from each sample were also analysed by Landcare Research Environmental Chemistry Laboratory using standard procedures to determine concentrations of organic C and total N (method 114), and Olsen P (method 124). Ammonium and nitrate were extracted using a modified version of method 118. Briefly, 2M KCl was used in a 1:10 soil: extractant ratio and the resulting slurry turned end-over-end for one hour, followed by filtration of the sample prior to analysis. Aboveground plant biomass from each sampling location, was also removed from a 20 × 20 cm grid centred on the soil core. Plant matter was dried at 60 °C and weighed (Rayment and Lyons 2011).

2.2.4 DNA sequence analyses

DNA was extracted from individual samples using MOBIO PowerSoil-htp 96 well Soil DNA Isolation Kits (MOBIO Laboratories Inc., USA) according to the manufacturer's protocol, but using a TissueLyser II disruption system (QIAGEN, Germany) to agitate sample solutions (30 revolutions per second, 2 minutes, twice).

To characterise bacterial community composition, the DNA from each soil sample was amplified and sequenced on an Illumina MiSeq sequencer (Illumina Inc., USA) following a standard protocol (Illumina 2013). Briefly, DNA fragments were amplified using modifications of the primers 341F (5'-**TCGTCTGGCAGCGTCAGATGTGTATAAGAGACAG**CCTACGGGNGGCWGCAG-3') and 785R (5'-**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACTACHVGGGTATCTAATCC-3'). These primers target the V3 and V4 region of bacterial 16S rRNA genes to provide a good combination of domain and phylum coverage (Klindworth *et al.* 2013) but are modified to also include Illumina adapter overhang nucleotide sequences (bold) required for downstream DNA sequencing. DNA was amplified using the standard protocol, which follows the thermocycling procedure: (1) 95 °C for 3 min; (2) 25 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; (3) 72 °C for 5 min. To ensure the accuracy of our sequencing approach to identify the correct taxa within samples, we also amplified the DNA of a 20-species 'Microbial Mock Community' (Community 'B' from BEI Resources, catalogue No. HM-782D, USA). Following amplification, PCR products were individually purified using AMPure XP reagents (Beckman Coulter, USA) and a Biomek 4000 liquid handling workstation (Beckman Coulter Inc., USA), according to the manufacturer's instructions. The concentration of purified amplicons was finally measured and recorded using a Qubit® dsDNA HS Assay Kit (Life technologies, USA) before submission to New Zealand Genomics Ltd. for sequencing. Briefly, the procedure followed by the sequencing facility, prior to DNA sequencing, was to attach a combination of Nextera XT A and B barcode dual indices (Illumina Inc., USA) to the DNA from each sample. This approach ensured that the DNA from each sample could be identified by its unique DNA barcode. After the DNA from up to 192 samples was pooled for DNA sequence analysis, sequence data were demultiplexed (i.e. assigned to the samples from which they originated) by the DNA sequencing facility. Our sequence analysis of the mock community DNA data (not shown) detected only DNA from the 20 species in the original mock community, suggesting that the barcoding and sequencing results of the Illumina MiSeq platform were reliable. All of raw sequences have been uploaded onto the SRA-NCBI database (BioProject ID: PRJNA338717).

2.2.5 Bioinformatic analyses

Paired end read DNA sequence data were merged and quality filtered using the USEARCH sequence analysis tool (Edgar 2013). After quality filtering, dereplication was performed so that only one copy of each sequence is reported; 'singleton' sequences represented by only one DNA sequence in the database were removed. Sequence data were then checked for chimera sequences and clustered into groups of operational taxonomic units based on a sequence identity threshold equal to or greater than 97% (hereafter referred to as 97% OTUs) using the clustering pipeline UPARSE (Edgar 2013) as described in Ramirez *et al.* (2014). Next, prokaryote phylotypes were classified to corresponding taxonomy by implementing the RDP classifier routine (Wang *et al.* 2007) with an 80% confidence cutoff in QIIME v. 1.6.0 (Caporaso *et al.* 2010) to interrogate the Greengenes13_8 database (McDonald *et al.* 2012); all sequences of chloroplast and mitochondrial DNA were removed. Finally, the DNA sequence data were rarefied to 5,500 randomly selected reads per sample and three samples per site.

To confirm the similarity of key bacterial taxa identified in the present study to those found at other study sites, we used the Nucleotide database from NCBI to search for closely related DNA sequences for inclusion in our phylogenetic analysis, which was completed using Geneious software (version 7.1.6; Biomatters Ltd, New Zealand). Briefly, we aligned all sequence data using the Geneious alignment tool, estimated evolutionary distances with a Jukes-Cantor Distance Model and constructed unrooted consensus phylogenetic trees from these distances using neighbour-joining. Bootstrap confidence levels were estimated from 10,000 replicated alignments of the data.

2.2.6 Quantitative data analyses

To assess the variation in bacterial community richness and composition, our analyses used either bacterial taxon richness (the relative number of different 97% OTUs at each site) or mean compositional similarity (calculated by constructing a Bray-Curtis distance matrix from the relative bacterial abundance data). Spatial patterns in bacterial

taxon richness and composition were plotted using the heatmap function within the R package ‘gplots’. Multivariate sample data were related to explanatory matrices of spatial and environmental data using distance-based redundancy analysis (db-RDA) and a forward selection procedure with the ‘capscale’ and ‘ordistep’ functions in the ‘vegan’ package in R (Oksanen *et al.* 2015). Variance partitioning procedures using db-RDA outlined in Borcard *et al.* (2011) were performed to indicate how much total variation in the bacterial community data be explained by groups of either (i) soil ‘resource’ variables, for example, concentrations of soil C, N and P, and above-ground plant biomass, which can both remove and input nutrients into the soil (ii) ‘non-resource environmental’ variables, that is soil temperature, moisture content, and pH, (iii) a combination of resource and non-resource environmental variables, and (iv) unexplained variance. The variance partitioning procedure computes canonical R^2 values analogous to the adjusted R^2 values produced in multiple regression (Peres-Neto *et al.* 2006). The components of variation associated with bacterial community variability at sample elevations and aspects were quantified and visualised using distance-decay curves within the R package ‘vegan’ (Oksanen *et al.* 2015). We plotted multivariate regression trees using the ‘randomForest’ package in R (Liaw and Wiener, 2002), to identify which individual resource and non-resource environmental soil attributes correlate most closely with the observed variation in bacterial community composition and richness across the study site. Finally, a correlation ‘heatmap’ was used to visualise the strength of correlation between each environmental factor using the R package ‘corrplot’.

Environmental variables were analysed individually using univariate analysis of variance (ANOVA), with P -values obtained by permutation. For these data, there were only two relevant factors: Aspect and elevation. Where appropriate, we also used paired or unpaired t -tests to detect differences in environmental variables between groups of samples collected at different elevations and aspects. The pairwise Bray-Curtis distance matrix calculated from the bacterial community data was analysed using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001; McArdle and Anderson 2001). Significant differences in bacterial community composition identified by the PERMANOVA procedure may be caused by average differences in compositional similarity among groups, or alternatively, by differences in the within-

group multivariate dispersion among groups. To quantify variation in bacterial community data within groups of study sites (e.g., to compare average community similarity of sample data collected within each elevation band), we used multivariate dispersion (MVDISP) index values, which calculate average Bray-Curtis distances among sample data. Permutational analysis of multivariate data dispersion (PERMDISP) routines were then used to confirm if within-group differences in multivariate dispersion varied significantly across elevation and aspect. MVDISP, PERMANOVA and PERMDISP routines were performed in PERMANOVA+, an add-on of the PRIMER6 package (Plymouth Marine Laboratory, UK). The PERMANOVA routine (i.e. permutational ANOVA) was similarly used for the statistical analysis of univariate data (e.g., taxonomic richness or soil carbon concentrations). We repeated our analyses for subsets of the bacterial community data to study variation in the relative abundance of the ten most abundant bacterial families, since dominant taxa typically have a larger influence on bacterial community composition.

Samples located on the sunnier aspect of the mountain ridge at an elevation of 900 m were heavily impacted by localised grazing and also by elevated concentrations of soil nitrogen. Thus, not surprisingly, the composition of bacterial communities in these samples differed markedly from that found at other sites across the elevation gradient (Appendix A Figure A1). For this reason, data from these two sample sites were not used in further analyses.

2.3 Results

We identified approximately 17,000 distinct bacterial operational taxonomic units (or OTUs of 97% DNA sequence similarity) from 2.2 million rarefied 16S rRNA gene sequences, representing 487 bacterial families across the study site.

2.3.1 Elevation and aspect patterns

Multivariate analysis of the bacterial community data (Appendix A Figure A1) revealed that across the entire study site, the gradient in elevation had a greater impact on both bacterial community composition and taxon richness than aspect (as assessed by the square root of the component of variation attributable to these factors in the PERMANOVA model; Appendix A Table A1, all $P < 0.001$). All interactions analysed in the model were statistically significant ($P < 0.001$). To further explore the hypotheses that bacterial community composition correlated with gradients in elevation (H1) and aspect (H2) we used spatial heatmaps to visualise the nature and extent of variability in community composition and richness (Figure 2.2). Bacterial community composition changed markedly across the elevation gradient (Figure 2.3; $y = 0.0003x + 0.30$, $R^2 = 0.99$). Bacterial community composition changed less in response to differences in aspect than to differences in elevation across the study site. The average difference in bacterial community composition comparing samples collected from sunnier (SU2) versus shadier aspects of the mountain ridge (SH2) that were separated by a fixed distance of 100 m, was 0.56 Bray-Curtis units. The same average difference in bacterial community composition (i.e., ~0.55 Bray-Curtis units) was only observed when comparing samples differing in elevation by 900 m or greater along the mountain ridge (Figure 2.1c). This difference in elevation corresponded to a flat surface distance of 4 km. This suggests that, comparing sample sites separated by fixed distances across the study site (e.g., ~100 m), variation in bacterial community composition changed more rapidly with variation in site aspect than site elevation. No clear pattern in bacterial community richness was observable across the whole elevation gradient. However, at higher elevations (i.e., above approximately 900 m), bacterial taxon richness was greater on the sunnier side of the mountain (i.e., 1263, 97% OTUs were detected per sample, on average), compared to on shadier aspects of the mountain ridge (mean 97% OTU richness = 1174; t -test p -value = 0.005).

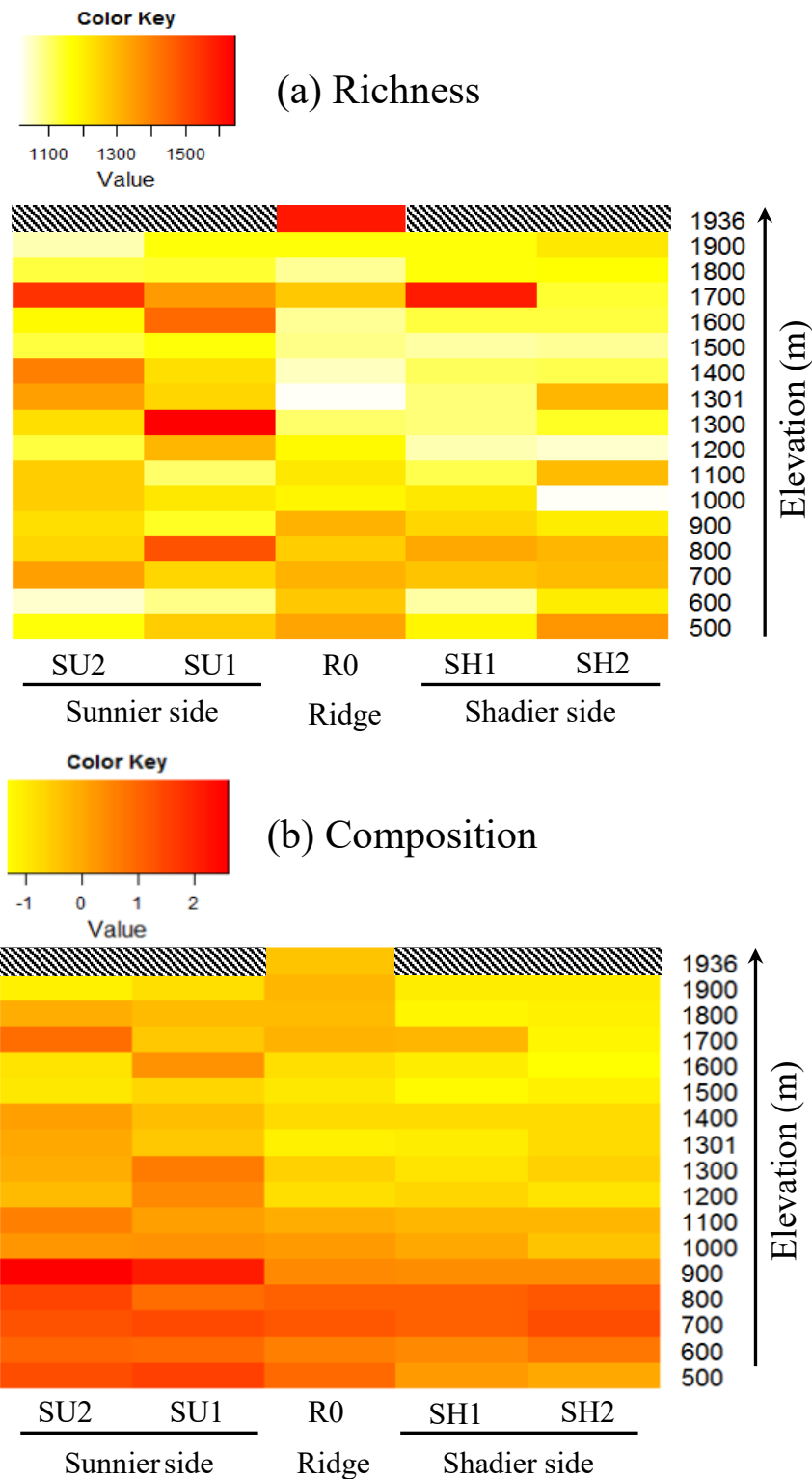


Figure 2.2. Variation in relative bacterial (a) taxon richness or (b) composition across the study site. Different rows represent data collected from different elevation bands; different columns represent data from different aspects. Sample data are assigned colours (a) across a gradient from red (highest average 97%OTU richness) to yellow (lowest average 97%OTU richness) or (b) across a gradient from red (highest 1D configuration score) to yellow (lowest 1D configuration score) after data reduction by non-metric multidimensional scaling of Bray-Curtis distance data. Sites at the top of the mountain where communities were not sampled are indicated by hatched lines.

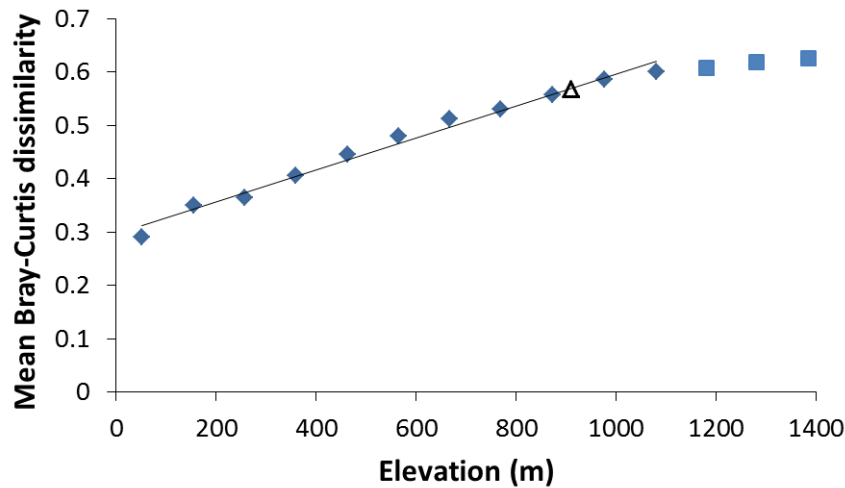


Figure 2.3. Scatterplot showing average Bray-Curtis dissimilarity (from Figure 2.2b) comparing sample data separated by different elevational distances. In the scatterplot, the linear trendline for the data is $y = 0.0003x + 0.30$ ($R^2 = 0.99$) using the first 11 data points (represented as diamonds). The hollow triangle shows the average distance among all samples collected from different aspects of the mountain, calculated by their mean Bray-Curtis dissimilarity.

We wished to confirm whether observed differences in bacterial community composition were caused by true variation in average bacterial community composition (i.e., the location of bacterial community data in multivariate space), or differences in community dispersion (i.e., differences in community variation comparing samples within the same site). Average MVDISP index values revealed an increase in the compositional variability of bacterial communities across sample aspects with increasing elevation (Figure 2.4; Regression p -value = 0.042). Variability in bacterial community composition among sample clusters was lowest for samples collected at 700 m (MVDISP = 0.095) and highest at an elevation of 1700 m (MVDISP = 1.50) (Figure 2.4; PERMDISP p -value = 0.002). Almost all of the data from higher elevation sites (> 900 m, except 1500 m, MVDISP = 0.53) were more variable (MVDISP > 0.80) than those from lower elevation sites (t -test p -value = 0.025).

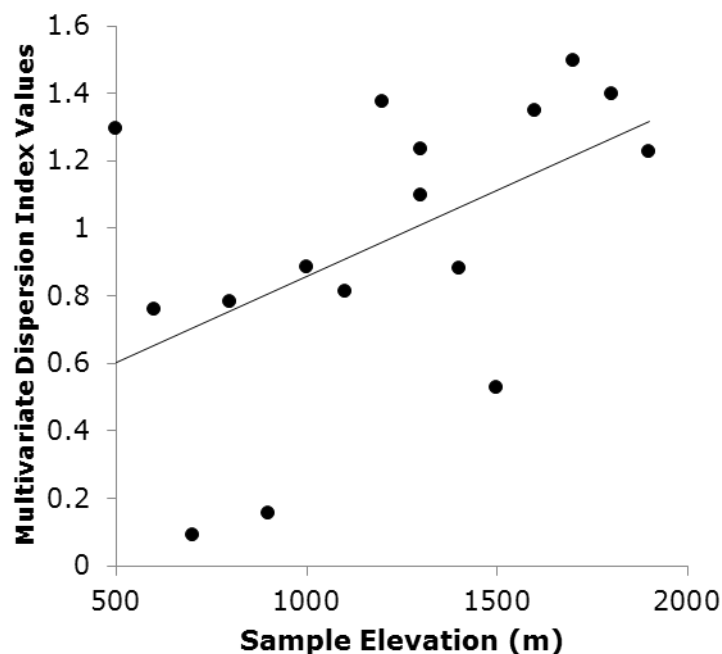


Figure 2.4. Scatterplot showing the relationship between sample elevation (m) and variability in bacterial community composition among samples collected at different clusters. Variability in bacterial community composition was quantified at each sample elevation using multivariate dispersion (MVDISP) index values, which describe the dispersion of sample data in multivariate space using a Bray Curtis distance (as in Appendix A Figure A1). Linear trend line for the data is $y = 0.0005x + 0.35$ ($R^2 = 0.26$).

The ten most dominant families represented 53% of the overall bacterial community and thus are expected to be important drivers of the overall changes in bacterial community composition observed across the site. The family *Chthoniobacteraceae* was most dominant across the study site (representing 13% of all taxa detected), followed by the family *Thermogemmatissporaceae* (10%) belonging to the phylum *Chloroflexi*. Sequences related to the *Thermogemmatissporaceae* were most abundant at higher altitude, representing 14% of DNA sequence reads over 900 m but just 2% of sequence reads below 900 m (t -test p -value < 0.001 ; see Appendix A Figure A2). The family *Koribacteraceae* (eighth most dominant overall) and Ellin6513 (10th most dominant overall) also were more abundant at high elevation representing 4.5% and 4.5% of DNA sequence reads detected over 900 m, respectively, but just 1.8% and 0.8% of sequence reads below 900 m (t -test p -values < 0.001). In contrast, members of the family *Gaiellaceae* (ninth most dominant) were more abundant at lower elevations; their average abundance was 1.9% and 6.4% at elevations above and below 900 m, respectively; t -test p -value < 0.001). At high elevation sites (i.e., above 900 m),

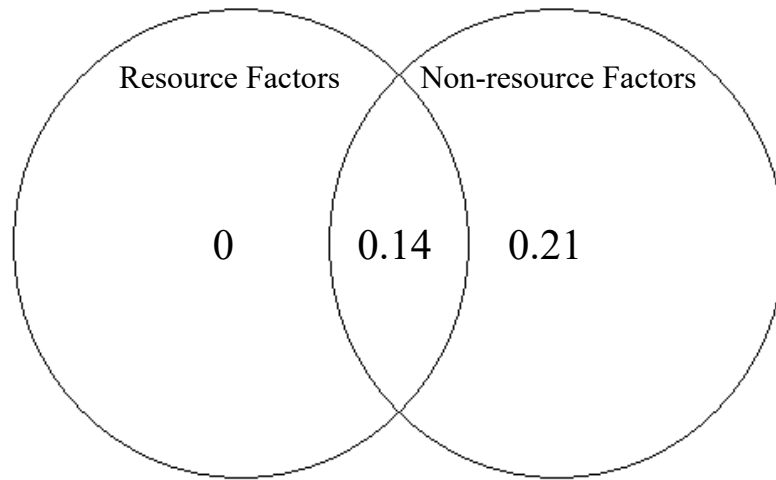
members of the family Ellin6513 were more abundant on shadier aspects of the mountain (mean abundance = 5.3%) than on the ridge or sunnier side at high elevation (mean abundance = 3.3%; *t*-test *p*-value < 0.001).

The family *Thermogemmatissporaceae* includes bacteria assumed to be adapted for growth at high temperature (King and King 2014; Yabe *et al.* 2011) and the ability to use substrates such as carbon monoxide as an energy source (King and King 2014). Since our study revealed a greater abundance of presumed thermotolerant *Thermogemmatissporaceae* at higher, cooler elevations, we explored the similarity of DNA sequences collected in the present study to known thermophile members of this family. Representative *Thermogemmatisspora* OTU sequences were compared to a variety of published gene sequences and their similarity plotted using a phylogenetic tree (Appendix A Figure A3). The OTUs found in the present study belonged to two distinct subdivisions. The majority were affiliated ($\geq 97\%$ sequence similarity) with established clades of the *Chloroflexi* previously detected in alpine environments (Costello and Schmidt 2006; Zinger *et al.* 2009).

2.3.2 Relative importance of resource versus non-resource environmental factors as determinants of bacterial community richness and composition

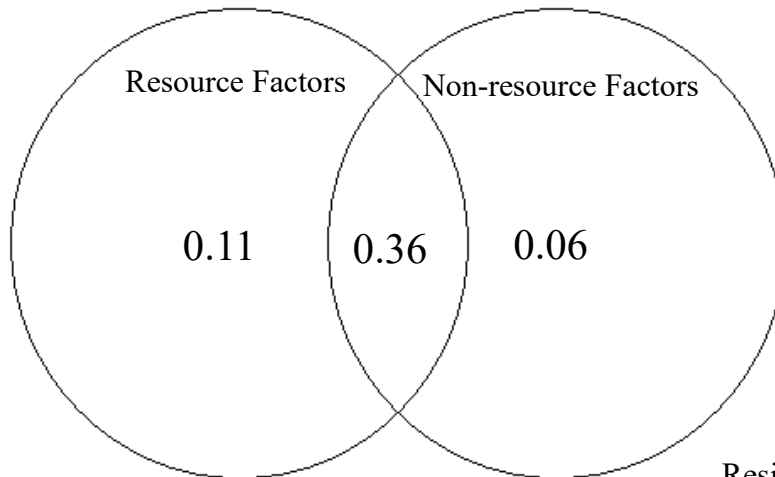
We used variance partitioning procedures to confirm that the group of explanatory variables categorised as ‘non-resource environmental variables’ (that is, soil pH, temperature, and moisture) independently explained the greatest amount of observed heterogeneity in bacterial community richness (Figure 2.5; 21%). ‘Resource variables’ (organic C, total N, NO₃-N, NH₄-N, Olsen P, and aboveground plant biomass) did not independently explain any variation in bacterial community richness. In contrast, variability in ‘non-resource environmental variables’ independently explained just 6% of the observed variation in bacterial community composition, assessed from Bray-Curtis measures of community similarity, compared to 11% explained by ‘resource variables’; the shared variance explained by both groups of factors accounted for the greatest component of the observed variation (36%).

(a) Richness



Residuals = 0.65

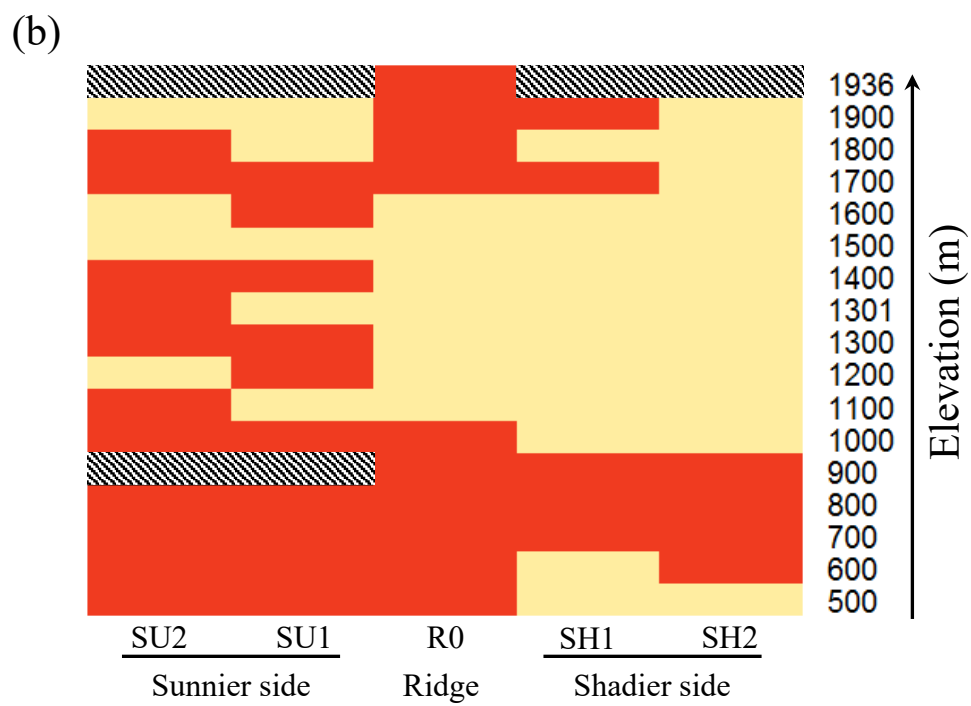
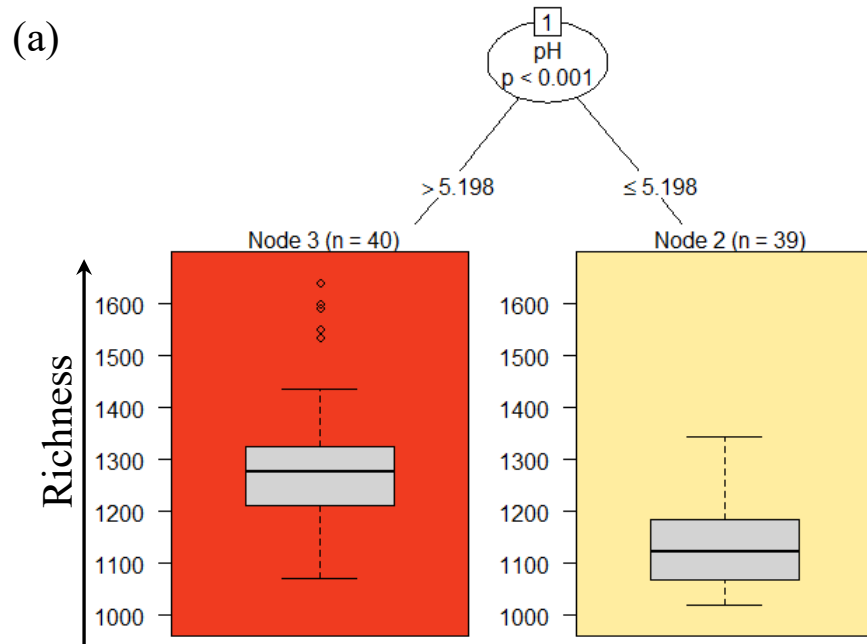
(b) Composition



Residuals = 0.48

Figure 2.5. Venn diagrams providing a graphical representation of the variance partitioning of bacterial community (a) richness and (b) composition (community Bray-Curtis similarity) between resource and non-resource environmental factors.

To identify individual resource and non-resource factors that contribute or relate most to the variation observed in bacterial community richness and composition across our study sites we used random forest analysis (Figure 2.6). The only variable to be consistently related to variability in microbial richness was pH. Indeed, the sample site with the greatest richness (SU1, 1300 m, richness = 1644) had a soil pH of 5.9, one of the highest pH values detected among our study sites. On the other hand, the sample with the lowest pH (SH2, 1200 m), of 4.7, had a richness of just 1019, the second lowest value across the study area. Overall, soil pH was more strongly correlated to changes in elevation (as assessed by the square root of the component of variation attributable to these factors in a permutational ANOVA model); a significant statistical interaction was also present between site aspect and elevation (Appendix A Table A2, all $P < 0.001$). Overall, pH was observed to be lower (i.e., closer to neutral) at lower elevation sites located on warmer aspects of the slope (Appendix A Figure A4). The results of random forest analysis confirmed interactions between the effects of resource and non-resource environmental factors in that pH had the strongest effect on the composition of bacterial communities, but in the sites with high pH (> 5.5), concentrations of total carbon were also a significant correlate of bacterial community composition. Besides the impact on whole bacterial communities, we confirmed significant correlations between soil pH and the relative abundances of dominant family members across the study site. The relative abundance of *Thermogemmatissporaceae* (the second most abundant family detected, 10%), *Koribacteraceae* (3.7%), Ellin6513 (3.4%) and *Gaiellaceae* (3.3%) were each correlated with soil pH (correlation coefficient (Spearman's Rho) = 0.47, 0.60, 0.68 and 0.52, respectively; p -values all < 0.001).



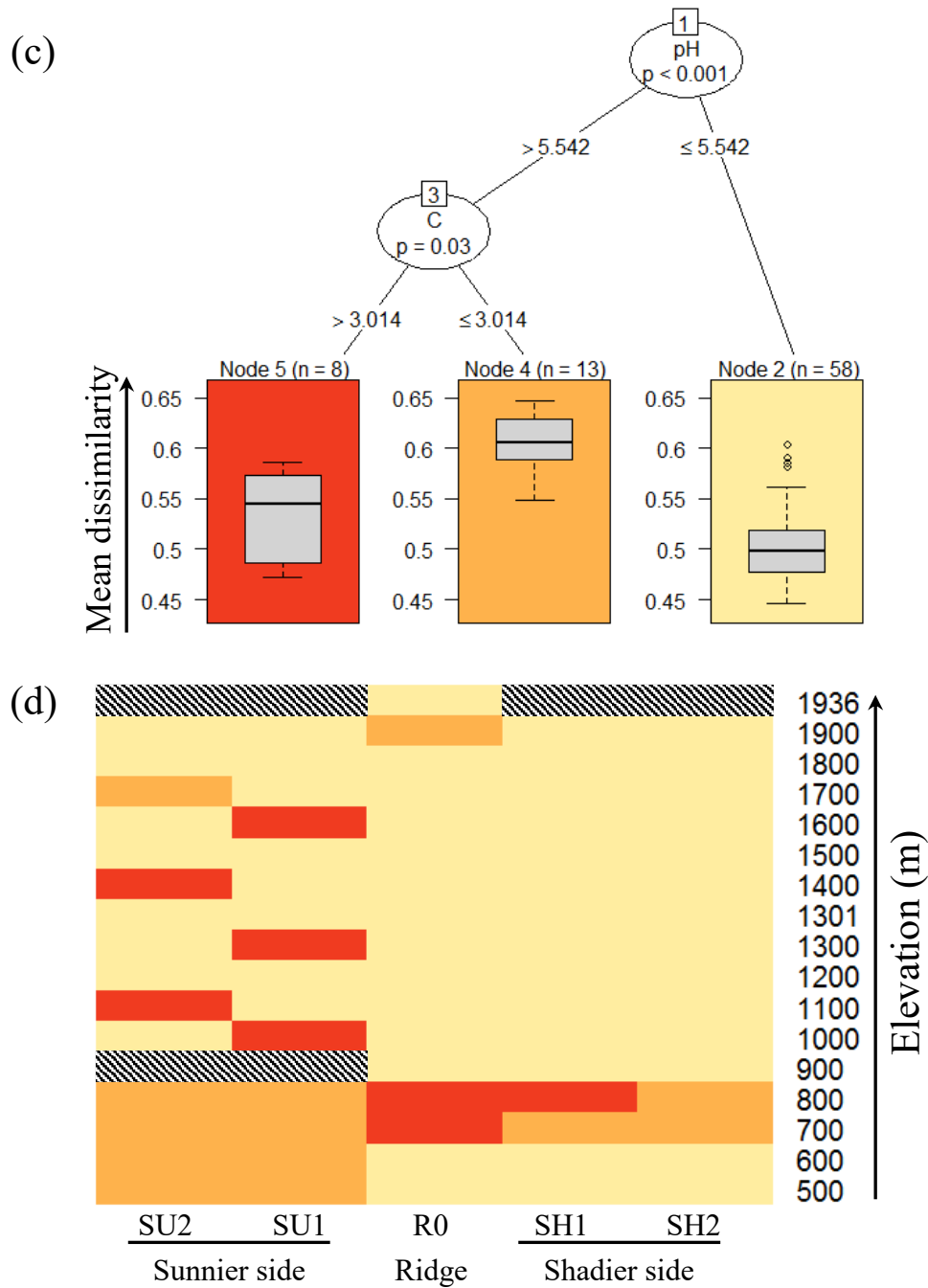


Figure 2.6. Multivariate regression tree of bacterial community (a) richness and (c) community composition associated with sampling location. The decision tree identifies variance in bacterial richness or compositional similarity caused by threshold values of elevation, resource or non-resource environmental factors. The values attached to each branch mark the criteria used by the regression tree to group samples based on differences in bacterial richness or composition. Corresponding box and whisker plots show richness of bacterial OTUs, or average composition (1D configuration score of non-metric multidimensional scaling plot) associated with each node of the data. (b, d) Graphical representation showing the spatial location of samples associated with each terminal node of the decision tree for bacterial richness and composition data, respectively. Bacterial richness refers to the number of distinct 97% OTUs identified within sample cluster, from the analysis of 27,500 DNA sequences. Sites where sample data were not collected or analysed are indicated by hatched lines.

2.4 Discussion

Across equivalent distances, variation in bacterial community composition across different aspects of a single mountain ridge was far greater than variation detected comparing samples collected at different elevations. Significant variation in the factors which are frequently suggested to be a primary determinant of the biogeography of macroorganisms (Gillman and Wright 2014), i.e., soil temperature and moisture availability, were observed across elevation and aspect gradients. However, variation in bacterial community composition and richness across the mountain microclimate gradient were more closely correlated with soil pH. Together, these findings highlight the complexity of microclimate impacts on soil bacterial communities, and the need to incorporate multiple factors, including site aspect and soil chemical attributes, into assessments of microbial community composition across elevation gradients.

Consistent with several other studies (Fierer *et al.* 2010; Shen *et al.* 2013), and in contradiction of our *Elevation Hypothesis* (Elevation H₁), we detected no significant decline in bacterial taxon richness with altitude. These findings contribute to a wealth of evidence indicating that bacterial communities exhibit fundamentally different responses to elevation than macroorganisms (Fierer and Jackson 2006; Lauber *et al.* 2009); gradients in the richness of both plant and animal communities are frequently observed with elevation (Aubry *et al.* 2005; Carneiro *et al.* 2013). Critically, and in support of our *Aspect Hypothesis* (Aspect H₂), we confirmed that, when comparing samples across equivalent distances, bacterial community attributes were more impacted by variation in sampling site aspect than elevation. That is to say that bacterial communities at the same elevation but separated by distances of just 100 m across aspects, were far more divergent, on average, than communities separated by elevational distances of up to 900 m. The cause behind the relatively strong impact of aspect compared to elevation is unclear. Although significant differences of over 2 °C were detected among adjacent slopes, equivalent to a ~300 m increase in altitude (Rorison *et al.* 1986), soil bacterial community composition and richness was nevertheless more tightly correlated with soil pH, leading to our third hypothesis (Environment H₃) being accepted.

Ours is not the first study to reveal the strong influence of soil pH on bacterial community composition (Fierer and Jackson 2006; Griffiths *et al.* 2011; Lauber *et al.* 2009), including across elevation gradients (Bryant *et al.* 2008). Soil pH has been identified as being a key predictive variable of bacterial composition in both soil (Fierer and Jackson 2006; Griffiths *et al.* 2011) and aquatic environments (Fierer *et al.* 2007). Changes in bacterial community composition may also be driven by additional soil factors that are, all-be-it indirectly, linked to soil pH. For example, pH can mediate the activity of extracellular enzymes involved in litter decomposition (Griffiths *et al.* 1995), thereby impacting soil carbon storage and transformation rates (Kemmitt *et al.* 2006). Soil pH is also a strong mediator of nitrogen mineralisation, which can occur across a wide pH range (Fu *et al.* 1987), but decreases under more acidic conditions. Elevational gradients in soil pH are commonly reported and are frequently attributed to increased rates of mechanical weathering that occurs up to elevations of ~2,000 m in the Southern Alps, beyond which persistent permafrost obviates the freeze-thaw cracking process (Hales and Roering 2016). Increased weathering causes soil acidification as base cations such as calcium, sodium, phosphate and magnesium are leached from the soil. Although significant gradients in soil temperature, moisture and resource availability were noted across our study site, our results contribute to a growing body of evidence that pH, possibly affected by different rates of weathering across the study site, is a dominant driver of bacterial diversity (Lear *et al.* 2009; Shen *et al.* 2013; Zhang *et al.* 2013).

A hypothesis commonly used by microbiologists is that ‘everything is everywhere – the environment selects’ (Baas-Becking 1934). However, the relative importance of environmental conditions, such as pH, for microbial community composition is scale-dependent. Many authors suggest that non-symbiotic populations of bacteria exhibit global distributions (Barberan *et al.* 2015; Brown *et al.* 2012). Nevertheless, any limitation to their movement will give rise to distance decay patterns in bacterial community composition. Therefore, even if bacteria are universally distributed, spatial variability in their community composition will reflect spatial patterns of their key environmental drivers. Where β -diversity patterns in microbial communities observed across space are not found to be related to variation on environmental attributes, they are hypothesised to result from dispersal limitation (Martiny *et al.* 2011). The decrease

in similarity of bacterial communities with elevation observed in this study is likely to result from a combination of stochasticity in bacterial dispersal and increasing environmental differences among samples at increasing distances; further experimental work is required to tease apart the relative importance of these effects.

It is noteworthy that members of the family *Thermogemmatisporaceae* were dominant in the community across our alpine study site. These spore-forming bacteria have been recovered from geothermal soils and biofilms (King and King 2014; Yabe *et al.* 2011) and also from soils surrounding natural gas vents (de Miera *et al.* 2014). Strains cultured from these environments are thermophilic, exhibiting growth optima > 50 °C (Yabe *et al.* 2011). Why do members of this apparently thermophilic family dominate the composition of communities across this alpine elevation gradient? Strong, negative correlation of their abundance with soil pH suggests that mechanisms of environmental selection operate among these communities. Interestingly, the abundance of *Thermogemmatisporaceae* was greatest in soils of lower pH, closer to the pH optima reported for members of this family (Yabe *et al.* 2011). However, direct evidence of cellular metabolic activity (e.g., using stable isotope probing or transcriptome-based methodologies) would be required to determine if these bacteria are indeed active within the soil of the study site. Another explanation is that not all organisms related to *Thermogemmatisporaceae* are thermophilic. Indeed, DNA sequences identified in the present study were similar to those found in other cool environments, including acidic alpine tundra soils of the Northern Hemisphere (Costello and Schmidt 2006; Zinger *et al.* 2009), indicating the widespread distribution of these taxa in alpine zones. However, microorganisms capable of forming resistant endospores are frequently found in environments that do not appear to support their metabolic activity. It is therefore conceivable that thermotolerant bacteria, such as members of the family *Thermogemmatisporaceae*, may be more resistant to long-range atmospheric transport and the extreme UV, low moisture and low nutrient conditions it provides. This notion is supported by studies that have identified resistant, but viable, spores from thermophilic organisms, particularly members of the *Geobacteraceae*, in samples from the upper troposphere (deLeon-Rodriguez *et al.* 2013) as well as within cold oceanic currents (Hubert *et al.* 2009; Muller *et al.* 2014). It is possible that these bacteria adhere to the ‘*Geobacillus paradox*’ which, owing to resistant properties and longevity, enable

quiescent cells to achieve surprisingly high population densities in environments that appear to be poorly suited for their survival (Zeigler 2014), such as soils at high elevation. However, the phylogenetic position of other thermophilic isolates or strains of the class *Ktedonobacteria*, such as *Thermosporothrix* (Yabe et al., 2010) and *Ktedonobacter* (Chang et al., 2011), need to be also carefully considered in further studies to validate the taxonomic identification of the OTUs belonging to the family *Thermogemmatissporaceae* observed in the current study.

2.5 Conclusion

We found greater differences in both the richness and composition of bacterial communities between samples taken on different mountain aspects, than between samples taken at equivalent distances at different mountain elevations. The findings of the present study lead us to conclude that to appropriately use elevation and aspect gradients as a natural laboratory for assessing the likely impacts of climate change on bacterial communities, we must account for local variation in abiotic conditions because, at least in this case, bacterial composition and richness were more closely related to local abiotic factors (soil pH) than to variation in temperature or resource factors. Further work is needed to better understand the mechanisms underlying microbial diversity patterns and the relative importance of both evolutionary and ecological processes as determinants of bacterial community structure and composition. In particular, the effect on bacterial communities of elevational gradients in pH and other soil factors must be quantified using gradients in elevation and aspect as proxies for climatic variability when predicting community responses to climate change.

Chapter 3

**Soil fungal communities form closer
network associations at lower elevation**

3.1 Introduction

Mountain gradients are commonly used to study the impact of environmental variables on community attributes including taxonomic richness (Gaston, 2000), because such gradients are characterised by dramatic changes in climatic (e.g., temperature (Korner, 2000)) as well as soil conditions (e.g., soil nutrient concentration (Guo et al., 2015)) across relatively short geographic distances. Numerous studies have reported elevational patterns from the analysis of a wide variety of plant (Smith, 1988; Vetaas and Grytnes, 2002; Lenoir et al., 2008; Randin et al., 2009; Asner and Martin, 2015) and animal (Willms, 1971; Kessler et al., 2001; Lee et al., 2004; Sekercioglu et al., 2008; Pellissier et al., 2012) community data. Most data from macroorganism communities are observed to follow one of two elevational patterns: richness/diversity decreases monotonically with increasing elevation (Gaston, 2000; Hillebrand, 2004), or peaks at intermediate elevations (McCain, 2004; Rahbek, 2005) as predicted by the mid-domain effect (Currie and Kerr, 2008). However, studies exploring elevation gradients in microbial community richness indicate that they respond to diversity drivers that are fundamentally different from those observed for plants and animals (Bryant et al., 2008; Fierer et al., 2010). Indeed, studies report inconsistent patterns in soil fungal community richness along elevation gradients, for example, a lack of pattern (Meier et al., 2010; Meng et al., 2013), unimodal pattern (Coince et al., 2014; Miyamoto et al., 2014), or a decrease in richness with increasing elevation (Lugo et al., 2008; Bahram et al., 2012; Liu et al., 2015). These inconsistent biogeographic patterns imply that soil fungal community richness and diversity attributes might not be driven by a single rule, but instead by a more complex set of ecological mechanisms.

Climatic variables, including temperature and precipitation are expected to be important determinants of fungal community patterns, because they influence not only fungal activity and dispersal, but also interspecific interactions, interactions with hosts, and trophic interactions (Gange et al., 2013; Andrew et al., 2016). However, climatic variables alone are rarely able to explain observed patterns of fungal community richness and diversity along elevation and latitudinal gradients (Meier et al., 2010; Bahram et al., 2012; Tedersoo et al., 2012; Tedersoo et al., 2014). More commonly, studies have reported that fungal community richness or composition is instead more

closely related to soil physicochemical attributes (e.g., total carbon, soil C/N, pH) (Tedersoo et al., 2012; Meng et al., 2013; Lanzen et al., 2016; Siles and Margesin, 2016), or an interaction of these with climatic factors (Coince et al., 2014; Miyamoto et al., 2014). Soil physicochemical variables might drive fungal populations based on variation in the nutrient preferences and acquisition strategies of different species (Goldfarb et al., 2011). For these reasons, we chose to study the influence of edaphic nutrient variables on soil fungal community composition and richness across a mountain elevation gradient to assess the relative importance of differing climatic, edaphic and biotic factors.

Fungal species are not found in isolation within complex environments and multi-species associations may be used to reveal functionally-distinct niche spaces shared by community members (Steele et al., 2011; Faust and Raes, 2012; Schimel and Schaeffer, 2012; Kara et al., 2013). Recent studies demonstrate that soil fungal communities and their functional role in soil carbon and nitrogen cycles (e.g., polycyclic aromatic hydrocarbon (PAH) biodegradation) are strongly structured by interspecific interactions, such as competition (Freilich et al., 2010; Pickles et al., 2012) and co-metabolism (Cerniglia, 1997; Peng et al., 2008; Thion et al., 2012), which ultimately may impact the functioning of entire ecosystems (Fuhrman, 2009) making co-occurrence patterns in microbial ecosystems of particular interest to study. Putative fungal-fungal interactions, as detected by co-occurrence networks, may be critical determinants or correlates of community attributes, such as diversity and ecosystem functioning (Deng et al., 2012) and to date have been largely ignored in most studies of microbial biogeography. As network co-occurrence analyses can reveal how particular fungi occur together and how these associations vary with different environmental conditions, such patterns the analyses of such patterns can be used to avoid the more reductionist view deduced from analyses of community richness, where each taxon is considered in isolation.

Analysis of co-occurrence network topologies can be used to reveal how taxa co-occur in niches and how these relationships change under different environmental conditions (Fuhrman, 2009). We sampled fungal communities every 100 m across an elevation

gradient of almost 1,500 m to address the following three hypotheses. First, we hypothesised that fungal community richness would decline with increasing elevation (Elevation H_1), since environmental conditions, such as temperature, may be considered to be more optimal for life at lower elevation due to increased energy-nutrient availability (Hawkins et al., 2003). Thereby, we further hypothesised that the number of overall interspecific co-occurrences within fungal communities would increase with decreasing elevation (co-occurrence H_2). Finally, based on the knowledge that fungi are major contributors of soil nutrient cycles and mediate the transfer of limiting nutrients to plant hosts, we predicted that soil physicochemical variables, e.g., soil nitrogen and ammonium would, in fact, be stronger correlates of fungal community network associations than climatic variables, i.e., temperature and soil moisture (soil physicochemistry H_3).

3.2 Materials and methods

3.2.1 Sample collection

We collected 405 soil samples from a continuous ridge on the north-eastern side of Mt. Cardrona, New Zealand (44.85° S, 168.95 E; see Figure 2.1 in Chapter 2). At 100 m elevation intervals from 500 m to 1900 m along the ridge we sampled soil at 15 elevations. Because the slope of this ridge is very shallow at 1300 m, we chose to sample two locations at 1300 m, a location at the upper edge of the ridge hereafter termed 1301 m and another at the lower edge hereafter termed 1300 m.

As climatic and environmental variables were expected to vary locally along the elevation gradient, we collected five soil replicate samples from each of five different locations at each elevation, one of the five locations (R0) at each elevation was located on the mountain ridge line, two (SU1, SU2) from the sunnier side of the ridge to the north, and another two from the shadier side of the ridge (SH1, SH2) to the south. All of these sample clusters were collected at 25 m intervals. We also collected a single cluster of samples at the summit of the mountain at 1936 m. Within each cluster, the five, 10 cm deep samples were collected along a 1.5 m transect using a soil corer. Thus,

a total of 405 samples were collected (16 elevations \times 5 sample clusters \times 5 site samples + 5 summit samples = 405) along a 1,400 m elevation gradient.

3.2.2 Analysis of soil physicochemical variables

During the summer (from February to March in 2014), we collected real-time temperature data at a depth of 10 cm below ground using ibutton temperature loggers located within each sample cluster (i.e., $n = 81$). To study soil physicochemical parameters (i.e., the concentrations of organic carbon, total nitrogen, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, Olsen phosphorus and soil pH), soil samples were analysed by the Landcare Research Environmental Chemistry Laboratory (Palmerston North, New Zealand) using standard procedures (Blakemore, 1987; Lachat Instruments, 1998b; a; Leco, 2003). We measured soil moisture gravimetrically by weighing samples before and after drying in an oven at 105 °C for 96 hours (Rayment and Lyons, 2011).

3.2.3 DNA sequence analysis

We used MOBIO PowerSoil-htp 96 well Soil DNA Isolation Kits (MOBIO Laboratories Inc., USA) to extract DNA from individual samples across the study site. We followed the manufacturer's protocol except we chose to use a TissueLyser II disruption system (QIAGEN, Germany) to agitate the sample solutions (30 revolutions per second, 2 minutes, twice). We characterised the fungal community composition of each sample by amplifying and sequencing the ITS1 (internal transcribed spacer) region of fungal nuclear DNA. Briefly, we followed the standard Illumina protocol (Illumina, 2013) but incorporated the primers of Bellemain et al. (2010). Hence, DNA fragments were amplified using the primers ITS1-F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGA AGTAA-3') and ITS2 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCG ATGC-3'), which were modified to include Illumina adapter overhang nucleotide sequences (underlined) required for downstream DNA sequencing. PCR reactions each contained 13 μL water, 10 μL 5 \times GoTaq Green Reaction Buffer (Promega, USA), 0.5

μL each of the forward and reverse primers (10 μM concentration) and 1 μL DNA template. Reactions were held at 94 °C for 3 min, before 35 cycles at 94 °C for 45 s, 50 °C for 1 min and 72 °C for 1.5 min, and finally extension at 72 °C for 10 mins. All PCR products were visualized on agarose gels and individually purified using AMPure XP reagents (Beckman Coulter, USA) and a Biomek 4000 liquid handling workstation (Beckman Coulter Inc., USA), according to the manufacturer's instructions. Concentrations of amplified product were quantified using a Qubit® dsDNA HS Assay Kit (Life technologies, USA) before submission to New Zealand Genomics Ltd. for sequencing. Briefly, the procedure followed by the sequencing facility, prior to DNA sequencing, was to attach a combination of Nextera XT A and B barcode dual indices (Illumina Inc., USA) to the DNA from each sample. This approach ensured that the DNA from each sample could be identified by its unique DNA barcode. Amplicons from all samples were composited together in equimolar concentrations and sequenced using an Illumina MiSeq instrument and 2 × 250 bp paired-end sequencing chemistry. Once sequenced, data were demultiplexed (i.e., assigned to the samples from which they originated) by the DNA sequencing facility.

3.2.4 Sequence processing and bioinformatics

We processed all raw DNA sequence reads using the USEARCH sequence analysis tool (Edgar, 2013). Briefly, demultiplexed sequences were merged and filtered for quality using default parameters. After the removal of replicate and singleton sequences, we checked for chimeric sequences and clustered DNA sequence reads into operational taxonomic units (OTUs) of 97% similarity using the clustering pipeline UPARSE. Based on the lowest number of sequences, we rarefied our data to 5,100 sequences per sample. One sample was discarded from the dataset as it comprised a lower number of reads. Each OTU was then identified to the lowest taxonomic level possible using the UNITE ITS database (Abarenkov et al., 2010).

3.2.5 Statistical analyses

We counted the number of different fungal OTUs at each site as fungal taxon richness. To visualise the overall change in fungal community richness along the elevation gradient, we averaged the data of fungal richness and plotted it with standard error bars at each elevation using the ‘ggplot2’ package in R (Wickham, 2009). Variation in fungal community composition was calculated by constructing Bray-Curtis distance matrices from the fungal data sampled from each location at each elevation. To visualise variation in the composition of fungal communities in our study, we used non-metric multidimensional scaling (NMDS) to plot the data using PRIMER 6 software (Plymouth Marine Laboratory, UK). Additionally, we quantified and visualised variation in fungal community composition, based on Bray-Curtis dissimilarity scores comparing communities sampled at different elevation, using distance decay curves constructed with the ‘vegdist’ function within the R package ‘vegan’ (Oksanen et al., 2015) including confidence intervals for the sample data using the ‘plotCI’ function within the package ‘plotrix’ (Lemon, 2006).

For environmental variables, samples were grouped by the factors ‘location’ and ‘elevation’. We evaluated the effects of individual climatic (temperature, and soil moisture) and soil chemical attributes (pH, C, N, C/N ratio, nitrate, ammonium and P), as well as spatial differences on the distribution of fungal community richness and composition at each sample location using the ‘lm’ function in R (Chambers, 1992). To explore which environmental variables have the strongest impact on the fungal community attributes, we selected the best-fitting model using Akaike’s Information Criterion (Akaike, 1974) adjusted for small sample sizes (AICc) to compare among a set of candidates (Burnham and Anderson, 2003). Such an approach is an acceptable method to study data when relatively little is known of the system (Symonds and Moussalli, 2011). To select the best fitted model, we ranked alternative candidate models based on ΔAICc . Akaike weights (w_i), also termed ‘model probabilities’ (Burnham and Anderson, 2003; Anderson, 2007), can be interpreted as the probability of the given model being the best approximation among the candidate model set. All AIC analyses were done using the ‘AICcmodavg’ package in R (Mazerolle, 2013).

To detect meaningful and robust microbial associations, it is essential to have detailed taxa data collected across a sufficiently large sample set, as without large numbers of samples it is difficult to determine whether differences in observed co-occurrence patterns are statistically significant (Barberán et al., 2012). Therefore, based on the observed elevational pattern of fungal community composition (the result of an NMDS plot; as later described in Figure 3.1b), we separated fungal data into three groups based on elevation (namely samples from ‘high-elevation’, 1936 m to 1500 m; ‘medium-elevation’, 1400 m to 1000 m; and ‘low-elevation’, 900 m to 500 m).

Identifying core taxa (or OTUs) is useful for unravelling the ecology of microbial assemblages, because these commonly occurring microorganisms are likely vital to community functioning (Shade and Handelsman, 2012). We used these core taxa/OTUs to produce networks (workflow in Appendix B Figure B1), thereby ensuring that differences in fungal co-associations observed at different elevations were not biased by differences in taxa richness or taxa only being present at high-, medium-, or low elevation, since taxa must be present at all three elevations to be included in the network association model. Briefly, core taxa were identified as the common and abundant taxa from bacterial assemblages for each of these three elevation groups. For each elevation group, we only retained data for OTUs found to be present in at least 50% of the samples, using the ‘compute_core_microbiome’ function within QIIME to avoid model biases that might be caused by rare taxa or those with patchy presence. Finally, we constructed a list of core taxa obtained from all three elevation groups, and used these taxa to construct co-occurrence network models.

To ensure that the fungal network patterns we observed across the mountain gradient were not simply caused by our of three elevation groups, we also subdivided the fungal community data into five groups based on elevation (i.e., rather than three: namely samples from ‘higher-elevation’, 1936 m to 1700 m; ‘high-elevation’, 1600 m to 1400 m; ‘medium-elevation’, 1301 m to 1200 m; ‘low-elevation’, 1100 m to 900 m; ‘lower-elevation’, 800 m to 500 m). We then used the same process to identify the core taxa present in samples representing these five elevation subdivisions, and to generate additional networks to study elevational variations in fungal co-occurrence.

3.2.6 Network analysis

To explore possible ecological interactions among members of the core fungal taxa present at different elevations, we followed the approach of Williams et al. (2014) to test for differences in the co-occurrence patterns of core OTUs across our study site. We generated a dissimilarity matrix consisting of Spearman's correlation coefficients representing the strength of co-occurrences between each pair of OTUs using pairwise comparisons across the entire dataset. As this Spearman's matrix represents the strength of correlation among fungal pairs, we only considered co-occurrence to be robust if the Spearman's correlation coefficient (ρ) was greater than 0.5 and also statistically significant ($p\text{-value} \leq 0.05$). The nodes (i.e., the connection points) in the constructed networks represent core OTUs, while the edges (i.e., linkages between individual nodes) correspond to a strong and significant correlation between nodes. We also calculated and compared one thousand random networks of equal size (i.e., same number of nodes and edges) generated by the Erdős–Rényi model for each network obtained by this study (Lupatini et al., 2014). This approach was used to determine the likelihood that our networks represent actual fungal co-occurrences, rather than random network patterns. We explored and visualized networks with the interactive platform Gephi (Bastian et al., 2009) using the Fruchterman-Reingold layout.

OTUs present in all three networks were identified as 'generalist OTUs' and highlighted in red text on our networks. For these generalist OTUs, if no taxon name could be assigned using the UNITE database, we ran manual BLASTn searches against the GenBank non-redundant nucleotide database (nt). Best-matching sequences were then used to identify closely related taxa. We relied on the similarity of identified sequences ($> 90\%$) in GenBank as the criterion for assigning OTU names. We also tested the reliability of fungal name assignments using the e -values of BLASTn results and discarded taxa assignments $< e^{-50}$. To determine the influence of environmental variables on fungal trophic diversity, we also assigned these fungal taxa to the trophic status used by Tedersoo et al. (2014).

3.2.7 Statistical analyses of network parameters

To describe the topology of the resulting networks, a set of measures (i.e., the total number of links, total number of nodes, average degree, average clustering coefficient and the number of the shortest paths) were calculated using network analysis tools within Gephi (Bastian et al., 2009). To determine whether network complexity decreases with increasing elevation, we calculated the total numbers of links and nodes, reflecting the total number of the co-occurrences and OTUs present in the networks, respectively. The other measures, including average degree, average clustering coefficient and the number of the shortest paths, were calculated to explore the robustness and efficiency of the obtained network systems. Average degree, one of the most commonly used network parameters, was calculated by summing the strengths of the links of each node (or OTU) with all of the other connected nodes in the network, and represents how strongly an OTU is connected to others (Zhou et al., 2010). The average clustering coefficient describes how well OTUs are connected with their neighbours on average (Watts and Strogatz, 1998; Ravasz et al., 2002). Average path length was calculated as the average number of steps in the shortest paths between each node to one of another node in the network (Faust and Raes, 2012). Shorter paths may increase the speed of the network's response to perturbations as suggested by Zhou et al. (2010) and Faust and Raes (2012). Together, the higher clustering coefficients and the shorter path lengths are key network properties in terms of system efficiency and robustness (Zhou et al., 2010).

To determine whether soil physicochemical variables would be more strongly correlated with fungal co-occurrences, a correlation matrix comparing the strength of relationship between network parameters and the data of soil physicochemistry, and climatic variables was calculated and plotted using the correlation heatmap package 'corrplot' (Wei and Simko, 2013). Finally, we estimated the difference in fungal co-occurrence across the elevation groups (i.e., 'high-elevation' from 1936 m to 1500 m, 'medium-elevation' from 1400 m to 1000 m, and 'low-elevation' from 900 m to 500 m) using Tukey's Honestly Significant Differences (HSD) tests. Unless otherwise stated, all analyses were performed in R v. 3.4.3 (R Core Team, 2015).

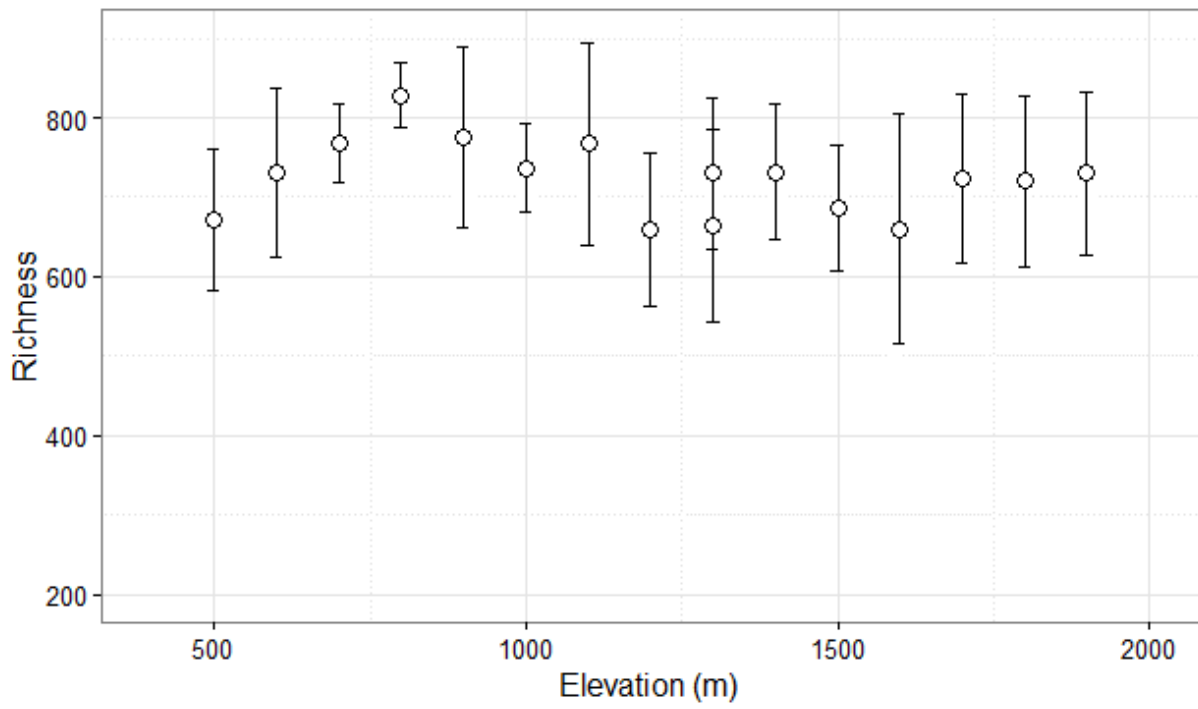
3.3 Results

3.3.1 Variation in fungal community composition and richness

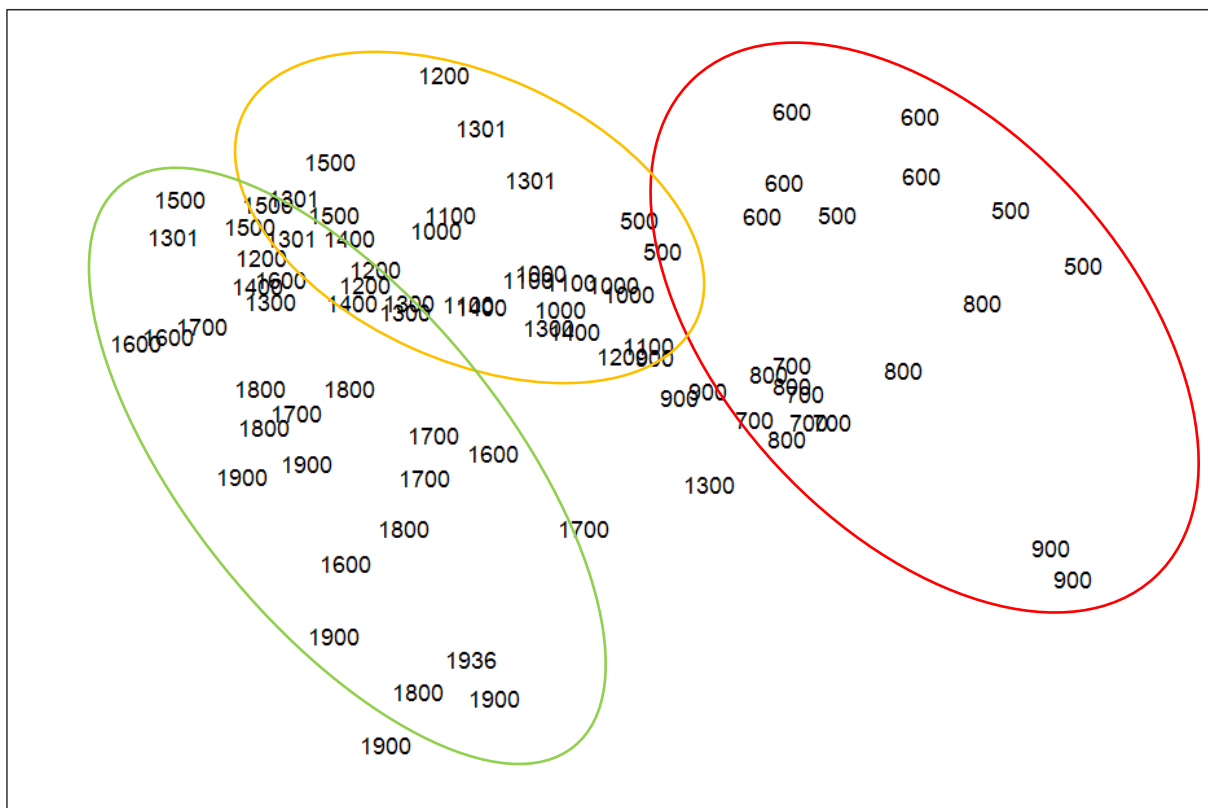
We recovered approximately 2 million fungal ITS1 gene sequences after quality-filtering and rarefaction. These sequences represented 12,105 distinct fungal operational taxonomic units (or OTUs of 97% DNA sequence similarity) across the study site.

In contrast with our hypothesis (Elevation H₁), fungal community richness did not vary significantly with elevation based on the result of linear regression (p -value = 0.26, multiple R-squared = 0.09; Figure 3.1a). However, fungal community composition varied significantly among the three elevational groups, namely, ‘high-elevation’ from 1936 m to 1500 m, ‘medium-elevation’ from 1400 m to 1000 m, and ‘low-elevation’ from 900 m to 500 m, (Tukey’s HSD, p -value < 0.01; Figure 3.1b). Fungal community composition also changed markedly with increasing elevational distance (Figure 3.1c; $y = 0.0003x + 0.47$, $R^2 = 0.92$). Additionally, significant differences in the fungal compositional data among the five elevational groups (i.e., samples from ‘higher-elevation’, 1936 m to 1700 m; ‘high-elevation’, 1600 m to 1400 m; ‘medium-elevation’, 1301 m to 1200 m; ‘low-elevation’, 1100 m to 900 m; ‘lower-elevation’, 800 m to 500 m) were observed (data not shown). These divisions were therefore used in later network analyses to test whether the fungal interspecific co-occurrences changed with elevation.

(a)



(b)



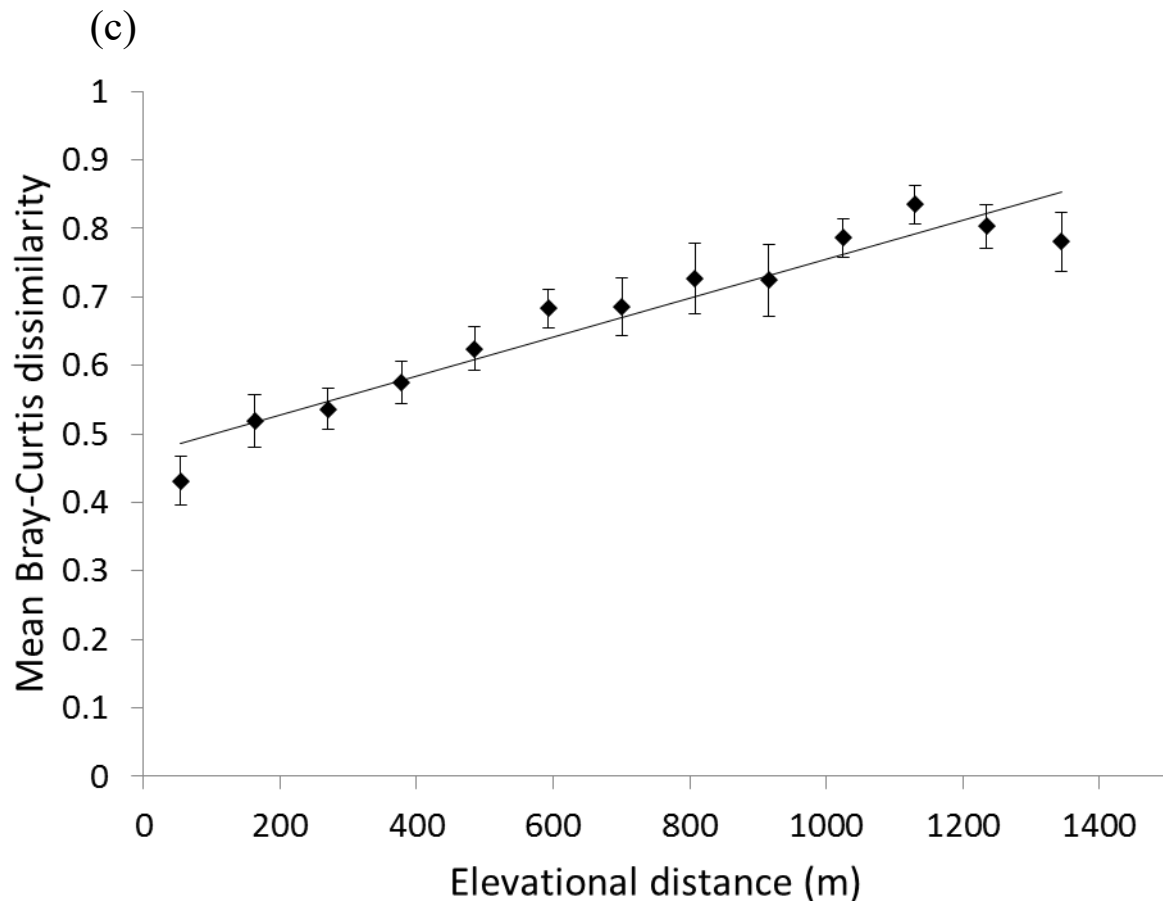
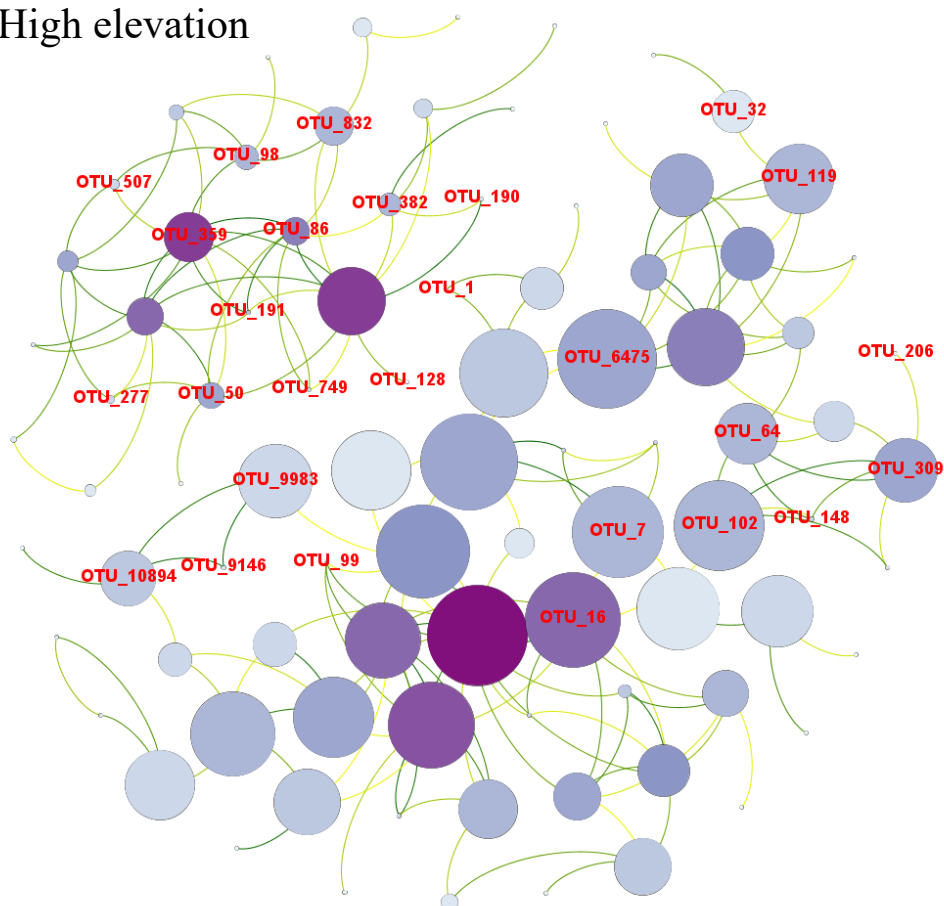


Figure 3.1. Variation in (a) average fungal taxon richness at each elevation, (b) community composition across the study site, and (c) average community similarity comparing samples separated by different elevational distances. The scatter plot (a) shows the average fungal richness at each elevation across our study site. Relative taxon richness was calculated from the rarefied fungal OTU table with 5,100 DNA sequences per sample. Because the slope of this ridge is very shallow at 1300 m, we sampled two locations at 1300 m. Plot (b) is constructed from non-metric multidimensional scaling of OTU data using a Bray-Curtis measure. Sample data closer to each other are expected to contain more similar fungal communities. Sample data are assigned numbers across a gradient from 500 to 1936 representing different elevations in metres (2D stress = 0.13). The high- (from 1936 m to 1500 m), medium- (from 1400 m to 1000 m) and low-elevation (from 900 m to 500 m) groups are respectively highlighted by the green, orange and red ellipses. (c) Scatter plot showing the average Bray-Curtis (dis)similarity comparing sample data separated by different elevational distance. Error bars indicate the standard deviation of the data. The linear trend line for the data is $y = 0.0003x + 0.47$ ($R^2 = 0.92$).

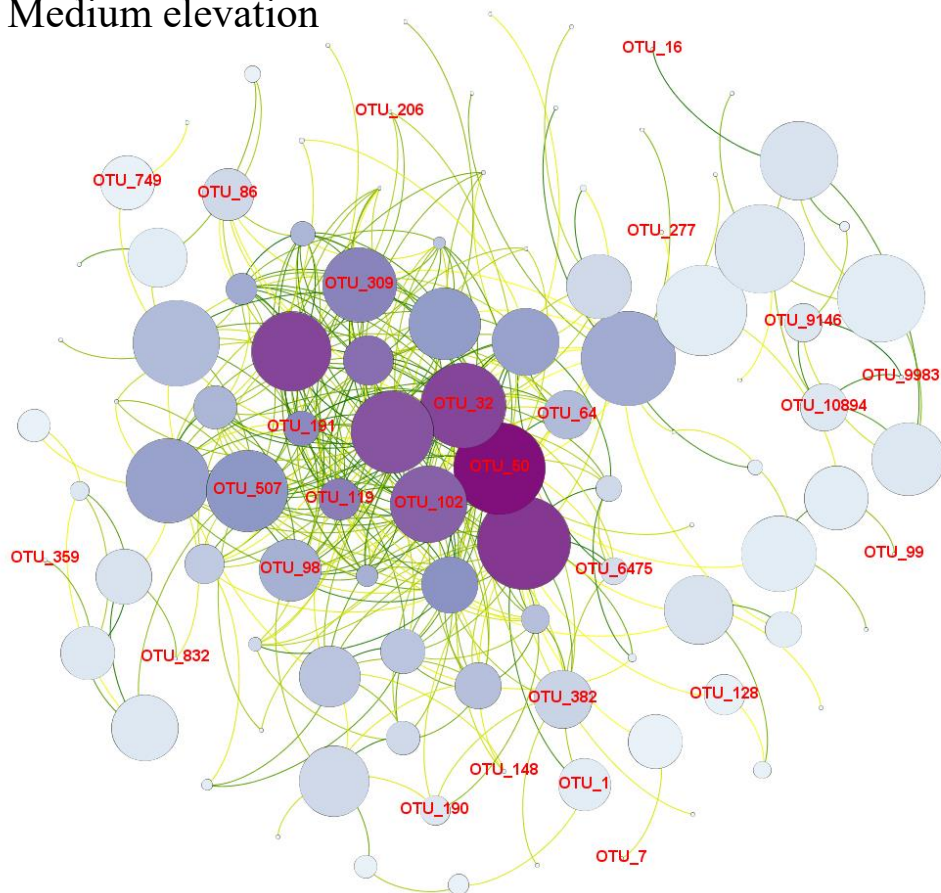
3.3.2 Elevational pattern in fungal co-occurrences

A core community was identified, consisting of 272 OTUs that occurred in more than 50% of the samples obtained from all three elevational groups. Comparison of the high, medium and low elevation divisions showed clear evidence of decreasing numbers of nodes, links and average degree with increasing elevation, which implies network complexity decreased with increasing elevation (Figure 3.2; total nodes: $y = 36.5x + 45$, $R^2 = 0.89$, total links: $y = 395x - 281.33$, $R^2 = 0.94$, average degree: $y = 4.0005x - 0.2177$, $R^2 = 0.99$). Likewise, the average clustering coefficient, showing how well OTUs were connected with their neighbours, was lower for the high and medium groups (average clustering coefficient: high group = 0.48; medium group = 0.49) than the low group (average clustering coefficient = 0.59), while the number of shortest paths increases with decreasing elevation ($y = 10763x - 7811$, $R^2 = 0.94$). Collectively, these patterns indicate that fungal taxa at lower elevation interacted more closely and with more complexity, supporting our hypothesis (co-occurrence H_2). These same broad patterns were observed when we subdivided our sample data into smaller intervals of elevation gradient (Appendix B Figure B2).

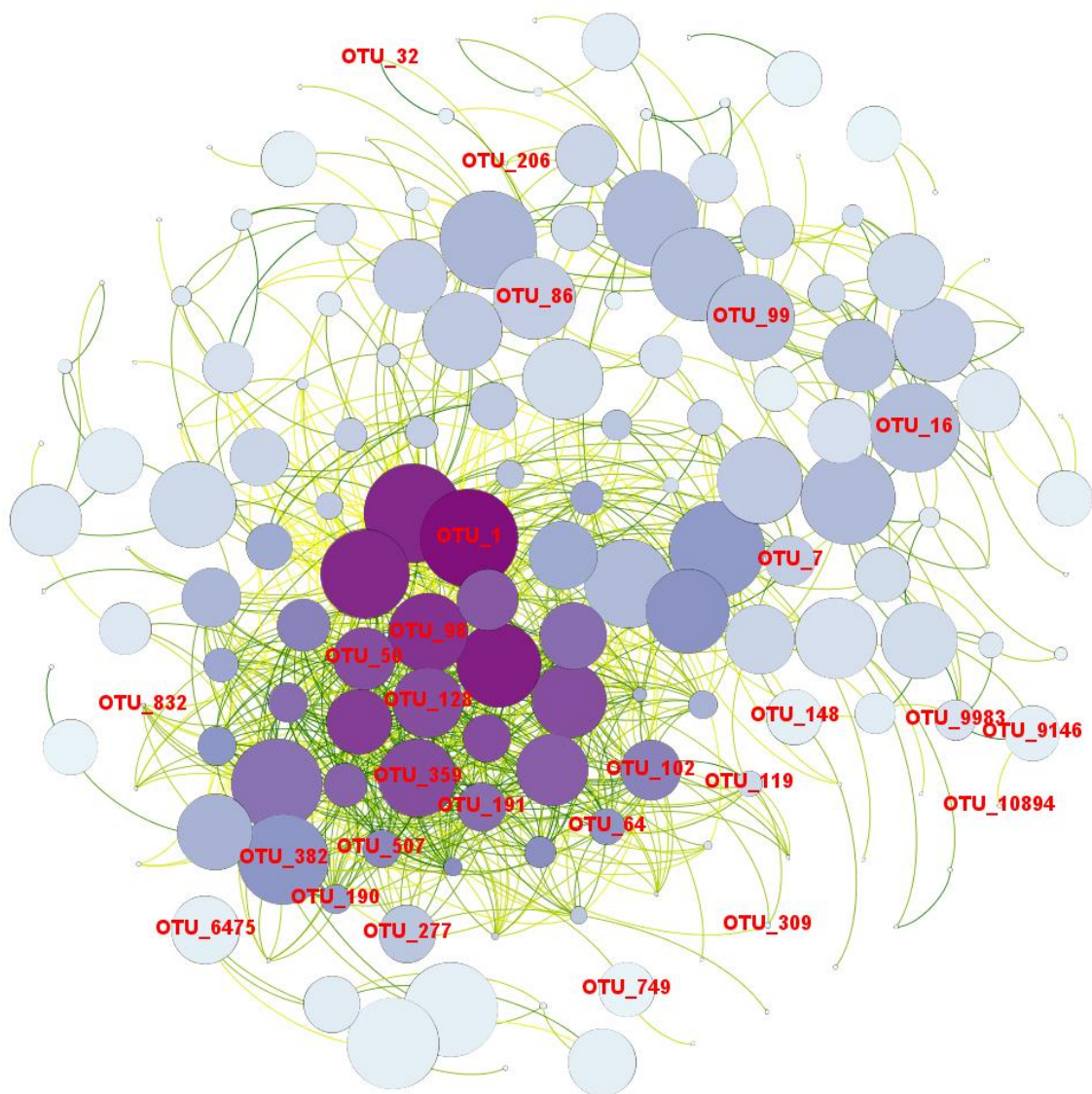
(a) High elevation



(b) Medium elevation



(c) Low elevation



(d) Bar charts comparing network parameters

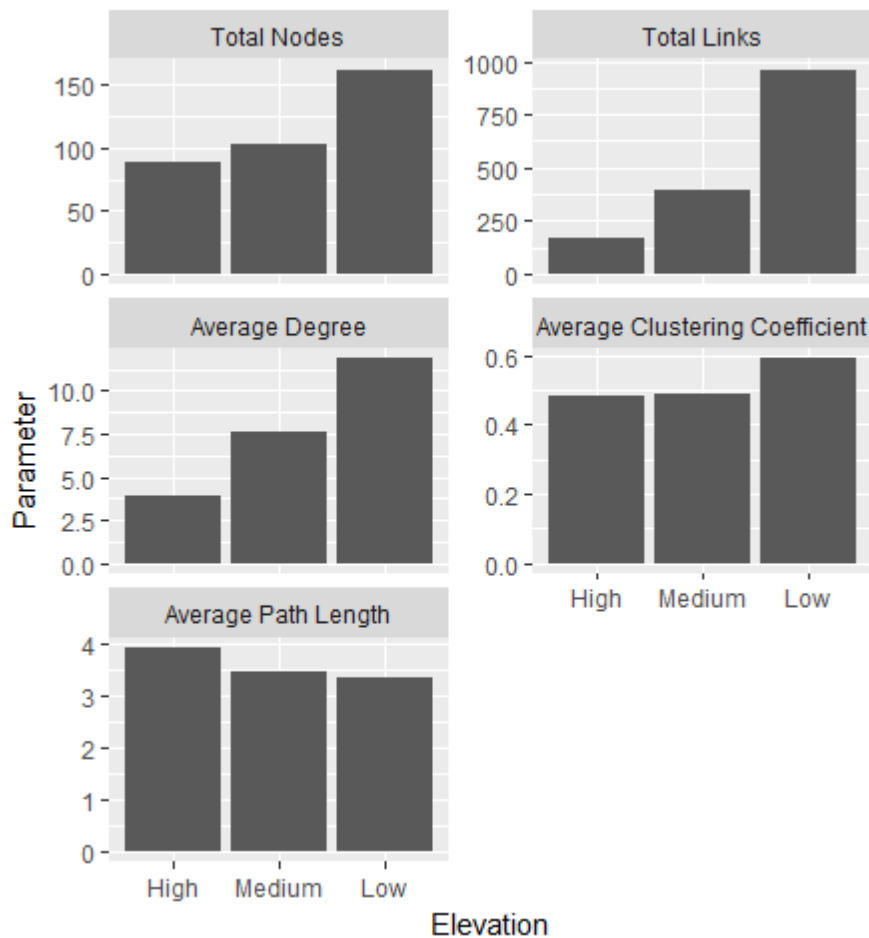


Figure 3.2. Network interactions of core soil fungal OTUs found in (a) high-elevation (from 1936 m to 1500 m), (b) medium-elevation (from 1400 m to 1000 m), and (c) low-elevation (from 900 m to 500 m) sites based on correlation analysis, and (d) bar charts comparing network parameters derived from (a), (b) and (c). A connection indicates a relatively strong ($\rho > 0.5$) and significant ($p\text{-value} \leq 0.05$) Pearson's correlation. Each node (i.e., circle) represents a core fungal OTU. The size of each node is proportional to the value of betweenness centrality. These nodes are assigned colours across a gradient from purple (the largest number of connections, or 'degrees') to light blue (the smallest number of connections). Lines connecting two nodes are coloured proportionally in relation to the Pearson's correlation between OTUs from yellow (weakest) to green (strongest). OTUs identified in red font are present in all three networks. The bar charts in (d) summarise key network parameters (the total number of links and nodes, average degree, average clustering coefficient, and average path length) associated with each of the three networks.

A total of 27 core OTUs occurred in all of three co-occurrence networks derived from the three elevational divisions (that is, high-, medium-, and low-elevation) and in each network were significantly correlated with other OTU nodes. The proportion of these ‘generalist OTUs’ (assigned red identities in Figure 3.2) was 30.3% of the OTUs detected in the co-occurrence pattern at high-elevation (from 1936 m to 1500 m), followed by 26.2% and 16.7% at medium- and low-elevation, respectively. To measure the relative importance of these generalist OTUs within each network, we calculated the ‘degree’ of each OTU, that is, the number of links with other nodes (OTUs) (Gonzalez et al., 2010; Vick-Majors et al., 2014). The average degree of these generalist OTUs was lower at higher elevation (Figure 3.3a). For example, these generalist OTUs at low elevation have more interactions (i.e., higher number of links per OTU) on average (Tukey’s HSD of degree, $p < 0.001$).

We sought to identify the trophic status of fungal generalist OTUs with interspecific co-occurrences in the networks. Almost 70.4% of the generalist taxa were identified as saprotrophs, while only one OTU was identified as a potential plant pathogen, *Neonectria* (Appendix B Table B1). The average degree of saprotroph OTUs (i.e. degree) decreased with increasing elevation, consistent with the pattern for the total community of generalists, as did the range of data variation (Figure 3.3b).

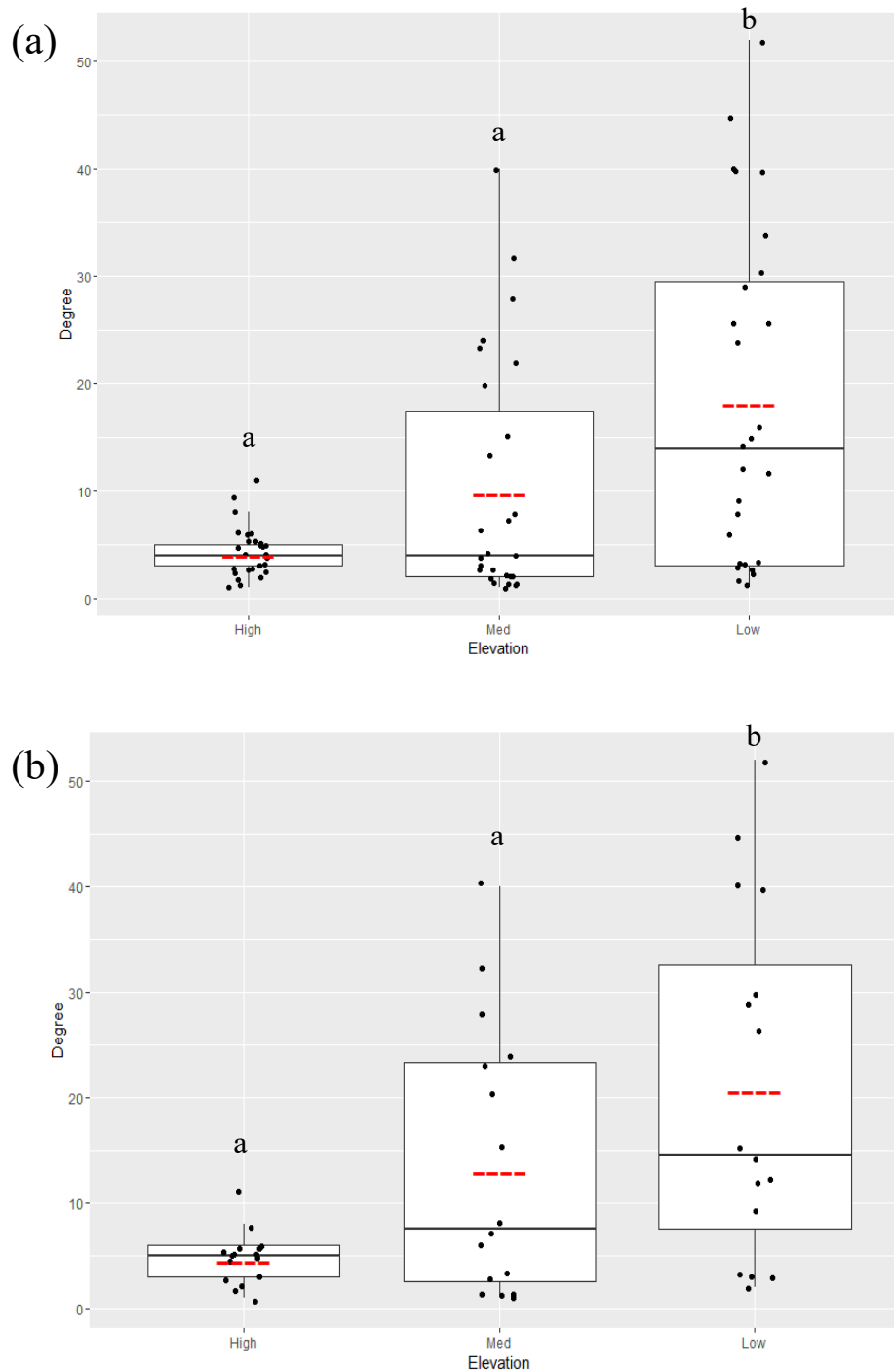


Figure 3.3. Boxplots of degree (that is, the number of links with other OTUs) by (a) all shared generalist OTUs and (b) generalist OTUs of saprotrophs. The horizontal line within the box corresponds to the median value, and the red dashed line indicates the mean value, with 25th – 75th percentiles as the box limits. The whiskers extend to the minimum and maximum data point and no more than 1.5-fold interquartile range. Statistical differences in OTU data between each elevational group were analysed by one-way ANOVA ($P < 0.05$) followed by Tukey's post-hoc tests ($P < 0.05$). Within each graph, clusters that do not share the same letter have significantly different means.

3.3.3 Environmental correlates of fungal community attributes

The results of comparison of AIC values indicated that the best fitting multi linear regression model was the one only composed of soil physicochemical variables, suggesting that these variables had the stronger influence on the fungal community richness, compared with climatic variables and site differences (Appendix B Table B2). This best model was well supported among the overall candidate models ($w_i = 1$), showing a significant relationship between fungal community richness and soil physicochemical variables ($p < 0.001$), with an R^2 of 0.44. The effect of soil pH on fungal richness was significant ($p < 0.001$), as well as the effect of ammonium ($p < 0.001$). Likewise, comparison of AIC model results indicated that variation in fungal community composition was also significantly related with soil variables (Appendix B Table B3; $w_i = 0.99$, $R^2 = 0.30$, $p < 0.001$), specifically, demonstrating a relationship between changes in community composition and ammonium ($p < 0.001$). Neither site differences (i.e., elevation and aspect differences), or climatic variables (i.e., temperature and soil moisture) were significantly related to the observed variation in either fungal community richness or composition. Overall, these results are consistent with our original hypothesis (soil physicochemistry H₃) that soil conditions would contribute most to differences in fungal community attributes.

A correlation heatmap comparing the strength of relationship among soil fungal network parameters and soil physicochemical parameters revealed similar spatial patterns between soil chemistry (i.e., nitrate, ammonium and phosphorus) and network complexity, i.e., the total number of edges/links and nodes/OTUs, average clustering coefficient and the number of shortest paths (Figure 3.4). This correlation heatmap indicates that fungal co-occurrences within communities are correlated more with changes in soil chemistry, rather than climatic conditions, based on their Pearson's correlation values.

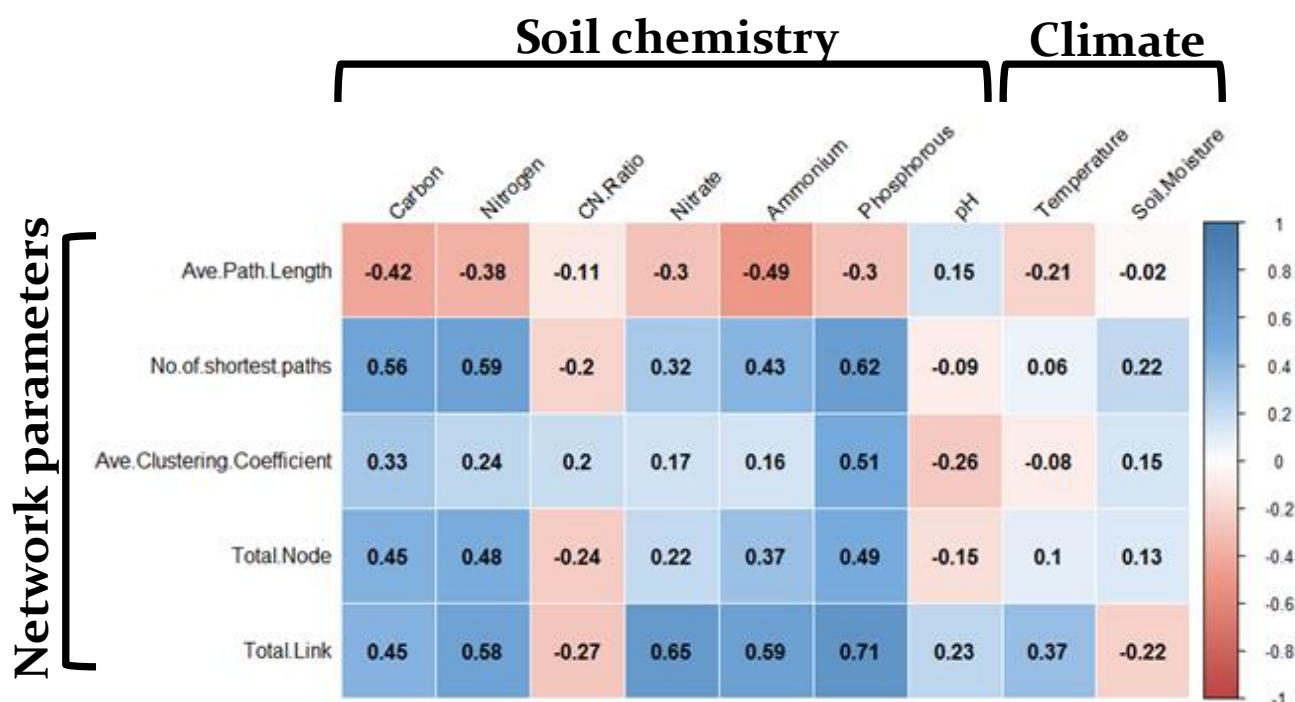


Figure 3.4. Correlation matrix heatmap showing the extent of correlation between network parameters and both climatic and soil physicochemistry data. The colour gradient from red to blue represents a proportional gradient from negative to positive correlation. Stronger colour intensities on the left side of this heat map illustrates that soil chemistry, namely nitrate, ammonium and phosphorous concentrations are more closely related to the network structure than climate factors.

3.4 Discussion

The nature of co-occurrences within soil fungal communities changed along this ~1,500 m alpine mountain elevation gradient, with a concurrent change in community composition, despite a lack of clear elevational pattern in richness. Variations in fungal community attributes and their co-occurrences along the gradient studied here were most closely related to soil physicochemical variables, including ammonium and pH, rather than climatic variables.

3.4.1 No elevational pattern observed in fungal community richness

In contrast to fungal composition and interspecific co-occurrences, we observed no significant change in the richness of soil fungal communities along the elevational

gradient, causing us to reject our first hypothesis (Elevation H₁). This observation is consistent with observations from prior studies (Meier et al., 2010; Meng et al., 2013; Counce et al., 2014) suggesting that fungal community structure may respond differently to macroorganisms across elevation gradients (Fierer and Jackson, 2006). This pattern implies that climatic factors, such as temperature, are more likely to play a secondary role in structuring the richness of soil fungal communities. Our results demonstrated that soil pH and ammonium were in fact, the two most important variables correlating with the observed changes in fungal richness. Other studies of fungal community richness over elevation/latitude gradients have found different results. For example, Tedersoo et al. (2012) indicates temperature and precipitation play substantial roles in shaping fungal richness on a global scale. Likewise, Pellissier et al. (2014) found an increase in fungal community richness related to lower temperature and higher soil moisture across a 2800 m elevation gradient at a regional scale. The inconsistency of our results compared to other studies is likely because we observed relatively little variation in temperature, as compared studies that have sampled more extreme latitude/elevation gradients.

3.4.2 Soil nutrient concentrations impact fungal interaction patterns

Changes in microbial community attributes could be driven by their nutrient-preference and strategies of nutrient-acquisition (Goldfarb et al., 2011), and therefore, some microorganisms are able to gain more advantage from having a higher nutrient acquisition ability as compared to the whole community. Based on nutrient-preference, these microorganisms may arrange in trophic groups and functionally distinct niches (Schimel and Schaeffer, 2012). Thus, investigating the co-associations of community members across environmental gradients can reveal important information on the underlying mechanisms of elevational patterns in microbial community composition (Barberán et al., 2012; Gilbert et al., 2012) and community interactions with environmental variables (Fuhrman, 2009). In the current study, an increase in the complexity and number of fungal co-occurrences with decreasing elevation was observed, supporting our *co-occurrence network hypothesis* (co-occurrence H₂). In addition, the observed fungal co-occurrence patterns were more strongly related to soil physicochemical variables (e.g., ammonium), rather than to climatic variables

(temperature and soil moisture). Considering the significant variability in fungal community composition and soil-nutrient resource (e.g., the concentration of ammonium) across our study site, the aforementioned increase in fungal co-occurrences is likely due to increasing competitive exclusion (Rajaniemi, 2003) or top-down predatory interactions (Worm et al., 2002). A recent study demonstrated that soil microbial community attributes vary in concert with their functioning, being related to, and with consequences for, variability in soil resource availability (Mau et al., 2015). This finding is consistent with our results, suggesting that an increase in the complexity of fungal networks is likely driven by variation in soil physicochemical attributes (e.g., concentrations of nitrate, ammonium and phosphorous), based on species nutrient preferences.

Current research indicates that soil abiotic variables, such as ammonium, impact fungal interactions within communities (Hiscox et al., 2016), as ammonium is utilized by most fungi (Rastin et al., 1990). More importantly, it is the major form of nitrogen for saprotrophs (Liaho, 1970; Lundeberg, 1970; Keller, 1996), which comprise the majority of our generalist taxa. Thus, when the limiting resource, such as the concentration of ammonium, increases, more species meet their minimum resource requirements (Rajaniemi, 2003) and species diversity typically decreases (Bakelaar and Odum, 1978; Clark and Tilman, 2008), as increasingly competitive organisms dominate. This hypothesis provides a mechanistic explanation for how fungal community richness and interactions change across elevation gradients. Our findings correspond to those of Baar and Stanton (2000), determining that variation in ammonium concentration leads to changes in the type and strength of fungal interactions. Interestingly, fungi generally prefer ammonium as an N source, rather than nitrate in pure culture (Rangel-Castro et al., 2002; Guidot et al., 2005), as well as field studies (Grenon et al., 2005; Clemmensen et al., 2008). This is likely because of its lower energy cost for assimilation, compared to the reduction of nitrate (NO_3^-) to ammonium (NH_4^+) (Chalot and Plassard, 2011). This is reflected in our results, observing that ammonium was significantly related to differences in both composition and richness of fungal communities. While little is known about the importance of fungal nutritional groups (Banerjee et al., 2016), our study highlights that non-random associations between fungi are likely mediated by soil resources in microbial ecosystems.

3.4.3 Soil nutrient concentrations impact elevational gradients in fungal community composition

In this study, a clear elevational pattern in fungal community composition was observed across our study site. Similar patterns have been observed in some previous studies, such as in tropical forest soils of the Peruvian Andes (Meier et al., 2010), Mediterranean grassland soil (Maggi et al., 2005), and Scots pine soil, Scotland (Jarvis et al., 2015). Consistent with our *Soil Physicochemistry Hypothesis* (Soil physicochemistry H3), the composition of fungal communities was more closely correlated with variation in soil chemistry (the concentrations of ammonium) than climatic variables (i.e., temperature and soil moisture). Indeed, fungal community structure is typically strongly related to soil chemistry (Bossuyt et al., 2001; Frey et al., 2004; Toljander et al., 2006; Allison et al., 2007), even among samples collected across diverse land uses (Lauber et al., 2008). This is probably because soil chemistry, such as concentrations of ammonium, can affect fungal community composition (Waldrop et al., 2006; Dennis et al., 2012; Kranabetter et al., 2015) and fungal biomass production (Jongbloed and Borstpauwels, 1990; Kranabetter et al., 2007; Rothstein, 2009). An increase in ammonium concentration could cause increasing in fungal growth and activity (Boberg et al., 2008). The change in ammonium concentration might also alter fungal community composition by affecting their trophic groups. For example, scientists found that a high ammonium concentration has negative influences on fungal decomposer communities via decreasing their biomass (Baldy et al., 2007; Duarte et al., 2009). In this case, a significant difference in concentrations of ammonium across the present study site was related to significant variability in fungal community composition, as the overall concentration of ammonium at medium elevation increased by ~40%, compared with concentrations at high elevation (Tukey test, $p = 0.007$; Appendix B Figure B3). Our results provide evidence of the association between soil nutrients and biogeographic patterns in soil fungal community composition, implying that fungal growth and activity are soil-nutrient altered.

3.4.4 Generalist taxa identified across the co-occurrence networks

We determined 27 generalist taxa present in three association networks; most of which were saprotrophs (Appendix B Table B1). Saprotrophic fungi are key determinants of decomposition (Schneider et al., 2012), carbon sequestration and nutrient cycling (Boddy and Watkinson, 1995; Hättenschwiler et al., 2005; Baldrian and Lindahl, 2011; Litchman et al., 2015). They are abundant particularly grassland in soils, in contrast to the abundances of ectomycorrhizal fungi and plant pathogens (Tedersoo et al., 2014). However, it is important to note that the sampling method we used here might detect less of the mycorrhizal species in soil. We only collected the top layer of soil and did not specifically collect soil adhering to the roots. Mycorrhizal species are commonly observed in the infected plant roots (Taylor and Bruns, 1999; Daniell et al., 2001; Vandenkoornhuysen et al., 2002), while saprotrophic species dominate in the litter layer (O'Brien et al., 2005). Together, this may cause the majority of the generalist OTUs in the present study to be deemed as saprotrophs.

3.5 Conclusion

We found significant differences in soil fungal community composition and taxon co-occurrences along an alpine elevation gradient. Change in community composition and an increased taxon co-occurrence at lower elevation were more closely related to soil nutrient conditions i.e., the concentration of ammonium, than to variation in climatic (i.e., temperature and soil moisture) variables. This is despite there being a dramatic decrease (that is, 7 °C) in the average below-ground temperature comparing data from the highest and lowest elevation sites. Thus, our results indicate that even substantial variation in climate may have a smaller impact on vital soil fungal community attributes and their interactions than the changing in soil conditions, be these natural or anthropogenic, for example greater soil fertilisation at lower altitude. Overall, these findings shed light on the contribution of soil physicochemical attributes to variation in fungal community structure and interactions. Exploring nutrient-driven shifts in fungal community diversity and function in soil will further enhance understanding of underground nutrient cycling and the likely impacts of climatic and environmental variables on soil microbial communities.

Chapter 4

**Use of ‘inferred metagenomics’ confirms
soil bacterial communities exhibit
functional biogeography across a mountain
elevation gradient**

4.1 Introduction

Soil microorganisms play critical roles in the functioning of entire ecosystems by regulating the cycling of carbon, nitrogen and the availability of other important soil nutrients and gases (Singh et al., 2010). To unravel the likely response of soil microbial communities to gradients in environmental and particularly climate conditions (Siles and Margesin, 2016; Wu et al., 2017), mountain elevation gradients are frequently used as ‘a natural climate laboratory’, as samples exposed to very different climate conditions can be collected in relatively close proximity. However, in numerous previous studies, inconsistent patterns in bacterial community composition and richness have been observed across elevation gradients (Bryant et al., 2008; Fierer et al., 2010; King et al., 2010; Singh et al., 2012; Shen et al., 2013; Zhang et al., 2013). These findings imply that variation in bacterial community structure might be more complicated than first thought, or perhaps more likely for communities of prokaryotes, that sufficient functional redundancy exists in these communities (Nannipieri et al., 2003; Sunagawa et al., 2015; Yan et al., 2017) that their responses to gradients in environmental condition are not easily explained using only phylogenetic and/or taxonomic information.

The rapid and ongoing development of high-throughput technologies has greatly facilitated bacterial community research (Riesenfeld et al., 2004; Eisen, 2007; Wooley et al., 2010; Bailey et al., 2013; Bergkemper et al., 2016; Yang et al., 2017). Critically, the emergence of metagenomics-based approaches in microbiology have helped overcome historical culture-based biases, which previously allowed the recovery of only a minority of the bacterial species present in soil (Amann et al., 1995; Ferrari et al., 2005). We are now capable of predicting the growth requirements (Pope et al., 2011; Walker et al., 2014) and stress tolerances (Mongodin et al., 2006; Yuan et al., 2012; Stuart et al., 2013; Trivedi et al., 2013) even of microorganisms that have never been grown in a laboratory environment. These advances are deepening our understanding of how soil bacterial diversity varies across natural environmental gradients and in response to treatment applications. For example, soil pH is widely observed to influence bacterial community composition and diversity (Fierer and Jackson, 2006; Bryant et al., 2008; Wang et al., 2012b; Shen et al., 2013; Hermans et al., 2017) even when

comparing communities across very different habitats (Fierer et al., 2012b). However, differences in the presence and abundance of specific functional genes were directly related to a broader variety of environmental variables rather than just pH, suggesting bacterial community functional and taxonomic attributes respond to environmental factors differently. This may occur as distinct taxa can share similar functional attributes, whereas highly related taxa may also have distinct environmental tolerances (Philippot et al., 2010). Nevertheless, functional trait-based approaches, which have been used by ecologists to study communities of macroorganisms for decades (McGill et al., 2006), remain less frequently applied to microbial communities to predict their distribution. The new era of high throughput metagenomics methods now provides microbial researchers the ability to catalogue distributions of multiple genes relevant to metabolic pathways, energetics and regulatory circuits to directly address changing microbial functional potential, across time and space (Yang et al., 2014).

Shotgun metagenomics is increasingly used to investigate the functional potential of complex environmental microbial communities (Tringe et al., 2005; Raes et al., 2011; Delmont et al., 2012; Wilkins et al., 2013; Leff et al., 2015). Still, it remains hard to effectively study the biogeography of microbial functional traits across large-or even local-scales, as cost limitations typically dictate the number of samples analysed (e.g., Fierer et al. (2012b), $n = 16$; Raes et al. (2011), $n = 25$; Leff et al. (2015), $n = 25$). This might lead to an incomplete understanding of microbial functional biogeography and impact predictions of how functional genes shape or respond to changes in community composition across environmental gradients.

Here, we investigate how genomic information, inferred from 16S rRNA gene sequence data, can be used to predict variation in bacterial community functional attributes derived from comparatively large sample numbers ($n = 81$). To achieve this, we chose to explore the genomic attributes of microbial communities across a mountain elevation gradient where significant differences in bacterial community composition were previously reported (Wu et al., 2017). We hypothesised that: (1) bacteria with larger genome size would be prevalent across the elevation gradient, as prior studies found that bacterial communities requiring resilience to more fluctuating environmental

conditions have larger average genome sizes (Matz and Jürgens, 2005; Bentkowski et al., 2015; Cobo-Simon and Tamames, 2017; Lear et al., 2017b); (2) the numbers of genes encoding for ‘cellular responses to stress’ would increase at higher elevation, as decreasing temperatures and increasing soil acidity with elevation across the study site might increase the environmental stress experienced by the bacterial communities.

4.2 Materials and methods

4.2.1 Sample collection and processing for 16S rRNA gene sequence analysis

Details of sample collection and the processing of soil for physicochemical and DNA sequence analysis is provided in Chapter 2. Briefly, we collected 405 soil samples from 81 locations along the north-eastern ridge of Mt. Cardrona, New Zealand (44.85° S, 168.95° E; see Figure 2.1 in Chapter 2). Because the slope of this ridge is very shallow at 1300 m, we chose to separate this elevation into two (one at the upper edge of the ridge hereafter termed 1301 m and another at the lower edge hereafter termed 1300 m). We collected samples from five different locations at each of 16 elevations from 500 m to 1900 m, with 100 m elevation intervals, as well as one single location at the summit of the mountain at 1936 m (16 elevations x 5 locations + one summit location = 81 locations). One of the five locations at each elevation was located on the mountain ridge line (R0), two to the north (SU1, SU2) on the sunnier side of the ridge, and another two to the south (SH1, SH2) on the shadier side of the ridge; samples identified by the number two (e.g., SH2) were those collected furthest from the ridge. The five samples collected at each elevation were separated by 25 m geographic intervals to identify the effect of fine-scale climatic and environmental variables on soil bacterial communities (i.e. related to slope aspect). Real-time temperature data was collected at a depth of 10 cm below ground at each location (i.e., n = 81) during the summer from February to March in 2014. At each of the 81 sampling locations, five soil samples (10 cm depth) were collected as replicates.

Soil physicochemical parameters, specifically concentrations of organic carbon, total nitrogen, NO₃-N, NH₄-N, Olsen phosphorus and soil pH, were analysed by the Landcare Research Environmental Chemistry Laboratory (Palmerston North, New Zealand) using standard procedures (Blakemore, 1987; Lachat Instruments, 1998b; a; Leco, 2003). We measured soil moisture gravimetrically based on the difference in the weight of each soil sample before and after drying in an oven at 105°C for 96 hours (Rayment and Lyons, 2011).

Soil DNA was extracted using PowerSoil-htp 96 well Soil DNA Isolation Kits (MOBIO Laboratories Inc., USA) as recommended in Lear *et al.* (2017a), with the DNA sampled from each site amplified and sequenced on an Illumina MiSeq following the standard protocol (Illumina, 2013). The V3-V4 region of the 16S rRNA gene was amplified using the 341F and 785R primer pair as described in Chapter 2. All of raw sequences were uploaded onto the SRA-NCBI database (BioProject ID: PRJNA338717).

Raw sequence data were processed using the USEARCH pipeline (Edgar, 2013). Briefly, paired-end sequences were merged and quality filtered using default parameters. After the removal of replicate and singleton sequences, non-chimeric sequences were clustered into operational taxonomic units (OTUs) using $\geq 97\%$ 16S rRNA gene sequence identity as a consensus threshold. As raw reads can vary by orders of magnitude even for samples from the same sequencing run, the number of sequences was rarefied to 5,500 per sample and yielded a total of ~17,000 distinct 16S rRNA gene OTUs identified across our study site.

4.2.2 Matching 16S rRNA genes to sequenced genomes

To identify those OTUs for which genomic information is already available to study their genomic traits, we adapted the method of Barberan *et al.* (2014). Namely, we extracted 16S rRNA gene sequences from the complete genomes derived from the June 2015 version of the NCBI genome database (NCBI Resource Coordinators, 2017) obtaining ~5,200 complete 16S rRNA genes as reference sequences. Then, we matched

the 16S rRNA gene representative sequences sampled from Mt. Cardrona against this subset of 16S rRNA gene reference sequences using USEARCH (Edgar, 2013) at $\geq 97\%$ identity to produce a database of ‘inferred genomes’ containing 16S rRNA gene sequences that matched sequences detected across the elevation gradient. The abundance of 16S rRNA gene OTUs related to the same genome was reported to yield the relative abundance of each ‘inferred’ genome in each sample.

Variations in community composition were compared using Bray-Curtis distance matrices using either all the 16S rRNA gene OTU data or the subset of OTUs for which genomic information was available. This was done using the ‘vegdist’ function within the R package ‘vegan’ (Oksanen et al., 2015). To determine whether biases introduced by our approach may influence determinations of bacterial community structure, we compared the similarity between Bray-Curtis matrices derived from these two datasets, using RELATE analysis to perform Spearman’s rank correlations in PRIMER 6 (Plymouth Marine Laboratory, UK). To visualise variation in the composition of bacterial communities derived from these two datasets, we used non-metric multidimensional scaling (nMDS) to plot the data using the R package ‘ggplot2’ (Wickham, 2009). Variation in bacterial community composition at different sample elevations was quantified and visualised using distance decay curves constructed using the ‘vegan’ R package (Oksanen et al., 2015).

Permutational multivariate analysis of variance (PERMANOVA) was conducted in PRIMER 6 software (Plymouth Marine Laboratory, UK) to test the effects of elevation and aspect differences (i.e., sunny versus shady aspect) on the genomic (i.e., genome size) and functional traits of the communities. Significance statistics (p -values) were generated using 9999 permutations of the data, and the proportion of variation explained by each explanatory variable noted as R^2 values.

4.2.3 Genome size

For the genomes extracted from 16S rRNA gene representative sequences, we calculated the sequence length of the whole genome in nucleotide base pairs and used it as the genome size of each taxon. Next, the genome size of the representative taxa identified in each site was weighted by the abundance of each taxon at each site to produce overall community genome size across our study site. To study which environmental variables were most strongly related to variation in overall community genome size, a correlation matrix comparing the strength of relationship between overall genome size and the environmental variables measured was calculated and plotted using the Spearman's correlation heatmap R package 'corrplot'. To explore whether bacteria with larger genome size were present across a wider range of elevations and greater number of different aspects than those with smaller genome sizes, we used Akaike's information criterion corrected for small sample sizes (AICc) to select the model with best-fitting from a set of candidates (Anderson et al., 1998). Three candidate models were conducted based on the variables, including the elevation range where OTU was present, the number of different aspects the OTU could be found, and the presence of OTUs at each combination of elevations and aspects. As OTUs are phylogenetically independent, we considered the genus information of each OTU as a random effect in the mixed effect model. The best-fitting model was selected on the basis of ΔAICc , R^2 , and AICc weight (Anderson et al., 1998), using the 'AICcmodavg' package in R (Mazerolle, 2013). Venn diagrams were further generated to represent differences in the number of unique/shared genomes across the aspect differences using the R package 'venn' (Mamakani et al., 2011).

4.2.4 Functional analyses

To determine the abundance of different functional annotations, we used HMMER v3.0 (Finn et al., 2015) to search the functional gene database FOAM (Functional Ontology Assignments for Metagenomes) for sequence homologs (Prestat et al., 2014). HMMER is a biosequence analysis software tool that uses Hidden Markov Models (HMMs) to detect remote protein homology (Eddy et al., 2015). Scripts and the workflow provided by FOAM were used to identify best hits in the database. For each genome, the best

KO (KEGG Orthology) hits were added to a count matrix of functional annotation counts as a single column. There are 19 major functional categories (e.g., identifying genes as encoding for fermentation, transporters, etc.), the highest level of functional categorization, which is primarily considered, although further hierarchy can be described when relevant. Then, the KO column was aggregated based on those 19 major categories to produce a count matrix with major FOAM categories for each genome. To compare variation in the functional traits of communities across the study site, the relative abundance of major functional categories at each site was used to generate a count matrix, weighted by the relative abundance of each genome.

Patterns in the relative abundance of functional traits at each elevation were plotted using the heatmap function within the R package ‘gplots’. As the samples on the summit were only collected at one location, these samples were removed to avoid the bias caused by the smaller sample size as compared with other elevations. To reduce the impact of extreme values while assigning the range of colours in the heatmap, the data obtained within each functional category was scaled to have mean zero and standard deviation using the ‘scale’ argument within the heatmap function. A dendrogram was also computed and ordered based on the means of taxon relative abundances at each elevation. To visualize the spread of functional trait data across two-dimensional space, a non-metric dimensional scaling (nMDS) plot was generated from a Bray-Curtis distance matrix of the data using ggplot2 and vegan packages in R. To detect whether there are relationships between elevation and the relative abundance of functional genes, linear regression analysis was performed for each of the 19 major functional categories using the ‘lm’ function within the R stats package. Dot plots were then used to represent proportional differences in the relative abundance of specific functional genes sampled at each elevation using the ‘ggplots2’ package in R. To study which environmental variables correlated most strongly with variation in the functional attributes of the soil bacterial communities, we used the ‘envfit’ function from the vegan package in R before fitting these onto an nMDS ordination plot.

4.3 Results

4.3.1 Inferred metagenomics analysis

From our analysis of bacterial 16S rRNA genes identified across the site we identified 400 OTUs for which complete genomic information were available, based on 97% DNA sequence similarity of their 16S rRNA genes to those in the genome database.

To explore whether bacterial community composition obtained from our ‘inferred metagenomics method’ reflects the composition of the broader bacterial 16S rRNA gene data, we used nMDS plots to visualise the community composition of both (Figure 4.1 and Appendix C Figure C1). The composition of the subset of the bacterial community for which genome data were available changed markedly across the elevation gradient (Appendix C Figure C2; $y = 0.0002x + 0.1657$, $R^2 = 0.96$); a similar pattern was evident comparing the composition of the total 16S rRNA gene data across the gradient (see Figure 2.3 in Chapter 2). We confirmed that data obtained from the two methods generated nearly identical patterns of bacterial community composition, comparing the Bray-Curtis similarity matrices for each dataset using a RELATE routine (Spearman’s $\rho = 0.94$, p -value < 0.001).

4.3.2 Variation in genome size across an elevation gradient

To examine if the bacteria with larger genome size would present at a wider elevation ranges and or greater number of different aspects, we used mixed effect model to detect the relationship between genome size and its prevalence across the elevation gradient and different aspects (Appendix C Table C1). The results showed a stronger positive correlation between bacterial genome size and its prevalence across the elevation gradient, suggesting that the bacteria with larger genome size appear to prevail across the elevation gradient. We found evidence of a significant negative relationship between the overall genome size of each bacterial community and elevation, but the model fit was very poor (Figure 4.2, p -value < 0.001 , $R^2 = 0.04$). It is noteworthy that both of two most dominant OTUs, which were most closely related to the genome of

the species *Bradyrhizobium* sp. S23321 (12.9%) and *Candidatus Solibacter usitatus* Ellin6076 (8.5%), have large genome sizes (7.2 Mb and 10.0 Mb, respectively). These two species appeared to be ubiquitous across our study site. Species with small genome sizes were more commonly found at low elevation, such as *Candidatus Tremblaya princeps* (with the smallest genome size, 0.1 Mb) and *Microbacterium testaceum* StLB037 (4.0 Mb) (Appendix C Table C2).

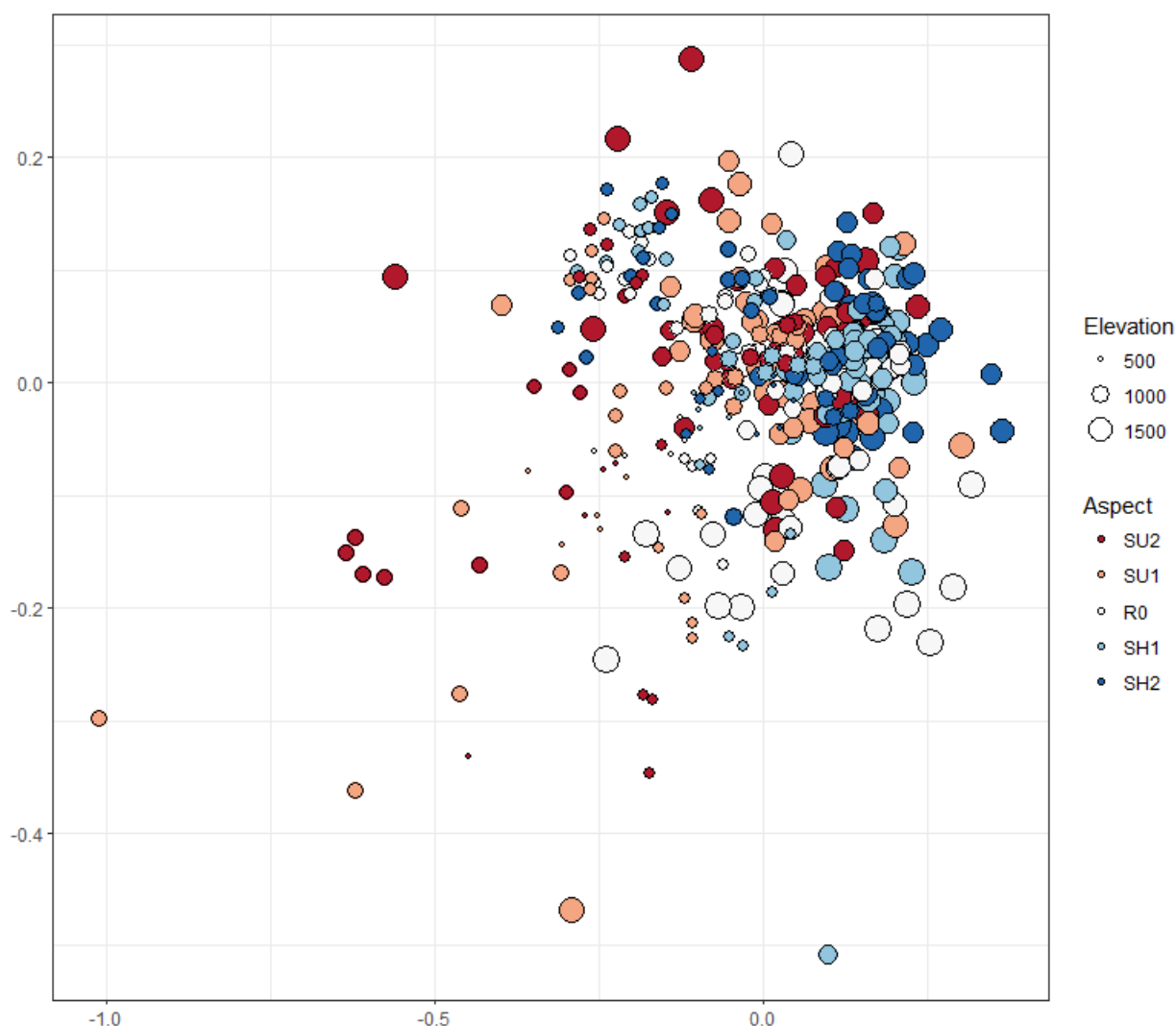


Figure 4.1. Variation in bacterial community composition across a mountain elevation and aspect gradient based on the analysis of the relevant 16S rRNA gene data subset obtained from ‘inferred metagenomic method’ across the study site. The non-metric multidimensional scaling (nMDS) plot was derived from a Bray-Curtis matrix of the data. The size of points increases with elevation. The colour of points are assigned based on the aspect difference at each elevation. The community composition obtained from this 16S rRNA gene data subset is highly similar with the one obtained from original, total bacterial 16S rRNA gene data (Appendix C Figure C1, $Rho=0.94$, p -value < 0.001).

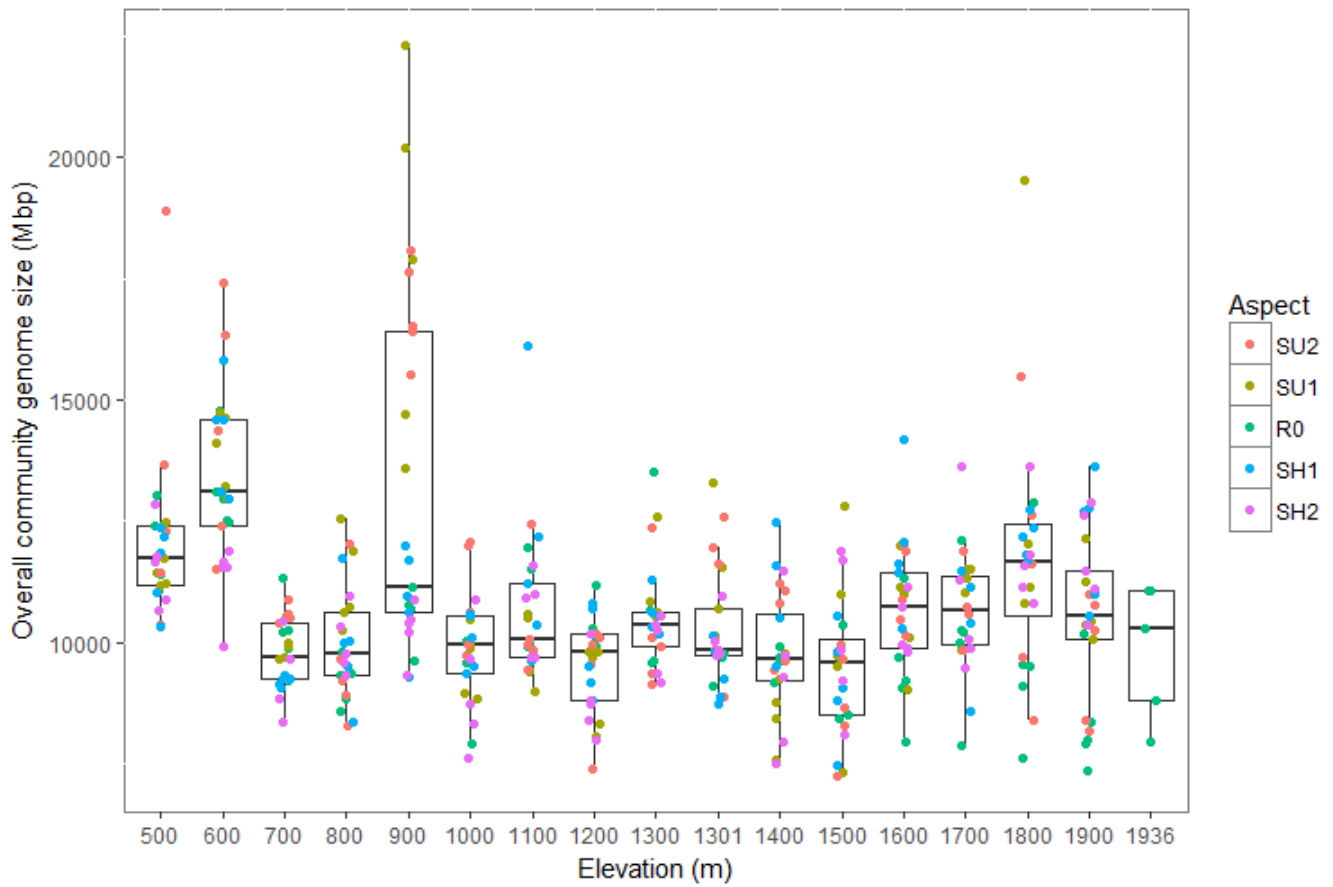


Figure 4.2. Boxplot showing overall community genome sizes obtained across the elevation gradient. Points represent overall community genome sizes collected from each sample site. Sample data are assigned colours based on their aspect at each elevation (R is ridge; SU are samples from the sunny aspect of the slope; SH are samples from the shady aspect of the slope). The horizontal line within the box indicates the median, boundaries of the box indicate the 25th- and 75th -percentile, and the whiskers extends from the hinge to the largest/smallest value no further than $1.5 * \text{IQR}$ (the interquartile range) from the hinge. Linear regression analysis shows a significant relationship between elevation and overall community genome size, but with poor model fit ($p\text{-value} < 0.001$, $R^2 = 0.04$).

The largest proportion of variance in predicted bacterial overall community genome size was attributed to differences in elevation (PERMANOVA $R^2 = 0.39$, p -value < 0.001), followed by differences in the interaction of elevation and aspect (PERMANOVA $R^2 = 0.23$, p -value < 0.001) (Appendix C Table C3). The environmental factors most strongly correlated with the predicted variation in overall genome size were phosphorus (Rho = 0.49, p -value < 0.05) and nitrate (Rho = 0.41, p -value < 0.05), followed by temperature (Rho = 0.31, p -value < 0.05) and soil moisture (Rho = -0.29, p -value < 0.05) (Appendix C Figure C3). Additionally, we studied the extent to which different genomes are unique to elevations and specific aspects or are shared between aspects. Most of the genomes obtained from the study site were identified across different slope aspects (Appendix C Figure C4; 272 shared genomes) and elevations (Appendix C Table C4). Counts of unique genomes derived from different aspects were markedly low (number of unique genomes: SU1 = 11, SU2 = 5, SH1 = 5, SU2 = 7, and R0 = 7). We detected < 7 unique genomes at most elevations (besides 900 m, which harboured 13 unique bacterial genomes).

4.3.3 Variation in functional traits across an elevation gradient

The Bray-Curtis distance matrices calculated from both the original 16S rRNA gene data and the genome representative subset of 16S rRNA gene data were both weakly but significantly correlated with the Bray-Curtis matrices calculated from the relative abundance of 19 major functional gene categories (genomic data: Rho = 0.488, p -value < 0.001 ; 16S rRNA gene data: Rho = 0.435, p -value < 0.001). The relative abundance of different categories of functional gene obtained from low elevation versus high elevation sites (e.g., comparing data from sites above and below 900 m) varies as can be visualised using a heatmap of the data (Figure 4.3). Indeed, for 13 out of 19 major categories of functional genes, the results of linear regression analysis show a significant association between elevation and the relative abundance of each functional category (Appendix C Figure C5; p -value < 0.001 , and $R^2 > 0.2$ in all cases). On average across the study site, the greatest relative abundance of functional genes belonged to the category ‘transporters’ (21%), followed by ‘amino acid utilisation biosynthesis metabolism’ (20%) and ‘cellular response to stress’ (19%). The relative abundance of these three functional traits differentiated data from sites at low elevation to those from

sites at higher elevation (e.g., comparing data collected above and below 900 m, *t*-tests *p*-value < 0.001 in all cases; transporters: average relative abundance \leq 900 m = 20.3%, > 900 m = 20.0%; amino acid utilisation: \leq 900 m = 22.1%, > 900 m = 20.5%; cellular response to stress: \leq 900 m = 17.9%, > 900 m = 18.9%). Meanwhile, the patterns observed from subsets of specific gene categories within these major functional traits were inconsistent (Appendix C Figure C6). For example, the relative abundance of functional genes within the major functional category ‘cellular response to stress’ differed across the elevation gradient. More specifically, genes responsible for ‘trehalose metabolism in response to cold stress’ were present in significantly lower abundance at higher elevation (> 900 m), as opposed to what was observed for the functional genes responsible for ‘response to oxidative stress’ (Appendix C Figure C6).

Spatial variation in bacterial functional traits were most closely related to elevation (PERMANOVA $R^2 = 0.41$, *p*-value = 0.0001) followed by differences in the combination of elevation and aspect (PERMANOVA $R^2 = 0.25$, *p*-value = 0.0001; Appendix C Table C5), although patterns related to the linear elevation gradient were not clearly discernible from an nMDS plot of the data (Figure 4.4). Nevertheless, fitting of environmental parameters to the resulting nMDS indicated that elevation correlated with the bacterial community functional traits. Although a few environmental variables (for example, soil pH, moisture, temperature and nitrate, etc.) showed strong correlation with functional nMDS coordinates as well, vectors related to those environmental variables lay across a similar trajectory with the one related to elevation. This indicates that variation in functional community composition was best explained by a combination of environmental variables along the elevation gradient rather than by any one explanatory variable alone.

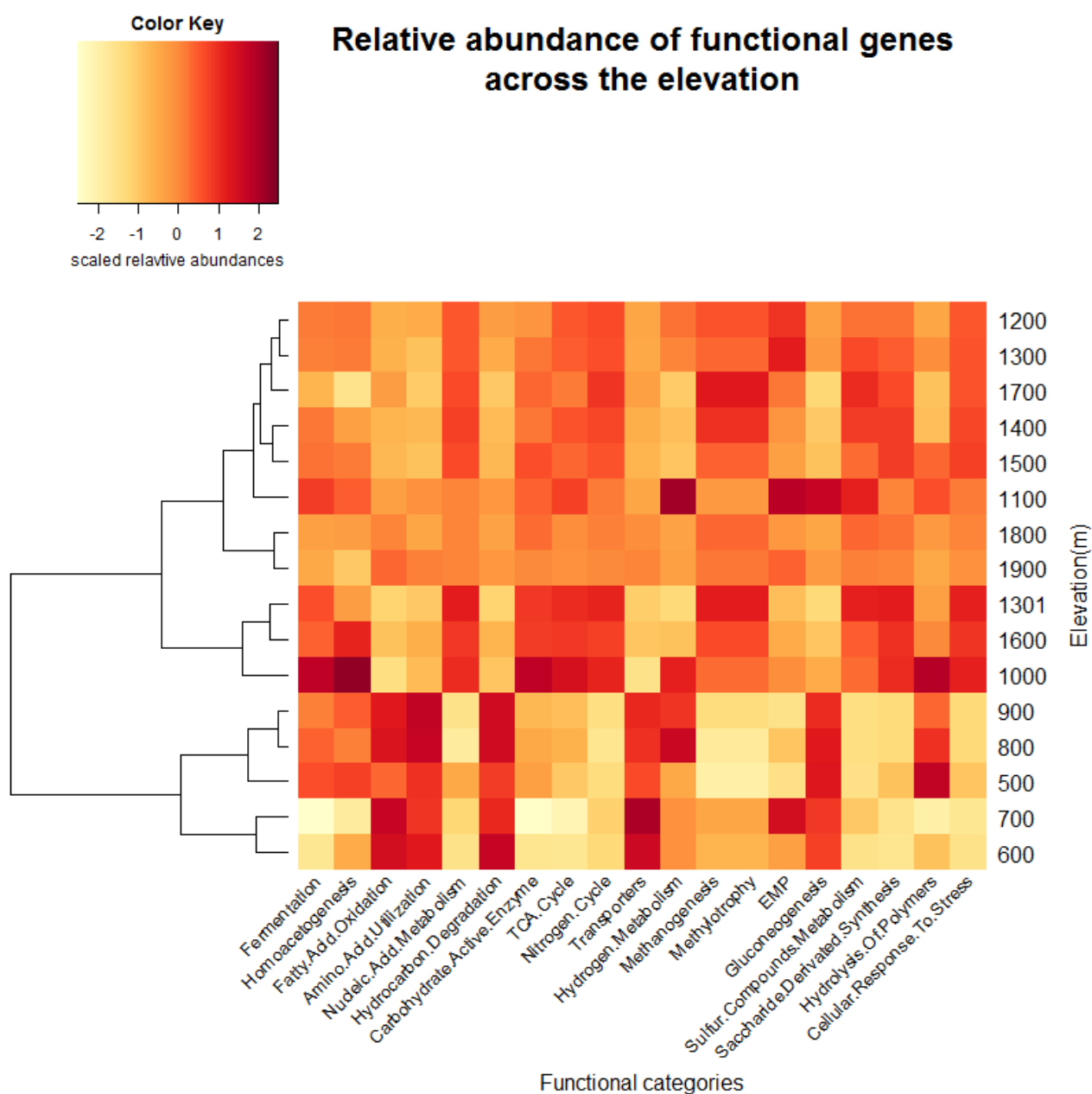


Figure 4.3. Variation in the average weighted relative abundance of 19 main functional categories obtained from genome data across the study site. Different rows represent data collected from different elevations; different columns represent data from different major functional categories. The data in the heatmap were assigned colours across a gradient from red (relative lowest abundance) to yellow red (relative highest abundance) based on their scaled relative abundance. The dendrogram was computed and reordered based on the mean of relative abundance at each elevation.

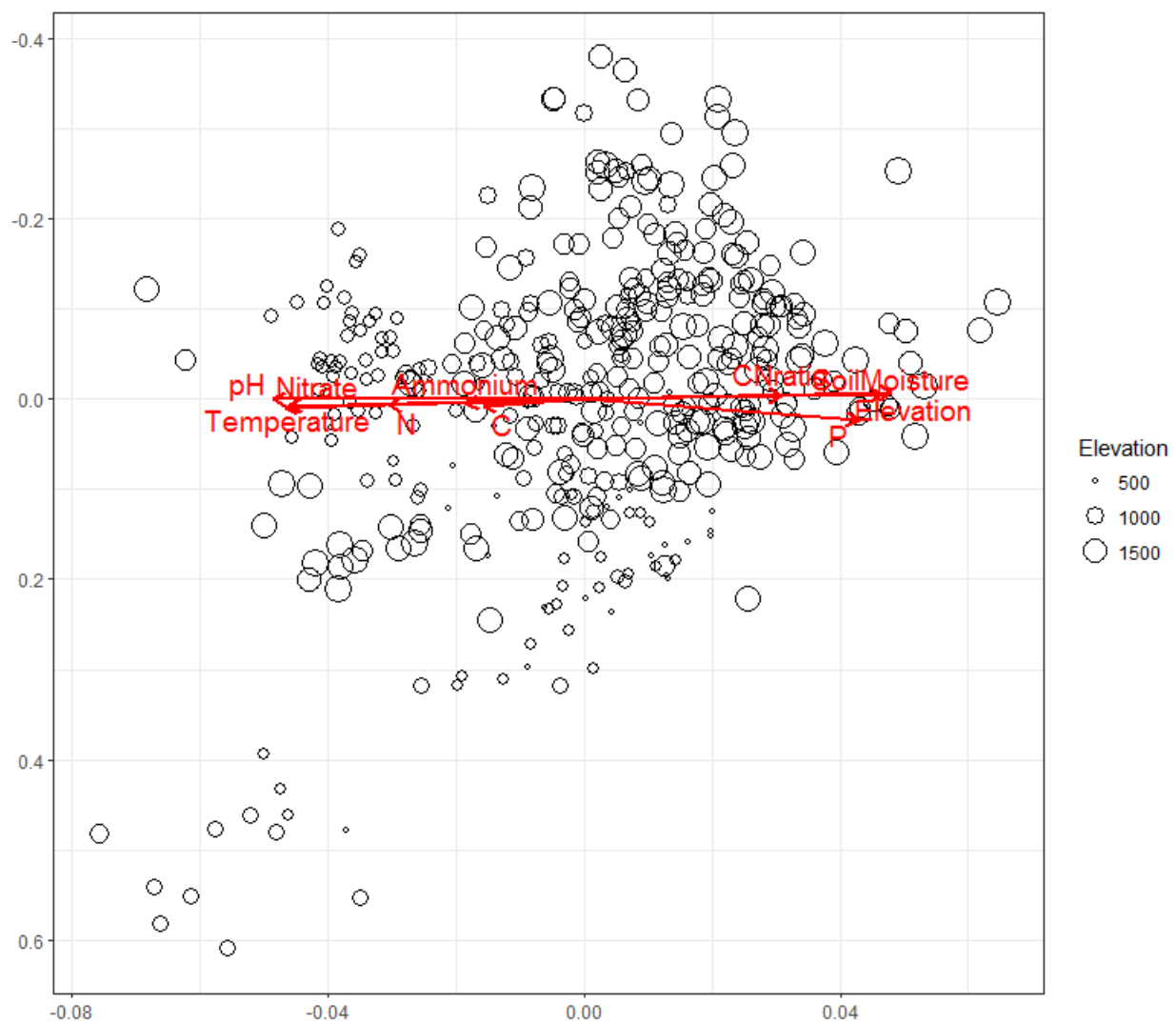


Figure 4.4. Variation in bacterial community functional composition based on Bray-Curtis dissimilarities of weighted relative abundances of major functional gene categories. The size of points increases with an increase in elevation. Vectors in the plot indicate fitted environmental parameters significantly correlated to nMDS coordinates.

4.4 Discussion

Bacterial community composition changed markedly across the elevation gradient. We identified a similar elevational pattern in bacterial community composition from the subset of our community for which genome data were available, as compared to the pattern obtained using the entire 16S rRNA gene dataset, suggesting that the subset of the community associated with our ‘inferred metagenomic method’ was largely reflective of that of the wider community.

Understanding how functional genes shape bacterial community structure across environmental gradients may help clarify their ecosystem contributions. For this reason we chose to examine the genome size and functional traits of soil bacterial communities already observed to exhibit compositional patterns across a ~1,500 m elevation gradient (Wu et al., 2017). Specifically, we expected to witness (1) widely prevalent bacteria with larger genome size across the elevation gradient; (2) increased numbers of genes encoding for cellular responses to stress at higher elevations and increased numbers of genes encoding for processes such as nitrogen fixation, correlated with observed elevational decreases in soil temperature and pH as well as an increase in C/N ratios.

4.4.1 Elevational patterns in genome size

We chose to investigate patterns in genome size across our study site, because previous studies have revealed a relationship between genome size and environmental parameters, including climate attributes (Litchman, 2010; Leff et al., 2015; Cobo-Simon and Tamames, 2017; Lear et al., 2017b). For example, a significant latitudinal gradient in bacterial genome size was observed by Lear et al. (2017b) with average genome size decreasing from samples collected from warmer to cooler latitude sites across New Zealand. These data were used to suggest that bacteria with larger genomes might be more successfully adapted to life in more variable climates as seasonal temperature variation increases towards cooler latitudes. Likewise, it is confirmed by our findings, showing that bacteria with larger genome size appear to prevail at a wider range of elevations. In previous studies, scientists have linked increases in bacterial

genome size to increased bacterial distributions and adaptability to changing environmental conditions (Konstantinidis and Tiedje, 2004; Barberan et al., 2014; Cobo-Simon and Tamames, 2017). However, most observations to date have been based on observations of the genome size of individual microorganisms rather than calculated for entire communities. Cobo-Simon and Tamames (2017) found significantly lower 16S rRNA gene copy numbers for bacteria inhabiting resource-poor environments, and with large genome sizes, indicating that those bacteria might successfully cope with harsh environmental conditions by both adopting strategically low growth rates and retaining a larger number of potential genes and related functions. It is evident that free-living bacteria inhabiting more variable and heterogeneous environments are often slow growing, oligotrophic *α-Proteobacteria* (Mitsui et al., 1997; Saito et al., 1998; Klappenbach et al., 2000). Our survey of soil microbial diversity found *Bradyrhizobium* sp. S23321, with a genome size of 7.2 Mb (Appendix C Table C2), to be both dominant and ubiquitous across our study site. The predominance of such organisms in high altitude soils support a hypothesis described in other studies suggesting that larger genomes are an adaptation to life in more heterogeneous or stressful environments (Konstantinidis and Tiedje, 2004; Guieysse and Wuertz, 2012; Barberan et al., 2014).

The biogeographic pattern in the overall community genome size observed in this study might also be explained by differences in the mean attributes of environmental factors, rather than their variation. Although soil pH and temperature were previously described to be key drivers in structuring bacterial communities along elevation gradients (Fierer and Jackson, 2006; Lauber et al., 2009; Wang et al., 2012b; Shen et al., 2013), it is not the case in regard to the spatial patterns we observed in bacterial community overall genome size. Soil conditions from 900 m to 500 m in the current study were also impacted by gradients in disturbance from livestock grazing (Wu et al., 2017) and it is noteworthy that highly elevated concentrations of total carbon, total nitrogen, phosphorous and nitrate were detected on the northern aspect (SU1 and SU2) of the mountain ridge at 900 m, while the soil pH was observed to be closer to neutral at lower elevation (Appendix A Figure A4 in Chapter 2). We identified a strong positive correlation between overall community genome size and both phosphorus and nitrate concentrations, implying overall genome size may increase in communities within

greater resourced environments. A reduction in the abundances of free-living bacteria with large genome size (*Bradyrhizobium* sp. S23321 and *Candidatus Solibacter usitatus* Ellin6076) at 900 m with elevated nitrogen and phosphorous was observed, in concert with an increase in the abundances of species with small genome size (for example, *Candidatus Tremblaya princeps* and *Microbacterium testaceum* StLB037; Appendix C Table C2). Previous studies indicated that both *Bradyrhizobium* sp. S23321 and *Candidatus Solibacter usitatus* Ellin6076 might be best suited to low-nutrient conditions (Ward et al., 2009; Lopez-Madrigal et al., 2011). Our results imply that free-living bacteria inhabiting more variable and heterogeneous environments might not be competitive in nutrient-rich habitats. As opposed to large genome species, an increase in the abundances of *Candidatus Tremblaya princeps* and *Microbacterium testaceum* StLB037 was obtained at 900 m and below (Appendix C Table C2). Many taxa with small genome size are symbiont bacteria, such as *Candidatus Tremblaya princeps* and *Microbacterium testaceum* StLB037, residing within mealybugs and plant leaves respectively (Lopez-Madrigal et al., 2011; Morohoshi et al., 2011). Nutrient enrichment and warmer environments might contribute to the increasing abundance of these taxa, by influencing their abundance of hosts.

Further, it also needs to be noted that there might be multiple matches between one genome of a particular bacterial specie and different OTUs observed in the study, due to the limited number of known genomes in the database and the length of 16S rRNA gene sequences. Here, we only assign the OTU with the name of the best matched species, but further investigation, such as shotgun metagenomics, needs to be done to further validate their taxonomic identification.

4.4.2 Elevational pattern in the relative abundance of functional traits

The correlation observed between the composition of the entire 16S rRNA gene OTU data, the subset of the 16S rRNA gene data for which whole genome data were available and variation in the composition of bacterial functional gene data provides evidence of a consistent relationship between bacterial taxonomic and functional composition. Similar findings have been reported by others across a variety of habitats such as coast,

ocean, forest, desert and grassland (Gilbert et al., 2010; Raes et al., 2011; Fierer et al., 2012b). Although shifts of individual functional gene structure may not necessarily be associated with variations in bacterial community structure, the overall potential functional attributes of communities appear to be predictable across elevational gradients, as inferred from their community taxonomic or phylogenetic attributes.

For most functional traits, a clear distinction in their relative abundances can be observed comparing sample site data collected above and below an elevation of 900 m, implying that particular bacterial functional traits may be controlled by, or at least related to, the environmental elevation gradient. For example, the relative abundance of functional genes encoding for ‘cellular responses to stress’, such as ‘responses to oxidative stress’, ‘responses to osmotic stress’ and ‘regulation of stress activated protein’, increased significantly with elevation, compared with those sampled at low elevation (Appendix C Figure C5s and Figure C6). This indicates that variations in bacterial composition and function might be impacted by, or at least associated with, environmental factors such as low temperatures, low soil pH, high UV irradiation and the availability of oxygen in soil. These findings are also verified by Yang et al. (2014) who found greater abundances of stress response genes encoding for adaptation to cold conditions at higher elevation. Rousk et al. (2009) revealed a dramatic decrease in bacterial growth with decreasing soil pH. In addition, for many environmental microorganisms, their minimum temperature of growth may be raised, when exposed to pH conditions lower than their optimal pH (Beales, 2004). Together, the combination of low temperature and low pH may combine to contribute to a higher level of stress to which bacterial communities at higher elevation are exposed.

Recent works have demonstrated how changes in functional traits can be used to explain why microbial communities vary across environmental gradients (Zhou et al., 2008; Edwards et al., 2013). Our nMDS plot illustrates that soil pH, moisture, nitrate and temperature were key correlates with the changing functional composition of these microbial communities, while C/N ratio, ammonium and total nitrogen were also influential in shaping community functional structure (Figure 4.4). The relative abundance of specific functional genes varied across the study site. For example, genes

responsible for components of the ‘nitrogen cycle’ were more abundant at higher elevation than those at low elevation (Appendix C Figure C5i). In particular, the abundances of genes encoding for ‘nitrification’ and ‘nitrogen fixation’ increased with elevation (Appendix C Figure C6); also correlated with an increase in soil C/N ratio observed with elevation (data not shown). Moreover, in addition to the importance of soil pH and temperature in structuring microbial community composition, as exhibited in previous studies (Lauber et al., 2009; Shen et al., 2013; Stark et al., 2014), significant shifts in community composition can also be caused by soil nutrient availability (Lozupone and Knight, 2007; Jesus et al., 2009). These previous studies determined that the taxonomic compositions of bacterial communities were significantly impacted by those environmental variables (namely, soil pH, temperature and nutrient inputs), whereas our study provides further details regarding how the composition of bacterial functional genes may similarly be impacted. Interestingly, correlations in the abundances of genes encoding different functional traits were evident in the present study. For example, the abundances of genes responsible for ‘saccharide and derivated synthesis’ had a significant linear association with elevation, corresponding with an increase in abundances of functional genes relevant to ‘carbohydrate active enzyme’ across the gradient (Appendix C Figure C5). A previous study indicates that a variety of carbohydrate active enzymes (CAZy) are widely involved in the building and breakdown of glycoconjugates, oligo- and polysaccharides (Cantarel et al., 2009); our results provide evidence of cooperation, or at least co-occurrence, between genes encoding for ‘carbohydrate active enzyme’ and ‘saccharide and derivated synthesis’.

While not all of functional traits or individual functional genes varied consistently across the environmental gradient (Appendix C Figure C5), the overall functional attributes of soil bacterial communities exhibited biogeographic patterns which could be interpreted across the environmental gradient.

4.4.3 Study limitations and future opportunities

There are a few technical limitations which must be considered carefully in the current study. First, with only 400 genomes relevant to our original 16S rRNA gene sequence

data, we are not able to capture the full extent of the genomic diversity of these microbial communities. However, by comparing the similarity between the Bray-Curtis distances calculated from the data of both all the 16S rRNA gene OTUs and the subset for which genomic information was available, our ‘inferred metagenomic method’ can be considered as a test case to generate a broad picture of the overall functional potential of microbial communities. Only ten years ago just 300 bacterial genomes had been sequenced (Binnewies et al., 2006). The number of bacterial genomes has since increased at least 100-fold (Land et al., 2015) and includes over 17,000 reference genomes now available from the NCBI genome database (<ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/bacteria/>). The increased availability of bacterial genome data will no doubt provide substantial additional opportunities for the exploration of microbial functional biogeography. Of particular interest, additional genome data will inevitably shed more light on the plasticity of bacterial genomes to discover the extent to which functional genetic attributes are correlated to taxonomy. At the present time, further analysis, such as quantitative PCR might be necessary to confirm the extent to which predicted patterns in the abundances of genes of interest reflect the true functional potential of bacterial communities.

4.5 Conclusion

Although investigations of the potential functioning of environmental microbial communities using DNA sequence data is increasingly common (Raes et al., 2011; Delmont et al., 2012; Wilkins et al., 2013; Cobo-Simon and Tamames, 2017), limited sample sizes, due to the high cost of metagenomic sequencing, might cause an incomplete understanding of the functional biogeography of communities across natural or applied environmental gradients. Across a mountain elevational gradient from 1936 m to 500 m, we assessed the reliability of the genomic information obtained using our own inferred metagenomic method. Our results show that free-living bacteria with large genome sizes appear to prevail in across the study site, which may reflect a community adaption to adverse and fluctuating environments. Furthermore, significant relationships between environmental variables and the spatial distribution of dominant functional traits were evident, suggesting environmental gradients in functional potential can be predicted using our methods. Our work highlights the potential for

using inferred genomic information, based on 16S rRNA gene data, to generate a general functional trait-based picture of microbial biogeographical pattern.

Chapter 5

Livestock exclusion reduces the spillover effects of pastoral agriculture on soil microbial communities in adjacent forest fragments

5.1 Introduction

Land-use conversion, particularly from forest to pasture, is a leading cause of plant and animal biodiversity losses globally, with negative consequences for terrestrial ecosystems and their functioning (Doran and Zeiss, 2000; Sala et al., 2000; Navarrete et al., 2010; Rodrigues et al., 2013). A growing body of evidence now documents how changes in land use also alters soil bacterial community attributes and the biogeochemical processes they carry out (Jangid et al., 2008; Wu et al., 2008; Ramirez et al., 2010; Rodrigues et al., 2013; Paula et al., 2014). Despite these knowledge advances, and the critical role of microbial life in regulating ecosystem function and soil biogeochemistry (Madsen, 2011), we have a poor understanding of the spatial impact of land use conversion. Previous studies reveal small organisms, such as insects, can penetrate long distances (> 1 km) into the adjacent habitats from agricultural lands, suggesting that the ecological effect of land use conversion might be more pervasive than has been generally appreciated (Blitzer et al., 2012). Since free-living bacteria are considered capable of widespread dispersal, we chose to explore to extent to which their community structure in natural forest fragments is affected by their proximity to adjacent pastoral land.

The homogenisation of communities and their respective functional diversity (Paula et al., 2014) has been reported following forest to pasture conversion (Rodrigues et al., 2013). This may be caused by the physical redistribution of soil, and associated microbial communities (e.g., via soil tillage (Anderson et al., 2017)), or via changes in above-ground diversity, since plant and soil microbial community members are known to affect one another (Prober et al., 2014). Further, changes in bacterial community composition associated with land-use conversion are frequently related to variation in soil physical and chemical properties, which, in turn have been related to fertilisation effects on soil (Garbeva et al., 2004; Wakelin et al., 2008) including soil pH (Fierer and Jackson, 2006; Griffiths et al., 2011; Wu et al., 2017), nitrogen (Di et al., 2009; Campbell et al., 2010; Fierer et al., 2012a) and phosphorus concentrations (Griffiths et al., 2011; Hermans et al., 2017). While the consequences of nutrients on receiving waterways have been well studied (Edwards et al., 2000), the effects of agriculture on adjacent terrestrial ecosystems remains largely unexplored.

Livestock grazing is a major biotic factor following forest-to-pasture conversion (Bokdam and Gleichman, 2000). Livestock excrete large numbers of enteric microorganisms into the environment, including pathogens (Hutchison et al., 2004), which can spread into adjacent forests either by the movement of livestock or by microbial transport processes including overland flow (Tyrell and Quinton, 2003). Manure and urine deposition provide significant sources of available carbon and nitrogen that may also be transported into adjacent ecosystems (Li et al., 2016). These inputs affect the diversity and functioning of bacterial communities in receiving environments (Cho and Kim, 2000), for example by accelerating nitrogen cycling (Kohler et al., 2005; Ma et al., 2006). Livestock also compact soil by trampling, which decreases air permeability and hydraulic conductivity (Yang et al., 2013). Therefore, it is crucial to evaluate the effect of livestock grazing on soil bacterial community attributes, within both grazed pastures and their adjacent ecosystems, to develop a better understanding of the implications of agriculture at a landscape-scale.

In this study, we investigate how pastoral land-use practices affect the richness and composition of bacterial communities in adjacent forest soils. First, we hypothesised that the richness of bacterial communities would be lower in individual samples collected from within grazed pasture, relative to those in adjacent forest soils (Richness H₁). We predicted this because plant and animal diversity is frequently reported to decline after forest conversion to agricultural land (Bierregaard, 2001; Soares et al., 2006) and the diversity of plants, animals and microbial life are intricately linked (Ter Steege et al., 2003; Zilber-Rosenberg and Rosenberg, 2008). Second, we investigated if the absence of a fence between pasture and forest systems increases the extent of biotic homogenisation between these land uses. This might occur directly because soil and microorganisms are transported across the study sites by stock animals, but also due to the indirect effects of the stock animals on the forest soil (e.g., soil compaction and nutrient additions). We hypothesised that the presence of a fence would increase the proportion of taxa occurring uniquely in the pasture of fenced farms, showing that fencing prevents migration of some pasture-associated taxa from grazed grasslands into adjacent forest soils (Fence H₂). Finally, apart from the effect of land use conversion,

how microbial communities in the adjacent forest soils respond to different pastoral land use intensities remains poorly studied. Hence, we hypothesised that bacterial community similarity would decrease along sampling transects (i.e. comparing communities in the forest versus pasture soil) in response to existing gradients in adjacent agricultural land use intensity (Land-use intensity H₃).

5.2 Materials and methods

5.2.1 Study area, soil collection and biogeochemical analysis

Details of the study area and procedures for sample collection and processing for biogeochemistry analysis are provided in Didham et al. (2015). Briefly, we collected a total of 531 soil samples during the Austral summer from December 2009 to March 2010 in the Waipa Region (37°49'S, 175°36'E) of the North Island, New Zealand (Appendix D Figure D1a). Samples were collected along transect lines from the interior of forest fragments into adjacent pasture for 11 fenced, and 10 unfenced, pastoral farms. Each farm was provided a number ranking corresponding to the intensity of pastoral agricultural practices as detailed by Didham et al. (2015), which we hereafter refer to as the 'land-use index'; the letters 'F' or 'U' preceding these numbers identify transects as bisecting fenced or unfenced forest fragments, respectively. Triplicate straight-line transects were demarcated 10 m apart running perpendicular to the pasture/forest boundary. Across each transect line, 10 cm deep soil samples were collected using a soil corer (2 cm in diameter) at seven distances of 46.5 m, 27 m, 9 m, 3 m, 0 m, -3 m, and -46.5 m (Appendix D Figure D1b; 7 distances × 3 repetitions × 21 farms = 441 samples). Negative values denote distances into the pasture, and positive values denote distance into the forest. The edge (0 m) was defined as the edge of the forest leaf-litter accumulation zone. In addition, three large natural forest reserves in the same region were selected as reference sites. These reference reserves were broadly similar in soil and vegetation type as the study sites but were not considered to be impacted by agricultural disturbances in the forest areas we sampled. Soil was collected in the natural reserves along triplicate straight line transects at distances of -46.5 m, -3 m, 0 m, 3 m, 9 m, 27 m, 46.5 m, 81 m, 243 m, and 420 m. The extension of the sampling lines along these reserve transects was intended to better describe spatial variation in

the composition of soil bacterial communities within the natural forest. A total of 90 soil samples were collected from three natural forest reserves (10 distances \times 3 repetitions \times 3 reserves = 90 samples). In total, there are 531 samples (441 samples collected from the study site + 90 samples collected from the reference site = 531 samples).

Soil biogeochemical attributes, specifically bulk density, soil moisture, pH, and concentrations of total C, total N, delta ^{15}N , total P, Olsen P, total Cd, total U, and C:N ratio were analysed as reported in Didham et al. (2015). Various farmer input measures (i.e., recent N input, recent P input, lime, and stocking rate) were also recorded, to account for the cumulative impacts of land-use practices occurring within circa five years prior to sample collection, by interviewing the landowners.

5.2.2 Bacterial 16S rRNA gene analysis

Soil DNA was extracted from individual samples across the study site using MOBIO PowerSoil®DNA Isolation Kits (MOBIO Laboratories Inc., USA) according to the manufacturer's protocol and as recommended in Lear et al. (2017a). We then characterised the bacterial community composition of each sample by amplifying and sequencing the V3-V4 region of bacterial 16S rRNA genes using the 341F and 785R primer pair as described in Wu et al. (2017). These amplicons were sequenced on an Illumina MiSeq platform following the standard protocol (Illumina, 2013).

All raw sequence processing was done using the USEARCH analysis tool (Edgar, 2013) and bioinformatics platform QIIME (Caporaso et al., 2010). Briefly, after merging the paired-end sequences and quality filtering by the default parameters, replicate and singleton sequences were removed. Non-chimeric sequences were clustered into groups of operational taxonomic units (OTUs) at 97% 16S rRNA gene sequence similarity. Then, OTUs were assigned to taxonomic groups by implementing the RDP classifier routine (Wang et al., 2007) to interrogate the Greengenes 13-8 database (McDonald et

al., 2012). Finally, each sample was rarefied to 5100 sequences, as unequal numbers of DNA sequences were returned among soil samples.

5.2.3 Bacterial community richness and composition

We counted the number of different 97% bacterial OTUs at each site as relative bacterial taxon richness. As species number (i.e., taxon richness) is scale-dependent (Harrison and Cornell, 2008; Giladi et al., 2011), we used three different scales to explore spatial patterns in relative taxon richness across the study site: (1) average sample-level richness, which is the OTU richness, on average, obtained from the individual soil cores; (2) overall site-level richness, which is the overall richness obtained from each habitat (i.e., pasture or forest) along transects passing through different farms or natural reserve sites; and (3) overall richness at the land-use level, which was calculated from the subset of data collected from different land use types. To visualise variation in bacterial richness, we plotted boxplots or bar charts of the data using the ‘ggplot2’ package in R (Wickham, 2009). Spatial patterns in the relative taxon richness of bacterial communities along sampling transects were plotted using the heatmap function in the ‘gplots’ package (Warnes et al., 2009). Changes in bacterial community richness among different land use types were examined using one-way analyses of variance (ANOVA) performed with the ‘aov’ function in R. T-tests were performed using the function ‘stat_compare_means’ within the R package ‘ggpubr’ (Kassambara, 2017).

Variation in bacterial community composition among samples was calculated by computing Bray-Curtis distance matrices from the OTU tables. Non-metric multidimensional scaling was then used to visualise variation in the composition of bacterial communities using the R package ‘ggplot2’. Stepwise regression analyses were used to identify environmental factors that could effectively explain changes in the composition of bacterial communities related to both the soil biogeochemical variables and the farmer input measures before the ‘envfit’ function in R was used to overlay significant environmental vectors on nMDS ordinations (Dixon, 2003). Variation in bacterial community composition along individual sampling lines was

quantified and visualised using distance decay curves constructed using the ‘vegan’ package in R (Oksanen et al., 2015).

To explore how environmental factors affect bacterial community richness and composition in the pasture and adjacent forest soils, Akaike’s information criterion corrected for small sample sizes (AICc) was used to select the best-fitting model from a set of candidates (Anderson et al., 1998). Candidate models were based on four explanatory variables: soil physicochemical variables (i.e., bulk density, soil moisture, pH, total C, total N, C:N ratio, C:P ratio, N:P ratio, delta ^{15}N , total P, Olsen P, total Cd, and total U), the presence of a boundary fence (i.e., fenced vs unfenced), the farming input (i.e., recent N input, recent P input, lime, and stocking rate), and land-use intensity (i.e., ‘land-use index’). The best-fitting model was selected on the basis of ΔAICc , R^2 and AICc weight (Anderson et al., 1998), using the ‘AICcmodavg’ package (Mazerolle, 2013).

5.2.4 Quantifying the impact of a boundary fence on bacterial communities in forest soils adjoining pastoral land

To visualise and assess variation in the composition of bacterial communities derived from the interior of grazed pastures (-46.5 m) and the interior of adjacent forest (46.5 m), these data were first plotted using an nMDS plot constructed using the R package ‘ggplot2’ and significant differences among data groups quantified using permutational analyses of variance (PERMANOVA). Permutational analysis of multivariate data dispersion (PERMDISP) routines were then used to confirm if bacterial community composition varied significantly among samples collected across different farms, with or without the presence of a boundary fence.

We used a regression modelling approach to assess the accuracy with which we can predict if a forest fragment is fenced, or not, based solely on analysis of the soil bacterial community composition. We employed a partial least squared regression (PLSR) algorithm (Wehrens and Mevik, 2007) to associate bacterial community profiles to the fence categories (i.e., fenced or unfenced). PLSR is a multiple linear regression method to summarise datasets with multiple, possibly correlated, variables into a set of linearly

uncorrelated principal components that describe the main variation observed in the dataset (Lau et al., 2015). The optimal number of principal components for the model was determined using bacterial community profiles from 203 randomly selected forest sites (the training dataset) across both 10 unfenced farms and 11 fenced farms. The bacterial taxonomic data, comprised of ~13,600 OTUs, was used to derive a regression model with ten predictor variables. To assess how well the model predicted the presence of a fence from the bacterial community data, an error matrix was constructed for the remaining 100 forest sites (the test dataset).

Apart from studying the effect of a boundary fence on overall bacterial community composition in the forest soils, we explored how the relative abundance of the top ten most abundant phyla varied across transects by plotting stacked bar charts using the ‘ggplot2’ package in R. We used distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999) to visualise the extent to which variation in the abundance of dominant phyla explained variation in total bacterial community composition across the sample transects. Multivariate multiple regression, using a distance-based linear model of 9999 permutations of the data (Anderson, 2004), examined the significance of relationship between changes in bacterial community composition and the abundance of the dominant phyla, based on Bray-Curtis dissimilarities. All multivariate procedures were carried out using the PRIMER v6 (Clarke and Gorley, 2005) and PERMANOVA + statistical packages (Anderson et al., 2008). To investigate the extent to which taxa normally restricted to the grazed pasture were present in the adjacent forest soils, and how this was impacted by the presence of a boundary fence, we developed a linear regression model to predict how far into the forest we could observe the expected abundance of specific taxa (typically detected in natural forest reserve without agricultural disturbance) using the function ‘lm’ within the R stats package.

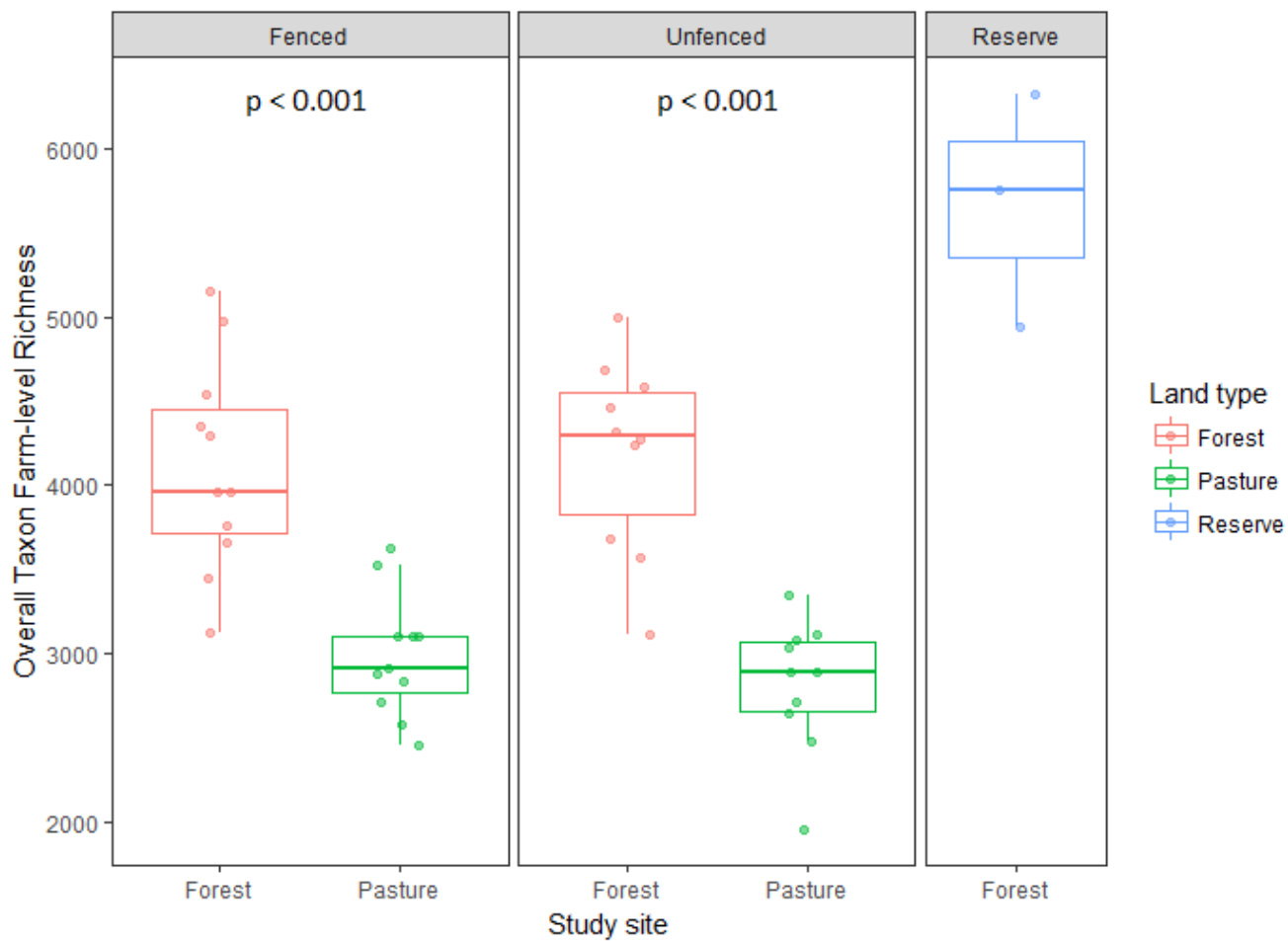
5.3 Results

We identified ~ 13,600 distinct bacterial OTUs of 97% DNA sequence similarity from 2.6 million rarefied 16S rRNA gene sequences across our study sites.

5.3.1 Changes in bacterial community richness and composition derived from soil under grazed pasture and adjacent forest

Contradicting our first hypothesis (*Richness H₁*), we found bacterial richness was higher, on average, in individual soil samples collected from the pasture than those from adjacent forest, but the difference was only significant for the fenced farms (Appendix D Figure D2; *t*-test, *p*-value = 0.008 in fenced farms, *p*-value = 0.49 in unfenced farms). There was no significant difference in the richness of bacterial communities from natural forest reserves, compared to communities in forests adjacent to either the fenced farms or the unfenced farms (Tukey's test, *p*-values > 0.05). In contrast, and consistent with the hypothesis (*Richness H₁*), when bacterial taxon richness was assessed at a larger spatial scale (i.e., the bacterial richness of individual sites, or land use type, Figure 5.1), significantly higher richness was obtained for the forest associated communities compared with the ones obtained from beneath pasture (*t*-tests, *p*-values < 0.001 for both the fenced and unfenced farms). Overall site-level richness was highest in natural forest reserves, while the overall richness of bacterial communities at the land use level was highest in forests surrounded by agricultural land. The results of linear regression show that bacterial richness per sample declined with distance along the fenced farm transects, by approximately 2 units \pm 0.4 (standard error) per meter into the forest (Appendix D Table D1, *p*-value < 0.001), although significant differences in bacterial richness were detected among different farms, regardless of the presence or absence of a fence separating land uses (all of ANOVA, *p*-value < 0.001). No significant distance gradient in per sample richness was observed for the unfenced farms (Appendix D Table D1, ANOVA, *p*-value = 0.232).

(a)



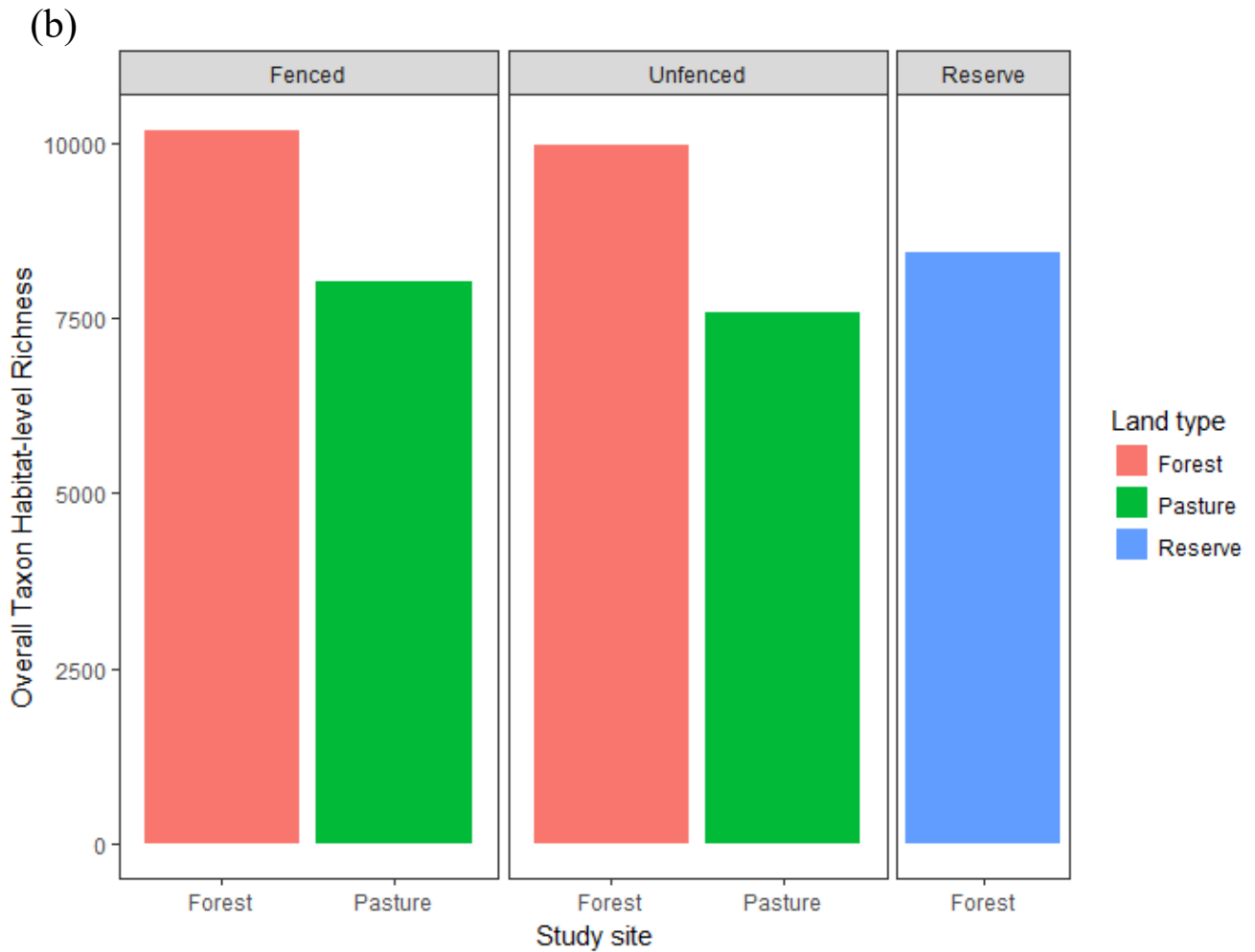


Figure 5.1. (a) Boxplot showing variation in bacterial overall site-level richness derived from different land types (i.e., pasture, forest and native forest reserve) and different fence categories (i.e., fenced versus unfenced) based on the richness of OTUs, rarefied to 5100 sequences per sample. The horizontal line within the box corresponds to the median value with 25th – 75th percentiles as the box limits. The whiskers extend to the minimum and maximum data point, but to no more than 1.5-fold interquartile range. Statistical differences in the data between pasture and forest were analysed by Student's *t*-test (in both case, $p < 0.001$; in fenced farms, average richness in forest = 4111.8 ± 188.3 (mean \pm standard error), in pasture = 2983.5 ± 108.8 ; in unfenced farms, average richness in forest = 4191.5 ± 180.6 , in pasture = 2812.2 ± 124.1 ; and average richness in reserve = 5674.7 ± 400.9). (b) Bar chart showing variation in taxon richness at the land-use level, derived from different land types (i.e., pasture, forest and native forest reserve) and different fence categories (i.e., fenced versus unfenced). We also mapped richness data along each transect line to visualise changes in the richness of bacterial communities transecting across pasture and forest as shown in Appendix D Figure D3.

Soil bacterial community composition among samples was affected by land use (i.e., pasture, forest and natural reserve, Appendix D Figure D4), as confirmed by the results of a PERMANOVA ($R^2 = 0.05$, $p < 0.001$). In addition, significant differences in bacterial community dispersion (i.e., variation in community composition comparing samples within the same site) were detected among these land uses (Appendix D Table D3, PERMDISP, $p < 0.001$). Variability in bacterial community composition among samples was lowest for samples collected from natural reserve. The highest variability in community composition was observed among samples derived from the fenced forest adjacent to grazed land, followed by those samples derived from the unfenced forest. Distance decay rates in bacterial community similarity (also referred to as turnover rates, which were measured by the slope of the linear regression) obtained from fenced sites were higher than those obtained from unfenced farms indicating a greater difference in community composition along transects passing through the fenced sites (Appendix D Figure D5). These results suggest that the bacterial population structure became more distinct with increased distance into the forest, when the forest was fenced off to keep livestock out. The environmental attributes that best explained the observed variation in bacterial community composition across the study site included multiple attributes that may be related to fertiliser inputs, such as concentrations of Olsen P, total P, delta ^{15}N and the ratio of C:P (Appendix D Figure D4).

5.3.2 The presence of a fence on the land use boundary affected the composition of bacterial communities in forest soils adjacent to pasture

Bacterial communities in different sites derived from the interior of forest soils (46.5 m) among all of 21 transects were more variable in their composition, compared with those sampled from the interior of the adjacent grazed pastures (-46.5 m; Figure 5.2). In particular, we observed greater differences among bacterial communities comparing data from the interior of grazed pastures (-46.5 m) and the interior of adjacent forest (46.5 m) if the farm was fenced compared to when the farm was unfenced (PERMDISP, p -value < 0.001); multivariate dispersion index values were 43.6 ± 1 [mean \pm SE] for the fenced farms and 39.9 ± 0.4 for the unfenced farms.

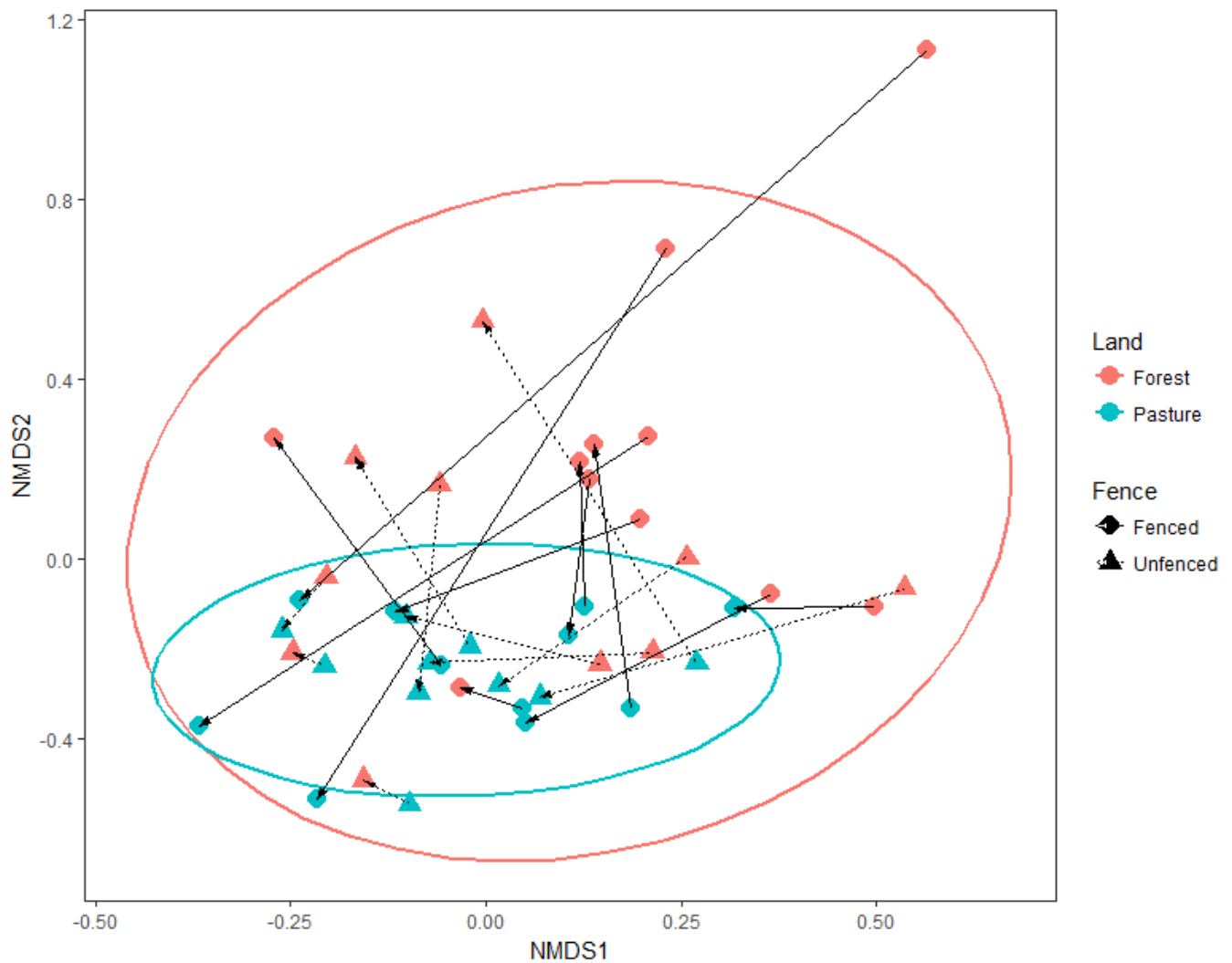


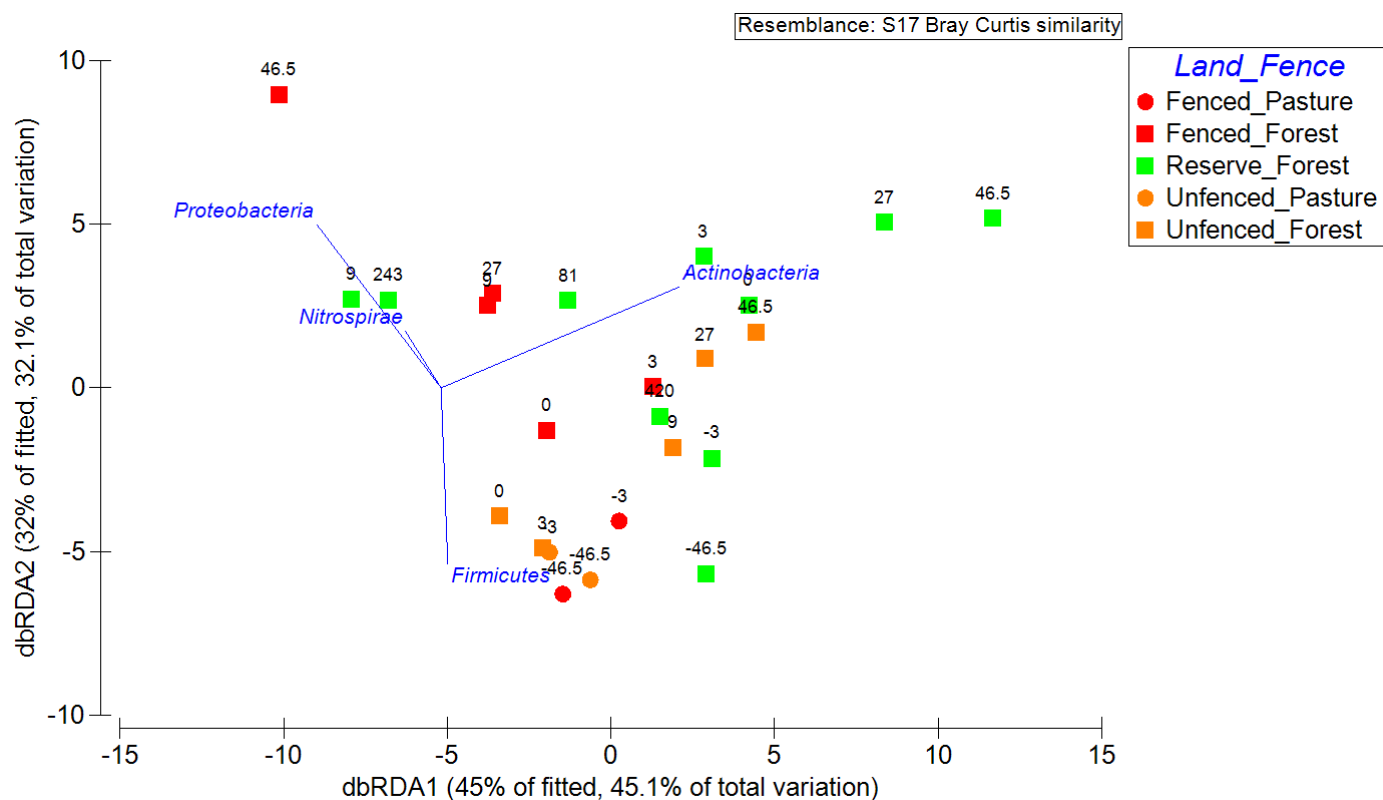
Figure 5.2. Non-metric multidimensional scaling (nMDS) plot representing variation in the composition of bacterial data comparing samples collected from the extreme ends of transects (-46.5 m and 46.5 m). The nMDS plot was derived from a Bray-Curtis similarity matrix of comparisons of 97% 16S rRNA gene data among sites. The colours of points are assigned based on the land use. The shapes of points indicate the fencing category (i.e., fenced versus unfenced) between the farm and the forest. The ellipses show the assumed multivariate t-distribution at the centre of each group of each land type at a 95% confidence level. The samples obtained from the same farm are linked by lines; the line types show the presence of a fence (i.e., solid line: fenced farms; dotted line: unfenced farms).

Since significant variation was detected between communities in the soil of fenced versus unfenced forest fragments. We used a predictive regression modelling approach (partial least squared regression) to assess the accuracy with which we are able to predict if a forest fragment is fenced, or not, based solely on analysis of the soil bacterial community composition. On average, the ten-component model explained ~93% of the variance in the bacterial community distribution and correctly predicted the presence or absence of a boundary fence with 88% accuracy (Appendix D Table D4). Furthermore, the sensitivity (true positive rate) of the model was ~89% ($n = 49$), while the specificity (true negative rate) of the model was ~87% ($n = 39$). The results show that the variance in soil bacterial community structure in the forest could be used to predict the presence of a fence on the land use boundary in a majority of cases, indicating that the presence of a fence on the boundaries to reduce the invasion of livestock does indeed have an important impact on the overall community structure of bacteria in the forest soil.

To further examine the influence of livestock movements on the occurrence of taxa normally restricted to the grazed pasture into adjacent forest, we first compared the number of the unique and shared OTUs between the land uses. A significant increase in the number of unique OTUs in the pasture emerged comparing samples collected from fenced farms compared to those collected from unfenced farms (Appendix D Figure D6, t -test, p -value = 0.04). To study how the composition of bacterial taxa change from grazed pasture into the interior of the forest, we also mapped the relative abundance of the ten most abundant phyla along transects passing through fenced, unfenced or natural forest reserve sites (Appendix D Figure D7). These results show a decline in the relative abundance of *Firmicutes* and *Nitrospirae* from the pasture (-46.5 m) into the interior of the forest (46.5 m) (t -test, *Firmicutes*, p -value < 0.01; *Nitrospirae*, p -value < 0.01), while an increase in the abundance of *Proteobacteria* and *Actinobacteria* (only in the unfenced farms) was also observed (t -test, *Proteobacteria*, p -value = 0.03; *Actinobacteria*, p -value \leq 0.05). Upon further investigation, we found that the relative abundance of the 20 most dominant phyla differed between fenced and unfenced farms (Appendix D Figure D8). The results show that samples collected from fenced farms were clustered based on their land use type (i.e., pasture or forest). Additionally, samples collected close to the boundary in unfenced farms clustered

together and were more similar to those collected in the interior of the grazed pasture (Appendix D Figure D8). The results of a distance-based linear model (DistLM) show that the phyla *Firmicutes*, *Nitrospirae*, *Proteobacteria* and *Actinobacteria* contributed most to the variation in the bacterial community attributes along the sampling line from the pasture into the forest (Figure 5.3).

To quantify the extent of the fence effect on the soil bacterial communities in the adjacent forest, we developed a linear regression model to predict how far into the forest we could observe the expected abundance of taxa strongly associated with land uses (that is, *Firmicutes*, *Nitrospirae*, *Proteobacteria* and *Alphaproteobacteria* (Class), as identified from previous analyses) (Table 5.1). Overall, based on the average taxon abundance obtained from natural forest as a proxy for its expected abundance, the results of the model show that without a boundary fence between different land uses, samples must be collected further into the forest to observe the expected abundance of pasture-associated taxa (*Firmicutes*, *Nitrospirae*, *Proteobacteria* and *Alphaproteobacteria* (Class)). This distance differed when the same approach was used for the fenced farms. For example, the expected abundance of the phylum *Firmicutes* in the fenced farms is expected to be observed at 27.5 m into the forest from the land-used boundary, while the distance in the unfenced farms is ~49 m into the forest. Likewise, the predicted distance into the fenced forest associated with *Nitrospirae* is 13 m, while it is about 39 m in the unfenced forest.



Results of Distance-based linear model (DistLM)

Phylum	<i>P</i>	Proportion of explained variation
Actinobacteria	< 0.001	0.44
Proteobacteria	< 0.001	0.36
Nitrospirae	< 0.001	0.28
Firmicutes	< 0.001	0.26

Figure 5.3. Distance-based redundancy analysis (dbRDA) ordination of the composition of soil bacterial communities along the transect line. Relative position of soil samples in the biplot is based on Bray Curtis similarities of relative abundances at the phylum level. The dbRDA axes describe the percentage of the fitted or total variation explained by each axis while being constrained to account for the differences in land use types and fence categories. Sample colours were assigned by their land type and fence category. Sample labels indicate the distance along the sampling line. Vectors indicate the weight and direction of those phyla that were best predictors of community composition based on the results of the distance-based linear model (distLM), shown in the table along with the plot.

Table 5.1. Results of a linear regression model and distance prediction for the expected abundance of dominant bacterial phyla (i.e., *Proteobacteria*, *Firmicutes*, and *Nitrospirae*) and the class *Alphaproteobacteria*. We chose to study these three phyla in particular† according to the results of the distance-based linear model (distLM), shown in Figure 5.3. Using the linear model, we predicted the distance needed to be travelled into the forest adjacent to fenced and unfenced farms for the abundance of these taxa to be similar as that observed in the soils of natural forest reserves. We used the average relative abundance of special taxa among the reserve sites as the expected abundance of these special taxa in the natural environment without human disturbance in the prediction model. To validate the prediction, we compared the predicted value in the table to the observed relative abundance of these taxa at the nearest site derived from the data.

Phylum/Class	Model	Linear regression model				Distance prediction		
		R ²	P	Coefficient (± Standard Error)	P	Mean relative abundance of the phylum in reserve samples	Distance in fenced farm (m) [mean relative abundance at the nearest site]	Distance in unfenced farm (m) [mean relative abundance at the nearest site]
Firmicutes	Distance ~ Farm + Relative abundance	0.50	0.021					
	FarmUnfenced			21.83 (13.08)	0.123	5.14	27.49 [3 at 27 m]	49.32 [4.78 at 46.5 m]
	Relative abundance			-8.77 (2.63)	0.007*			
	Intercept			50.26 (15.72)	0.008*			
Nitrospirae	Distance ~ Farm + Relative abundance	0.61	0.006					
	FarmUnfenced			26.13 (11.85)	0.049*	0.57	12.96 [0.62 at 9 m]	39.10 [0.41 at 46.5 m]
	Relative abundance			141.24 (34.07)	0.002*			
	Intercept			-67.74 (18.96)	0.004*			
Proteobacteria	Distance ~ Farm + Relative abundance	0.46	0.033					
	FarmUnfenced			17.65 (13.10)	0.205	13.63	-12.79 [13 at -3 m]	4.86 [13.3 at 3m]
	Relative abundance			5.12 (1.66)	0.011*			
	Intercept			-82.56 (29.70)	0.018*			

Alphaproteo- bacteria (Class)	Distance ~ Farm + Relative abundance	0.49	0.026					
	FarmUnfenced			15.66 (12.51)	0.237	10.90	-4.98	10.68
	Relative abundance			11.02 (3.42)	0.008*		[9.03 at -3 m]	[10.32 at 9m]
	Intercept			-125.07 (41.27)	0.011*			

Asterisks (*): The p -value is significant (p -value ≤ 0.05).

Dagger (†): The linear regression model of Actinobacteria was not statistically meaningful ($p > 0.05$).

5.3.3 Soil nutrient concentrations impact bacterial communities in the forest soil

To determine if agricultural land use intensity has an impact on soil bacterial communities in adjacent forests, we used AIC comparisons to select the best model among different candidate models constructed with either bacterial richness or composition data, compared to a variety of environmental variables (Appendix D Table D5 & D6). No significant impact of land use intensity was found for the richness or composition of forest soil bacterial communities. However, our results suggest that the richness of bacterial communities in the forest increased with increasing concentrations of total Cd or soil pH (Appendix D Table D5). Meanwhile, community composition was most closely correlated with total P and total U (Appendix D Table D6). We also found that the bacterial richness and composition in the farming pasture were driven by soil variables related with fertiliser, (e.g., N, Olsen P, *etc.*) (Appendix D Table D7).

5.4 Discussion

Soil bacterial community richness and composition changed markedly along transects from the grazed pasture into the interior of adjacent forest fragments. Notably, the richness of bacterial communities was scale-dependent. Communities were more homogeneous within the pastoral land, as compared to within the forest soil, with the result that overall, bacterial community diversity was lower in the pastoral land. Meanwhile, bacterial community composition was more variable among samples derived from within fenced forest, compared with these derived from within unfenced forest fragments. When the forest fragments were not protected by a boundary fence, soil samples needed to be collected up to 50 m further into the forest to observe the expected abundance of pasture soil-associated phyla (i.e., *Firmicutes*, *Nitrospirae*, *Proteobacteria* and *Actinobacteria*). Variation in soil bacterial community composition was most affected by variables commonly associated with soil fertilisation practices (e.g., total Cd, total P, total U and soil pH). Together, these findings highlight the complex effects of agricultural land use on soil bacterial communities in the forests adjacent to pastoral land, and the need to incorporate understanding of multiple factors,

including fertilisation practices and livestock grazing, into assessments of the effect of land-use conversion on broad-scale variation in soil microbial diversity.

5.4.1 Land-use conversion results in a net diversity loss in pasture soils

Consistent with our first hypothesis (*Richness H_1*), the overall bacterial richness representing each farm site or land use type was higher in the forest, as compared to in the pasture soils, likely due to greater spatial/environmental heterogeneity in the forest soils. Our findings suggest that soil bacterial communities in the grazed pastures are more homogeneous than those in forest soils, reducing overall bacterial diversity in the pastoral land. The greater bacterial taxon richness observed within individual soil samples from the grazed pasture may result from microbial diversity being stimulated by live-stock associated factors, including diversification via organism and substrate introductions from faeces and urine, promotion of rhizosphere activity as a result of grazing, and also the mixing and dispersal of microbial communities through trampling (Kohler et al., 2005; Patra et al., 2005; Sørensen et al., 2009). Our study found increasing taxon richness (i.e., alpha diversity) within individual samples and decreasing dispersion in community composition among samples (i.e., beta diversity) in the grazed pastures, implying an uncoupling of alpha and beta diversity after land-use conversion. Both Rodrigues et al. (2013) and Gossner et al. (2016) similarly report increases in alpha diversity and decreases in beta diversity of soil microbial communities after forest to pasture conversion, indicating increased biotic homogenisation (Olden et al., 2004). These results imply a loss of locally rare taxa, which are thought to contribute a greater amount towards microbial community dynamics and stability than is apparent from their low proportional abundances (Shade et al., 2014). Furthermore, with increasing sampling effort, we found that the taxon richness obtained in the forest eventually surpasses that obtained in the pasture, indicating that demonstrated increased biotic homogenisation in the pasture soils further contributed to a net loss of local microbial diversity, which could leave the system more vulnerable (i.e. less able to respond) to future disturbance events (Olden et al., 2004).

5.4.2 A boundary fence protects the integrity of soil bacterial communities in forest fragments adjacent to pastoral land

Livestock exclusion is a common management tool for the conservation and restoration of remnant native reserves (Spooner et al., 2002; Lindsay and Cunningham, 2009). Mitigating the effects of grazing animals is important because their presence affects soil ecosystems by decreasing plant litter, soil aeration, increased nutrient inputs through urine and manure deposition (Yamulki et al., 1998; Ma et al., 2006; Radl et al., 2007; Wolf et al., 2010), and increasing the transfer of soil and gut-associated microorganisms (Cho and Kim, 2000; Hutchison et al., 2004; Wellington et al., 2013). We provide evidence that livestock exclusion plays an important role in protecting the integrity of bacterial communities in forest soils because the observed biotic homogenisation supported our second hypothesis (*Fence H₂*), evidenced by lower turnover rates of bacterial community similarity with distance, and more pasture-associated taxa in the adjacent unfenced forest soils. Our regression modelling approach was able to predict the presence of a boundary fence with near 90% accuracy, and we further confirmed that forest sites located near to the boundary of grazed pastures were more colonized by microbes related to livestock movement. Together, these results indicate that variation in microbial community structure in forests without a boundary fence, is caused by the presence of livestock, particularly at sites closest to the grazed pastures. Overall, our findings suggest that restricting livestock movements into forest, could have positive influences for maintaining the integrity of forest soil bacterial communities.

Livestock invasion appears to be correlated with abundances of some key phyla. We witnessed a decreasing relative abundance of pasture-associated taxa (i.e., *Firmicutes*, and *Nitrospirae*,) with distance from the interior of grazed pasture into the adjacent forest. Our observations were confirmed by a previous study, demonstrating an increase in the abundance of *Firmicutes* and a decrease in the abundance of *Proteobacteria* (e.g., β -, Δ -, γ -*Proteobacteria*) in grazed pasture soils as compared to forest soils (Jangid et al., 2008), whereas Yang et al. (2013) report an increase in the abundance of virulence, stress and antibiotics resistance genes derived from a wide range of bacterial phyla (e.g., *Firmicutes*, *Proteobacteria* and *Actinobacteria*) consequent of grazing. Livestock

faeces comprise bacteria representing multiple families in the *Firmicutes*, *Actinobacteria* and *Proteobacteria* (Lu et al., 2003; Mao et al., 2015; Tanca et al., 2017). Therefore, that the relative abundance of these organisms increased even in the forest soils adjacent to fenced pasture, indicates that hydraulic activity is capable of transporting livestock-related microorganisms and agricultural nutrients further into the forests (Lim and Flint, 1989; Champagne et al., 2000; Maule, 2000). Overall, our study has shown clear evidence of the migration and dispersal potential of livestock-associated taxa from grazed pastures into adjacent forest fragments, and the effect of livestock exclusion on protecting the integrity of soil bacterial community structure in the forests surrounded by agricultural lands. The positive effect of livestock restriction has been previously confirmed, showing that the occurrence of pathogens, such as *Campylobacter* spp. belonging to phylum *Proteobacteria*, decreased significantly in watersheds with restricted livestock movements (Sunohara et al., 2012). In our study there was no occurrence of any known pathogen in high abundance (< 15 reads per sample in all cases) either in the grazed pasture or adjacent forest soil.

5.4.3 Soil bacterial community structure is influenced by fertilisation practices

We were not able identify significant relationships between land-use index values and the richness or composition of soil bacterial communities in either the grazed pastures or adjacent forests. Nevertheless, we observed that the richness and composition of forest soil bacterial communities were most closely associated with soil variables related to fertilisation practices.

Concentrations of total Cd and soil pH were positively related to variability in microbial richness. Soil pH is well recognised as a key determinant, or correlate of bacterial community attributes (Fierer and Jackson, 2006; Griffiths et al., 2011; Hermans et al., 2017; Wu et al., 2017), including in agricultural systems (Jangid et al., 2008; Shange et al., 2012). Decreases in soil pH are commonly reported following fertiliser application (Fox and Hoffman, 1981; Liu et al., 2012), also reducing nutrient availability and microbial biomass in agricultural soils (Bardgett, 2005). The composition of bacterial

communities in the present study was correlated with concentrations of total P and total U. Prior studies have found total fertiliser P inputs into agricultural soil to be linearly associated with concentrations of soil Cd and U, both of which are frequently elevated in farmland soils, so they could be used as sensitive cumulative markers of soil phosphorus dynamics (Schipper et al., 2011; McDowell, 2012; McDowell et al., 2013). The impact of accumulated soil Cd and U has been observed to extend into adjacent forest fragments (Stevenson, 2004). The toxic impacts of heavy metals, such as Cd and U, on soil microorganisms have been reported in several studies (Renella et al., 2005; Cardenas et al., 2008; Xu et al., 2010). Similarly, owing to their integral role in mediating the availability of P in the soil, microbial community composition is commonly observed to be related to P fertilisation practices (Wakelin et al., 2009; Mander et al., 2012), for example as *Pseudomonadaceae*, *Enterobacteriaceae* and the *Actinobacteria* play roles in soil P-solubilisation (Hamdali et al., 2008; Oliveira et al., 2009; Hui et al., 2011; Richardson and Simpson, 2011). At our study site, Didham et al. (2015) provide evidence of the transfer of phosphorous from grazed pastures into adjacent forest fragments, increasing concentrations of total P, U and Cd in the forest soil. Shifts in soil properties induced by fertilisation practices likely drive the observed response of microbial communities, including in forest soils surrounded by agricultural land. Overall, our study shows that fertiliser disturbance in grazed pasture and its transfer into the adjacent forest, likely shapes bacterial community structure in forest soils after the conversion of adjacent land to pasture.

5.5 Conclusion

Biodiversity losses caused by forest-to-pasture land use conversions are a global issue. Here, we confirm significant impacts of land changes on soil microbial community composition that are not restricted to the underlying soil, but also affect the composition of communities up to 50 m away under adjacent land uses. We observed a net loss of bacterial diversity caused by the biotic homogenisation of microbial communities after forest-to-pasture conversion. We also demonstrated that boundary fences used to restrict livestock movement are useful for protecting the integrity of soil bacterial communities in forests surrounded by the grazed pastures. The changes in bacterial community attributes we observed in the forest soils were most closely correlated with

variation in soil attributes frequently associated with fertilisation practices (e.g., total Cd, total P, total U and soil pH) and the invasion of livestock. Overall, our study suggests that agricultural practices associated with grazing and fertiliser use can have a sphere of influence that extends well beyond the management unit of individual farms to impact the composition and therefore presumably the functioning of microbial communities in adjacent forest ecosystems.

Chapter 6

General Discussion

Advances in molecular biology, such as next-generation sequencing technologies, allow us to survey uncultivated microorganisms in the environment, revolutionising our ability to describe microbial communities with regard to their presence, abundance, distribution and the functional roles they perform. In this thesis, I used next-generation sequencing to explore how both bacterial and fungal communities vary along environmental gradients, including across a mountain elevation gradient and across different land use types. Firstly, using DNA gene sequencing of bacterial and fungal genes, I investigated the biogeography of soil microbial communities across broad (i.e., along a ~1,500 m mountain elevation gradient) and fine sampling scales (i.e., across sunny and shady aspects of a mountain ridge), to simultaneously examine whether bacteria and fungi respond similarly to gradients in environmental conditions. Then, to deepen our understanding of the impact of environmental variables on the functioning of microbial communities beyond simple biogeographic patterns of microbial community richness and composition, network analyses and an ‘inferred metagenomics’ method were used to respectively study community co-occurrence patterns and their potential functional traits. To address variation in a wider range of environmental conditions, I finally shed light on how land-use conversion influences the diversity and composition of bacterial communities in agricultural pastoral soils and the soils of adjacent forest fragments.

6.1 Elevational patterns in bacterial and fungal communities

Mountain elevation gradients have served as a heuristic tool and ‘natural laboratory’ for the field of microbial biogeography for decades (Bryant et al., 2008; Fierer et al., 2011; Singh et al., 2012; Liu et al., 2015; Collins et al., 2018). The environmental variables driving the biogeographic distribution of microbial taxa along mountain elevation gradients can be complex (Wu et al., 2017). It is of importance to investigate whether distinct biogeographic patterns can be distinguished between bacteria and fungi across the same environmental gradient, as the intrinsic properties and functional attributes of fungal communities are different from those of bacterial communities. It is perhaps unlikely bacteria and fungi share similar patterns, because previous studies have shown differences in their sensitivity and responses to changes in the environmental conditions for these two taxonomic groups (Berlemont et al., 2014;

Koyama et al., 2014; Liang et al., 2015). Interestingly, no clear elevational patterns were observed for either bacterial (Chapter 2) or fungal (Chapter 3) taxon richness along a continuous mountain ridge along an elevation gradient of ~1,500 m in this thesis. These observations were consistent with observations from previous reports, revealing no significant decline in the taxon richness of bacterial (Fierer et al., 2010; Shen et al., 2013) and fungal (Meier et al., 2010; Meng et al., 2013; Counce et al., 2014) communities with altitude. Likewise, bacterial community composition varied significantly with elevation, with a concurrent change in fungal compositional data. However, numerous previous studies have described inconsistent elevational patterns observed for both bacteria and fungi, for example decreases in diversity/richness (Bryant et al., 2008; Lugo et al., 2008; Bahram et al., 2012; Liu et al., 2015), no trend (Fierer et al., 2010; Meier et al., 2010; Meng et al., 2013), or unimodal patterns (Singh et al., 2012; Counce et al., 2014; Miyamoto et al., 2014) in both bacterial and fungal community composition with increasing elevation. These findings suggest that the richness and diversity attributes of microbial taxa along the elevation gradient might be driven by complicated ecological mechanisms, rather than a single rule. Together, these results contribute to a wealth of evidence illustrating that microbial communities exhibit fundamentally different responses to elevation than macroorganisms (Fierer and Jackson, 2006; Lauber et al., 2009), as gradients in the richness of macroorganisms, such as plant and animal communities, are commonly found with elevation (Smith, 1988; Lee et al., 2004; Aubry et al., 2005; Randin et al., 2009; Carneiro et al., 2013; Asner and Martin, 2015). Additionally, I found that when comparing samples across equivalent distances, bacterial community composition was more affected by variation in sampling site aspect than elevation. Even though the cause behind this observation is unclear, these findings highlight the need to incorporate knowledge of multiple spatial factors, such as site aspect differences, for the appropriate use of elevation gradients as a proxy to study the influence of spatial and environmental gradients on microbial community attributes.

Although broadly similar elevational patterns in both bacterial and fungal community richness and composition were observed, further analyses showed that these communities responded differently to spatial variation in environmental variables. Soil pH was most strongly correlated with changes in bacterial community composition and

richness; soil pH is widely reported as being a major influence on bacterial community attributes (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011; Hermans et al., 2017). However, apart from pH, concentrations of ammonium played more substantial roles in shaping fungal community richness and composition. This result is consistent with other studies, indicating that fungi generally tolerate wider pH ranges for growth, and therefore their community attributes are more strongly affected by soil nutrient attributes (Rousk et al., 2010; Zinger et al., 2011). Together, my findings suggest that soil physicochemical variables, rather than climatic variables, have a crucial influence on shaping microbial community structure and diversity, even across a ~1,500 m elevation gradient, corresponding to a temperature gradient of ~9 °C (McCain and Grytnes, 2010). The divergent environmental drivers simultaneously detected as being of importance for bacteria and fungi indicate that the distributions and diversity of different taxonomic groups might not be simply driven by one simple rule, but instead by the different underlying ecological mechanisms.

Overall, my results suggest that responses of bacteria and fungi to elevation are fundamentally different to those of macroorganisms and the biogeography of bacterial and fungal communities were controlled by differing environmental variables. These findings contribute towards a deeper understanding of how microbial communities respond to variation in environmental conditions, such as climate change, and shed light on the need for further studies exploring related shifts in the functional and trophic traits of these communities.

6.2 Beyond simple biogeographic patterns in richness and composition: functional traits and co-occurrence patterns

If sufficient functional redundancy exists in microbial communities, their responses to gradients in environmental conditions might not be easily explained by only phylogenetic and/or taxonomic information (Nannipieri et al., 2003; Sunagawa et al., 2015). The emergence of metagenomics-based approaches in microbiology provides a powerful tool to investigate the functional potential of complex environmental microbial communities (Ferrari et al., 2005; Raes et al., 2011; Delmont et al., 2012;

Leff et al., 2015). Indeed, increasing usage of shotgun metagenomics currently provides microbiologists the capability to detect distributions of functional genes relevant to metabolic pathways, energetics and regulatory circuits to directly address changing microbial functional potential in complex natural environments (Yang et al., 2014). However, the prohibitive cost of this approach has hindered its widespread use, particularly in the study of biogeography of microbial communities across large- or even local-scales. Consequently, this might cause an incomplete understanding of microbial functional biogeography. To fill this knowledge gap, I have verified that ‘inferred metagenomics’ approaches, using bacterial 16S rRNA gene sequencing data, can provide a general functional trait-based picture of bacterial biogeographic pattern, in Chapter 4. The results of my inferred metagenomics analysis revealed that bacteria with larger genome size appear to prevail at a wider range of elevations across my study site, which is consistent with previous studies, showing an increase in bacterial genome size correlates with increased bacterial distributions and adaptability to environmental fluctuations (Konstantinidis and Tiedje, 2004; Barberan et al., 2014; Cobo-Simon and Tamames, 2017). Additionally, analyses of putative functional traits provide evidence that some critical environmental variables (e.g., soil pH, temperature and C/N ratio) contribute to the prevalence of community functional traits, including genes encoding for ‘cellular responses to stress’, ‘nitrogen fixation’ and ‘nitrification’. For example, consistent with the findings of Yang et al. (2014), I determined a greater abundance of functional genes encoding for ‘cellular responses to stress’, which may provide adaptation to low temperature, low pH and high UV irradiation at high elevation. Overall, in Chapter 4, I demonstrate how ‘inferred metagenomics’ approaches can be used as a test case to provide a broad overview of spatial variation in the functional potential of soil bacterial communities, based on bacterial 16S rRNA gene data, before investing a large amount of money to fund shotgun metagenomics surveys.

It is evident that microbial species are not found in isolation within complex environments, and their interspecific interactions might be critical determinants or correlates of community attributes, such as diversity and ecosystem functioning (Steele et al., 2011; Deng et al., 2012; Faust and Raes, 2012; Kara et al., 2013). In particular, functionally-distinct niche spaces shared by microbial community members could be distinguished by their interspecific interactions, as detected by co-occurrence networks

(Steele et al., 2011; Faust and Raes, 2012). Hence, apart from investigating bacterial functional traits, putative fungal-fungal interactions, as detected by network co-occurrence analysis, were also used to study how fungal co-occurrence patterns change across an elevation gradient. These co-occurrences could provide important information on the underlying mechanisms that determine elevational patterns in community attributes (Barberán et al., 2012; Gilbert et al., 2012). Given that the significant variability in fungal community composition and soil-nutrient resources (e.g., decreasing concentrations of ammonium at greater elevation) occurred across my study site, the observed increase in the complexity of fungal networks with decreasing elevation were likely due to increasing competitive exclusion (Rajaniemi, 2003) or top-down predatory interactions (Worm et al., 2002). My results suggest that interspecific co-occurrences may be mediated by variation in soil nutrient concentrations, based on different nutrient-preferences among taxa. Soil nutrients such as ammonium, are widely utilised by most fungi (Rastin et al., 1990), and are often resource limiting. As resources increase, more species meet their minimum resource requirements (Rajaniemi, 2003) and species diversity typically decreases (Bakelaar and Odum, 1978; Clark and Tilman, 2008), because increasingly competitive organisms dominate. My findings correspond with the observations of Baar and Stanton (2000), revealing that variation in ammonium concentrations correlate with changes in both the type and strength of fungal interactions. Overall, based on the evidence supporting nutrient-driven shifts in fungal community diversity and function, my research provides a mechanistic explanation for how the richness and composition of fungal communities, and their interactions, change across elevation gradients.

In general, these findings highlight the potential for using ‘inferred metagenomics’ methods and network co-occurrence analyses to conduct a general picture of functional microbial biogeographic patterns and trophic traits. I show how such methods can enhance our understanding of microbial processes, including ecosystem nutrient cycling processes which cannot be understood from traditional assessments of biogeographic patterns in microbial richness and composition. Collectively, it is of particular importance to study the functional traits and co-occurrence patterns in microbial ecosystems to aid in developing and testing hypotheses regarding how

functional traits shape or respond to changes in microbial community structure across environmental gradients.

6.3 Spatial patterns in the bacterial community structure of forest soils are impacted by agricultural disturbances in adjacent land uses

Previous studies confirm that small organisms can disperse long distances (> 1 km) into adjacent habitats (Blitzer et al., 2012), suggesting that the spatial impacts land use conversion can be extensive. Considering the capability of microorganisms for widespread dispersal and their critical role in regulating ecosystem function and soil biogeochemistry (Madsen, 2011), the extent to which soil bacterial community structure is affected by their proximity to adjacent agricultural land remains unclear. In the final chapter of my thesis, I chose to study how pastoral land management practices affect soil bacteria, not only in underlying agricultural soils but in adjacent forest fragments. We characterized soil bacterial community composition along transects spanning the pasture-forest boundary of 21 farms, to generate novel insights into the broader impact of agricultural disturbances on soil microbial community attributes within the soils of adjacent forest.

The key effects of agricultural land use on soil bacterial communities in pastoral land and adjacent forest fragments are expected to be diverse, including fertilisation and livestock-associated factors. An increase in bacterial taxon richness in grazed pastures was observed, likely driven by the impacts of livestock grazing causing bacteria and substrate introductions from their faeces and urine and also the mixing and dispersal of microbial communities through trampling (Kohler et al., 2005; Patra et al., 2005; Sørensen et al., 2009). Reduced spatial variation in community composition (i.e., beta diversity) and increases in bacterial taxon richness (i.e., alpha diversity) within the grazed pastures suggests increased biotic homogenisation (Olden et al., 2004), supporting the outcomes of prior studies by Rodrigues et al. (2013) and Gossner et al. (2016). Such increased biotic homogenisation in the pasture soils after land-use

conversion was found to further contribute to a net loss of local (i.e. per sample) microbial diversity, which could leave the system more vulnerable (i.e. less able to respond) to future disturbance events (Olden et al., 2004).

In the forest adjacent to pastoral land, I found that boundary fences used to restrict livestock movement are useful for protecting the integrity of bacterial communities in forest soils (Chapter 5). It is evident that more pasture-associated taxa, for example *Firmicutes* which are associated with livestock faeces (Mao et al., 2015; Tanca et al., 2017), were present in forest soils that were not fenced off from the adjacent pasture. These taxa were observed up to 50 m into the soils of adjacent forests, but only 27 m into the soils of fenced forest fragments. These results suggest that livestock invasion and/or hydraulic activity is capable of transporting livestock-related microorganisms and agricultural nutrients into the forests (Lim and Flint, 1989; Champagne et al., 2000; Maule, 2000). The observed response of bacterial community composition was likely driven by shifts in soil properties induced by P fertilisation practices. Such findings are consistent with observations from a prior examination of the same sample sites that reported the transfer of phosphorous from grazed pastures into adjacent forest fragments, increasing concentrations of total P, U and Cd in the forest soil (Didham et al., 2015).

In summary, in Chapter 5, my study suggests that agricultural practices associated with grazing and fertiliser use can have a sphere of influence that extends well beyond the management unit of individual farms, impacting the composition and therefore presumably the functioning of microbial communities in adjacent forest ecosystems.

6.4 Future research priorities

6.4.1 Are biogeographic patterns in microbial communities at local-, regional- and even continental-scale impacted by the same environmental mechanisms?

In Chapter 2 and 3, similar elevational patterns in the richness and composition of both bacterial and fungal communities along a mountain ridgeline were evident, being most strongly correlated with soil physicochemical variables, rather than climatic conditions. In particular, soil bacterial community attributes were largely driven by changes in pH, while fungal community attributes were most strongly related to changes in soil nutrient availability. Hence, these findings lead us to conclude that to appropriately use elevation and aspect gradients as a natural laboratory for examining the likely influences of climate change on microbial communities, microbiologists need to also account for more local-scale variation in soil conditions. Further work is needed to better understand the mechanisms underlying microbial diversity patterns and the relative importance of both evolutionary and ecological processes as determinants of microbial community structure and composition. For example, microcosm experiments would be a powerful research tool to explore the likely responses of soil microbial communities to dramatic further environmental changes, such as climate change. Microcosms could be placed at each elevation along a mountain ridgeline using consistent undisturbed soils. Therefore, scientists could study the influence of climatic conditions on soil microbial community attributes better by simplifying the complexities of changes in soil properties across geographic scales. Additionally, in prior studies, it has been documented that the biogeographical patterns observed in microbial community structure were not random at the regional or continental scale in different habitats, such as in soils (Fierer and Jackson, 2006; Tedersoo et al., 2014; Ma et al., 2016) and streams (Fierer et al., 2007; Lear et al., 2013; Lear et al., 2017b). Collectively, there is a clear need for further studies to test whether there are differences in microbial assembly mechanisms for different taxonomic or functional groups at different spatial scales. A thorough integration of microbial biogeography across different spatial scales is likely to provide a more comprehensive understanding of how communities respond to gradients in environmental variables.

6.4.2 Advancing understanding of microbial distributions via in depth assessments of functional biogeography

In Chapter 3, I highlighted the existence of significant differences in fungal community composition and taxon co-occurrences along an elevation gradient, mainly driven by soil nutrient conditions, implying nutrient-driven shifts in fungal community diversity and function in soil. However, the technical limitations of network co-occurrence analysis must be carefully considered, as the putative co-occurrence patterns observed only represent statistical correlations among taxa, instead of a direct actual microbial interaction in the ecosystem (Ma et al., 2016). Therefore, future investigations of microbial interactions are needed, validated by literature or experiments, for example using microscopy to confirm symbiotic relationships (Lima-Mendez et al., 2015). Furthermore, it is also essential to study the implications of variations in microbial co-occurrence patterns for changes in microbial composition and also functional potential to further enhance our understanding of how microbes interact with each other within community and their functioning in the ecosystem. An example of such a study is provided by Li et al. (2015), who demonstrated that a combination of metagenomic approaches and network analysis tools can provide novel visualisation and analytical strategies for comparative metagenomics to profile microbial community functioning. Network-based comparisons of metagenomics data can be used to examine, as well as to generate hypotheses, for ecological investigations of microbial functioning.

Using an inferred metagenomic method (in Chapter 4), I demonstrated significant relationships between environmental variables and the spatial distribution of bacterial functional traits as well as the community adaption to adverse environments, evidenced by the greater prevalence of free-living bacteria with large genome sizes. My research provides relevant insights into the potential for using inferred genomic information, based on 16S rRNA gene data, to generate a general functional trait-based picture of microbial biogeographical patterns. However, one must be cautious when inferring the functional potentials from this method, as it is unable to capture the full extent of the genomic diversity of these microbial communities. Using further analysis, such as

quantitative PCR or a microarray-based metagenomics tool such as GeoChip (He et al., 2007; Tu et al., 2014; Yang et al., 2014), would be beneficial to confirm the extent to which predicted patterns in the abundances of genes of interest reflect the true functional potential of bacterial communities. Now, the increasing usage of metagenomics is providing an unprecedented wealth of functional genes directly from nature (Hugenholtz and Tyson, 2008). With continuous declines in sequencing costs, the emergence of functional biogeography, the study of the spatial distribution of functional diversity, can bridge species-based biogeography and ecological science. This emerging field may shed new light as to the drivers of multifaceted diversity, including species, functional and phylogenetic diversities, and even ecosystem functioning (Violle et al., 2014). Studies integrating trait-based approaches to microbial ecology would enhance our ability to associate microbial diversity with ecosystem processes, which would provide a deeper mechanistic understanding of the functional role of microbial biodiversity in maintaining multiple ecosystem processes and services (Krause et al., 2014).

6.5 Concluding remarks

The spatial distributions of species and their function traits to cope with different environmental conditions across both space and time are fundamental ecological attributes (Barberan et al., 2014). Using next-generation sequencing technologies, I examined a number of biogeographic patterns in both bacterial and fungal community richness, composition and functional attributes across distinct environmental gradients, including along gradients in elevation and land use. My research confirms that using gradients in elevation and land-use types as proxies for variability in climatic and soil physicochemical conditions is a useful tool for the analysis of environmental drivers and correlates of microbial biodiversity encompassing multiple taxonomic groups, including both bacteria and fungi. Additionally, I have highlighted that inferred metagenomic methods and network co-occurrence analyses have much potential to enable comprehensive and efficient trait-based analyses, to gain a better understanding of the structure and dynamics of microbial communities. Although these approaches have limitations, there is still great scope and many potential applications to provide a complementary view-point to microbial biogeography and

ecosystem functioning. The potential applications of shotgun metagenomic methods will enable much improved understanding of the spatial and temporal biogeography of soil microbial communities and the vital functioning roles that they perform.

APPENDIX A

**Aspect has a greater impact on alpine soil bacterial
community structure than elevation**

Figure A1. Similarity in the composition of bacterial sequence data obtained across all sample elevations and aspects. The plot is constructed from non-metric multidimensional scaling of OTU data using a Bray-Curtis measure. Sample data closer to each other are expected to contain more similar bacterial communities. Sample data are assigned colours across a gradient from red (samples from sunny side) to yellow (samples on the ridge) through to blue (samples from shady side) and the size of sample markers increases with elevation (there are three example markers shown in the figure legend for samples collected at 500 m, 1000 m and 1500 m elevation). Highlighted on the plot (contained by an ellipse in the top right corner) are sample data obtained from sites impacted by a localised high abundance of sheep (sites SU1 and SU2 at 900 m elevation). 2D stress = 0.11.

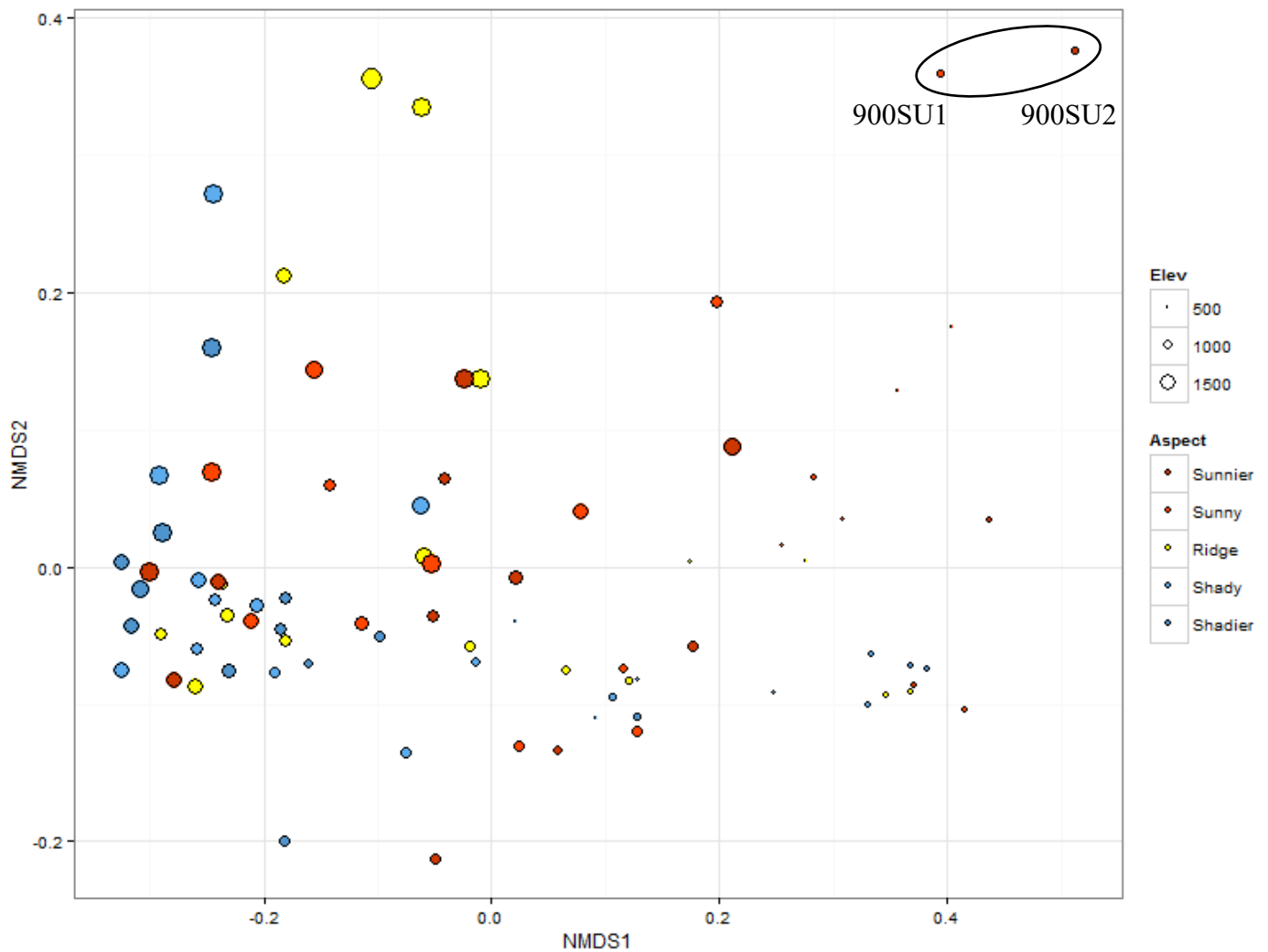
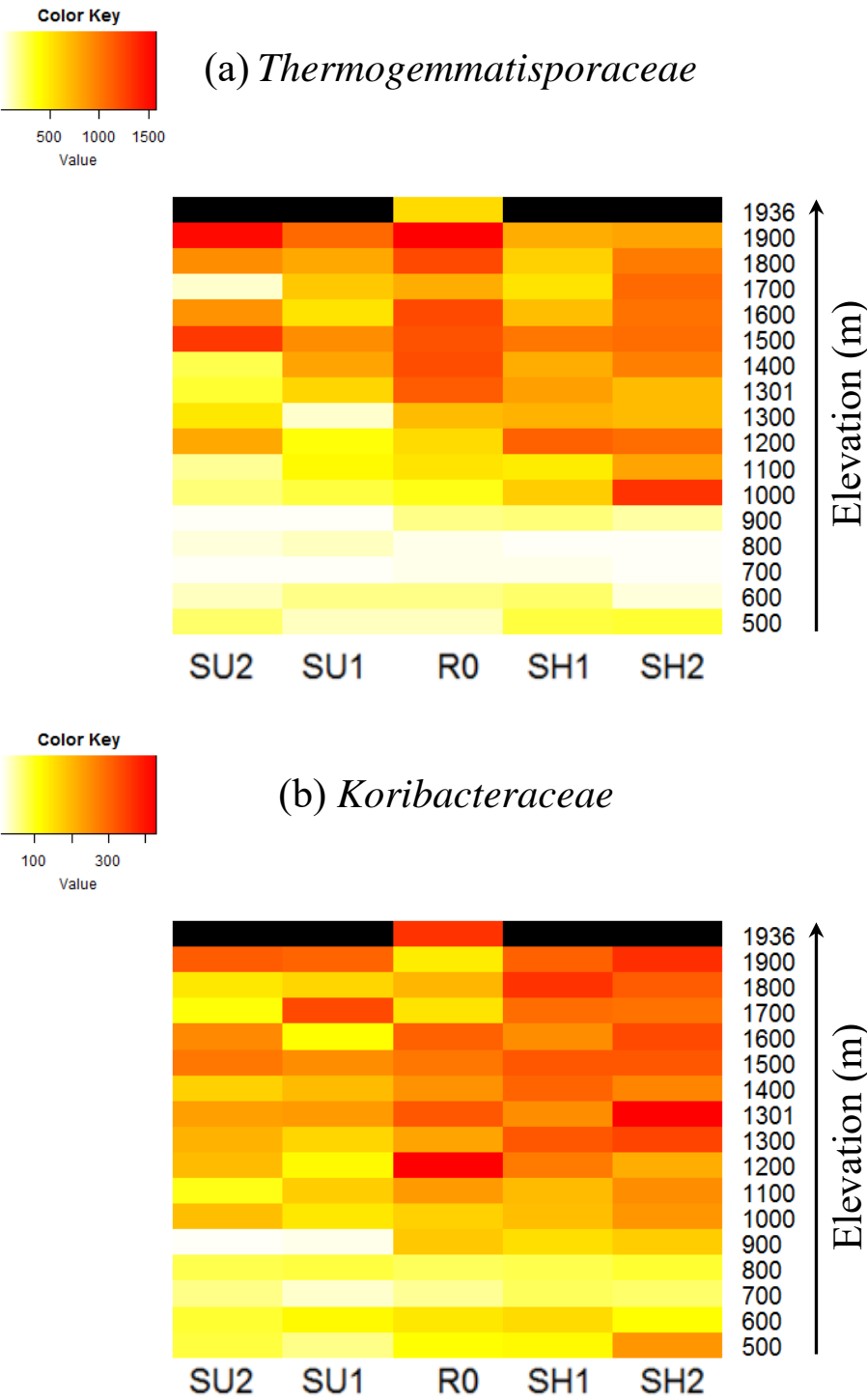
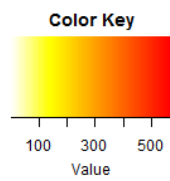
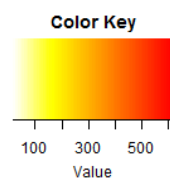
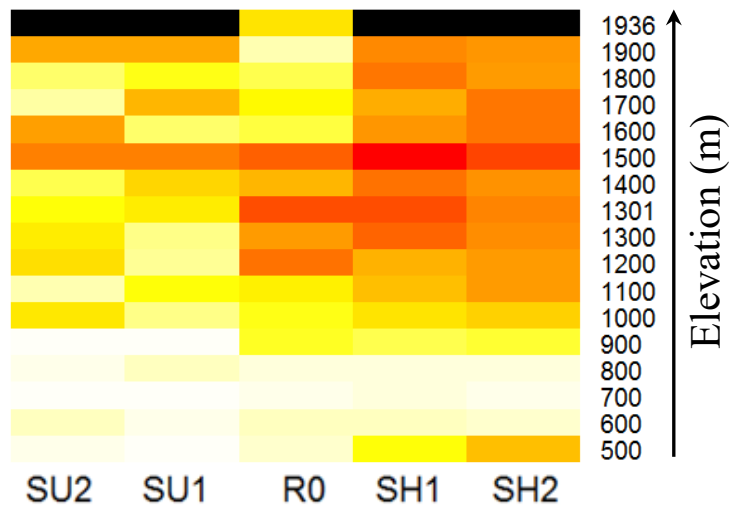


Figure A2. Variation in relative abundance of the family (a) *Thermogemmatisporaceae*, (b) *Koribacteraceae*, (c) Ellin6513, (d) *Gaiellaceae* across the site. Sample data are assigned colours across a gradient from red (highest relative abundance) to white (lowest relative abundance). Different rows represent data collected from different elevation bands; different columns represent data from different aspects. Sites at the top of the mountain where no sample data were collected are shown in black.





(c) Ellin6513 (*Acidobacteria*)



(d) *Gaiellaceae*

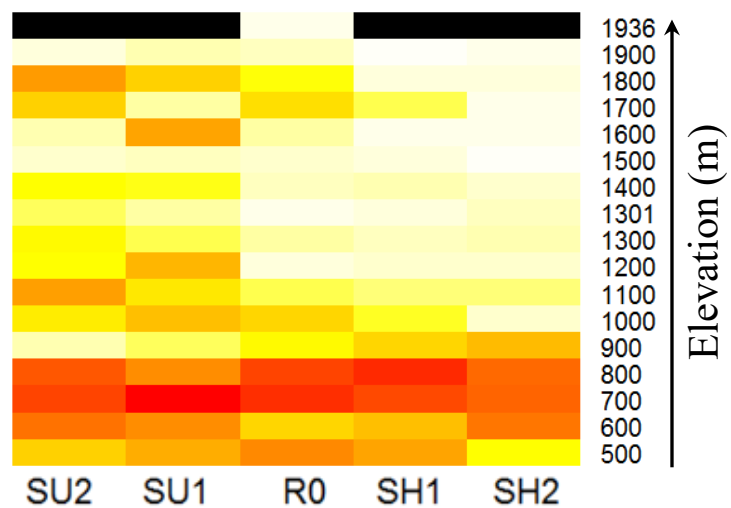


Figure A3. Unrooted Jukes-Cantor neighbour-joining consensus phylogenetic tree showing the position of some representative OTUs belonging to the family *Thermogemmatissporaceae* and environmental 16S rRNA gene clones from Genbank (Herein, saturated alpine tundra soil clones are *Chloroflexi*-related sequences). Bootstrap support values (10,000 resamples) are represented by the following symbols: ● $\geq 90\%$; ○ $\geq 80\%$ at each internal branch. The scale bar represents 0.1 change per nucleotide position. Accession numbers are given in parentheses.

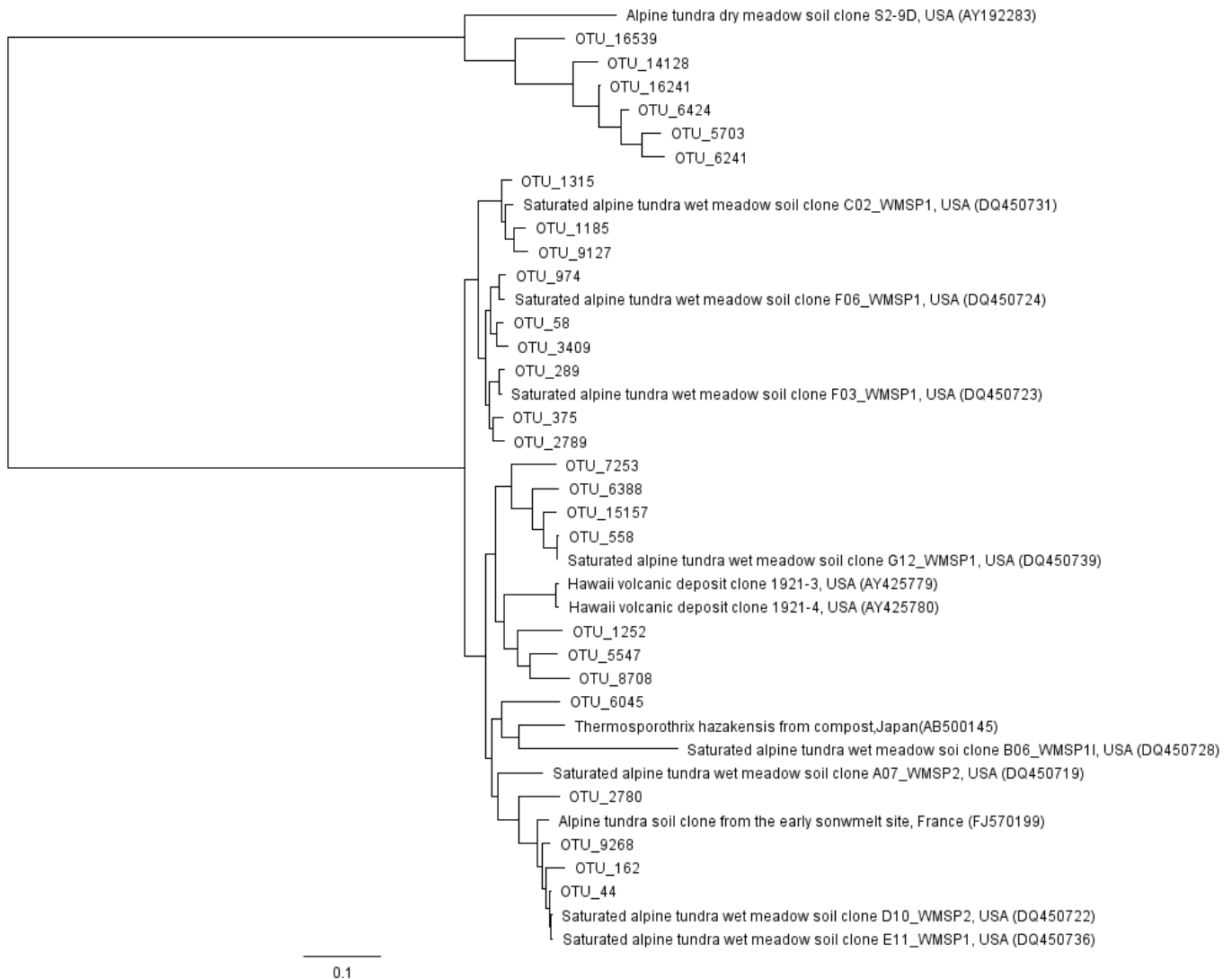
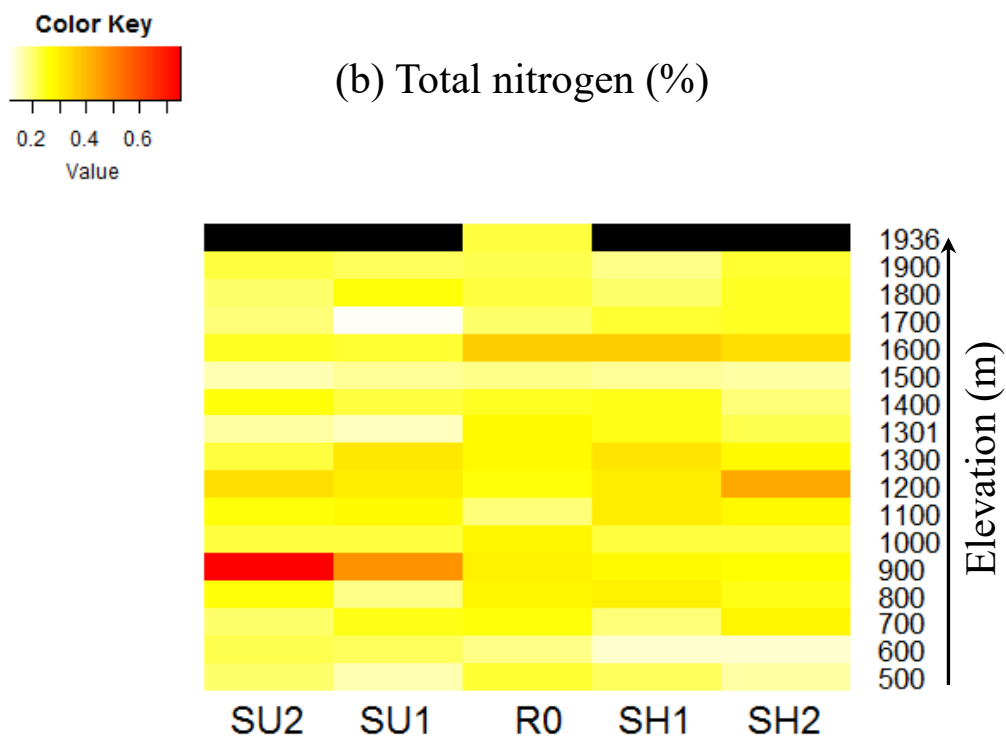
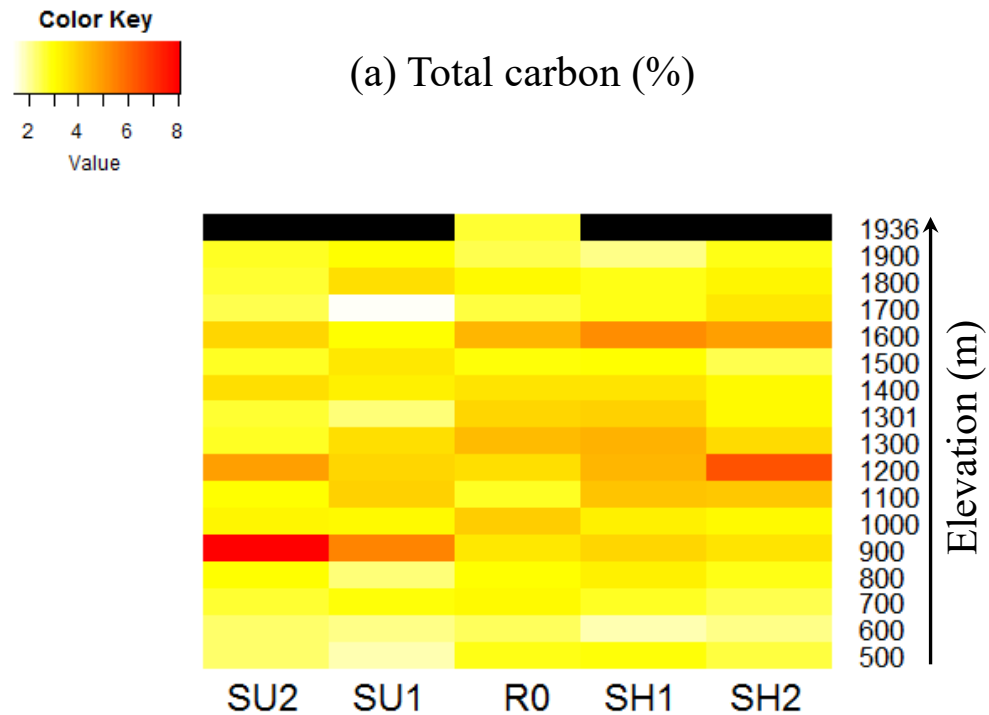
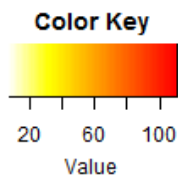
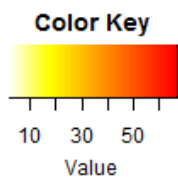
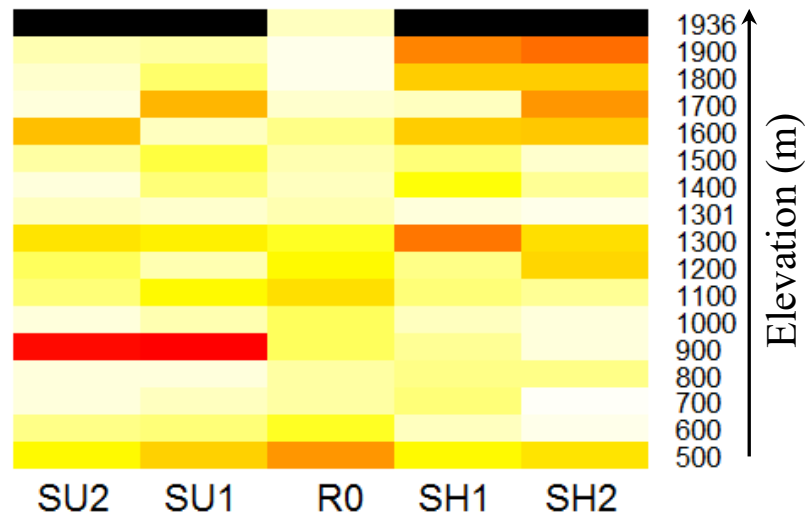


Figure A4. Variation in relative abundance of (a) total carbon (%), (b) total nitrogen (%), (c) phosphorus (mg/kg), (d) soil moisture (%), (e) soil pH, and (f) plant biomass (gram) across the site. Sample data are assigned colours across a gradient from red (highest relative abundance) to white (lowest relative abundance). Different rows represent data collected from different elevation bands; different columns represent data from different aspects. Sites at the top of the mountain where no sample data were collected are shown in black.

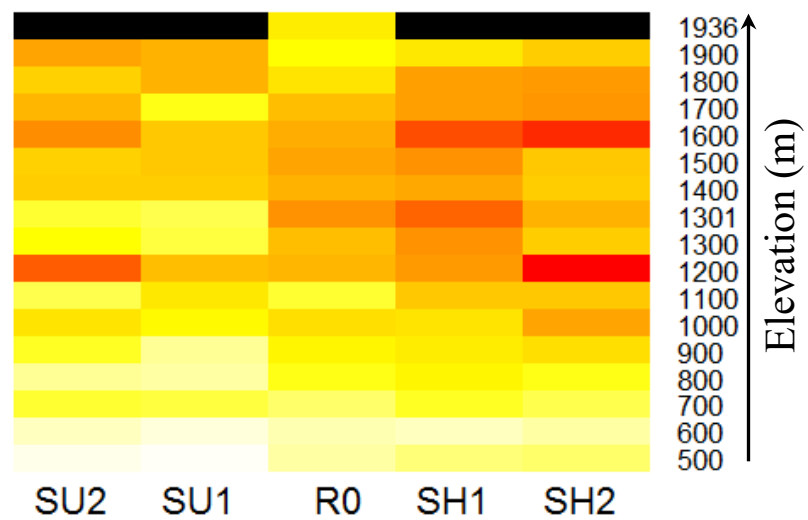




(c) Phosphorus (mg/kg)



(d) Soil moisture (%)



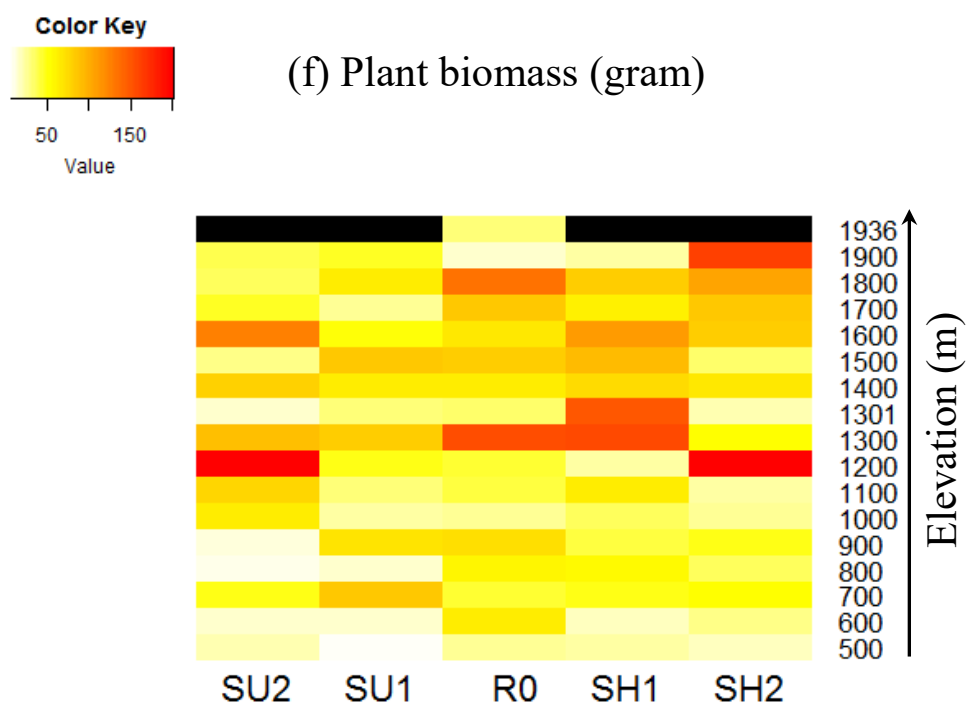
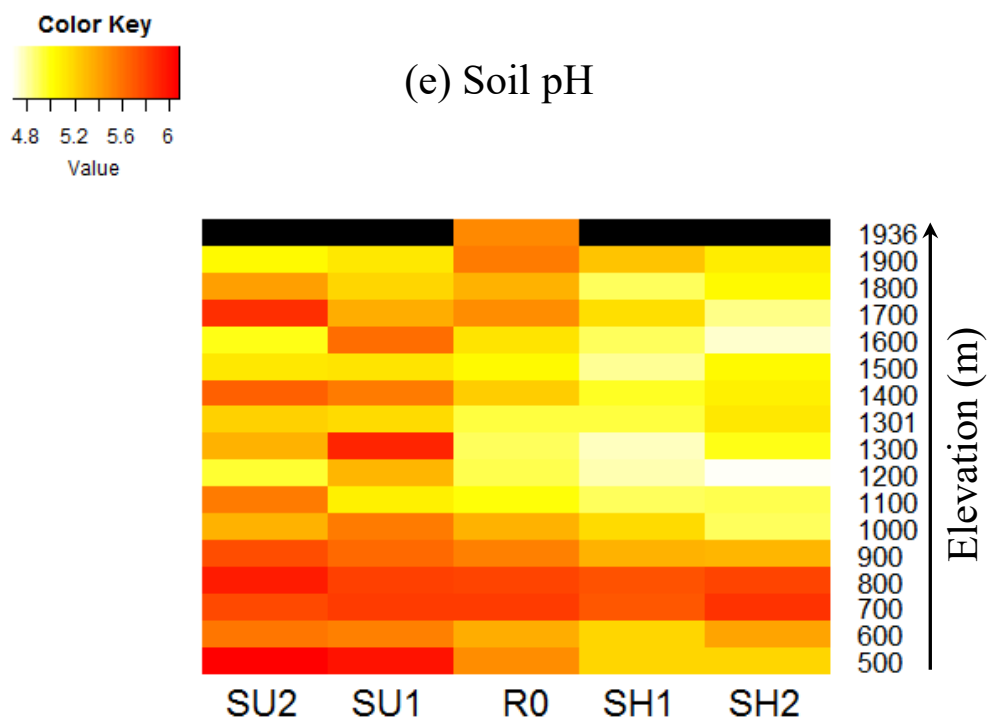


Table A1. Results of permutational ANOVAS for (a) richness, and (b) composition of bacterial communities in response to elevation, aspect and the interaction between these two factors. Df = the degrees of freedom; SS = sum of squares; MS = mean sum of squares; Sq.root = square root; Pseudo-*F* = *F* value by permutation. Bold face indicates statistical significance ($P < 0.05$); *P*-values are based on 9999 permutations (i.e. the lowest possible *P*-value is 0.0001)

Source of variation	Df	SS	MS	Sq.root	Pseudo- <i>F</i>	<i>P</i> (perm)
(a) Richness of bacterial communities						
Elevation	16	1184.4	74.025	1.695	11.209	0.0001
Aspect	4	234.57	58.642	0.812	8.879	0.0001
Elevation x Aspect	60	1441	24.017	1.876	3.637	0.0001
Residual	320	2113.4	6.604	2.570		
Total	400	4946.2				
(b) Composition of bacterial communities						
Elevation	16	2.418 x 10 ⁵	15113	24.626	17.173	0.0001
Aspect	4	31647	7911.8	9.433	8.99	0.0001
Elevation x Aspect	60	1.804 x 10 ⁵	3007.1	20.735	3.417	0.0001
Residual	320	2.816 x 10 ⁵	880.07	29.666		
Total	400	7.368 x 10 ⁵				

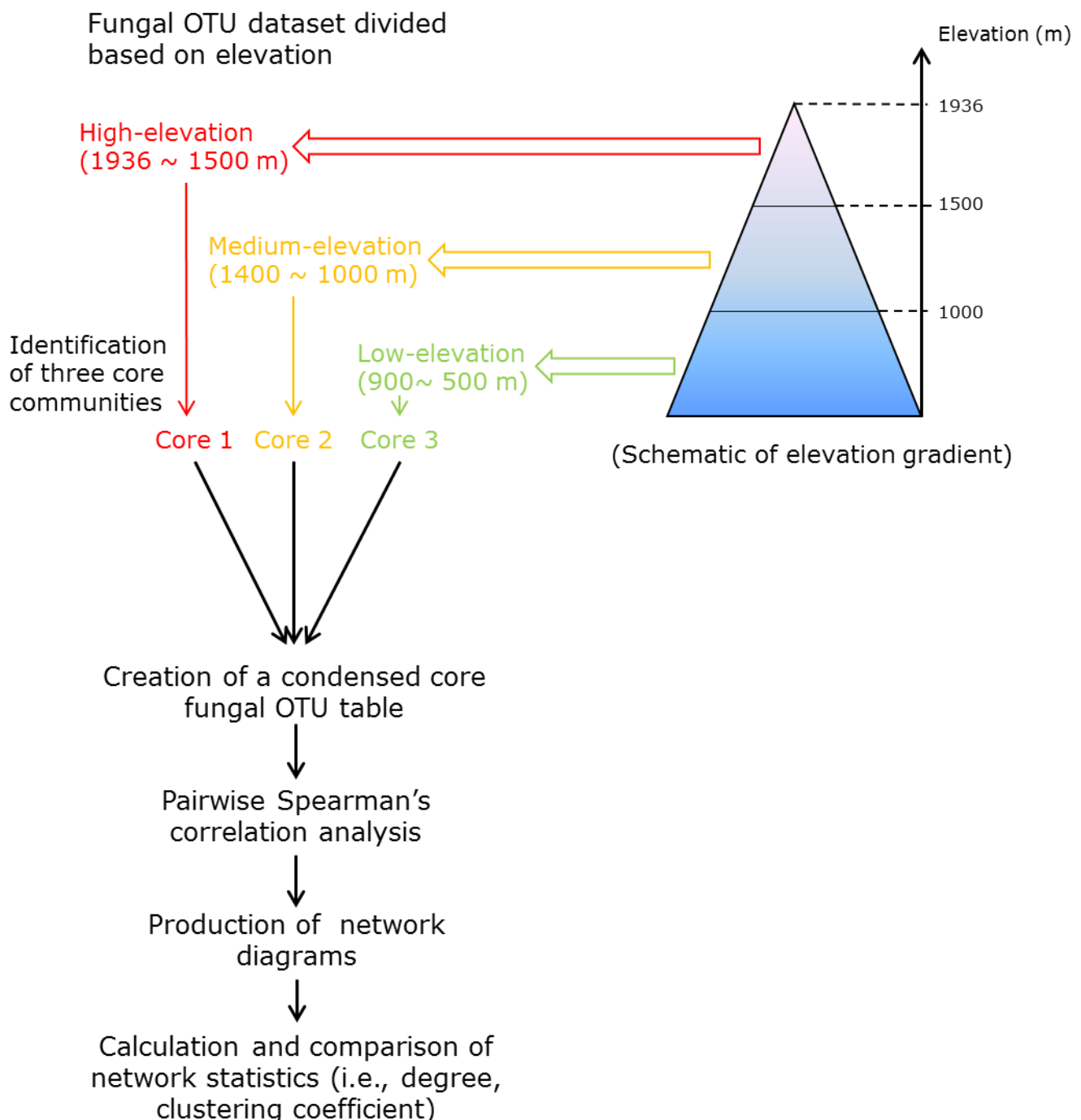
Table A2. Results of permutational ANOVAS for soil pH in response to elevation, aspect and the interaction between these two factors. Df = the degrees of freedom; SS = sum of squares; MS = mean sum of squares; Sq.root = square root; Pseudo- F = F value by permutation. Bold face indicates statistical significance ($P < 0.05$); P -values are based on 9999 permutations (i.e. the lowest possible P -value is 0.0001)

Source of variation	Df	SS	MS	Sq.root	Pseudo-F	P(perm)
Elevation	16	572.06	35.754	1.227	81.525	0.0001
Aspect	4	233.17	58.292	0.856	132.92	0.0001
Elevation x Aspect	60	262.15	4.3692	0.891	9.963	0.0001
Residual	320	140.34	0.4386	0.662		
Total	400	1212				

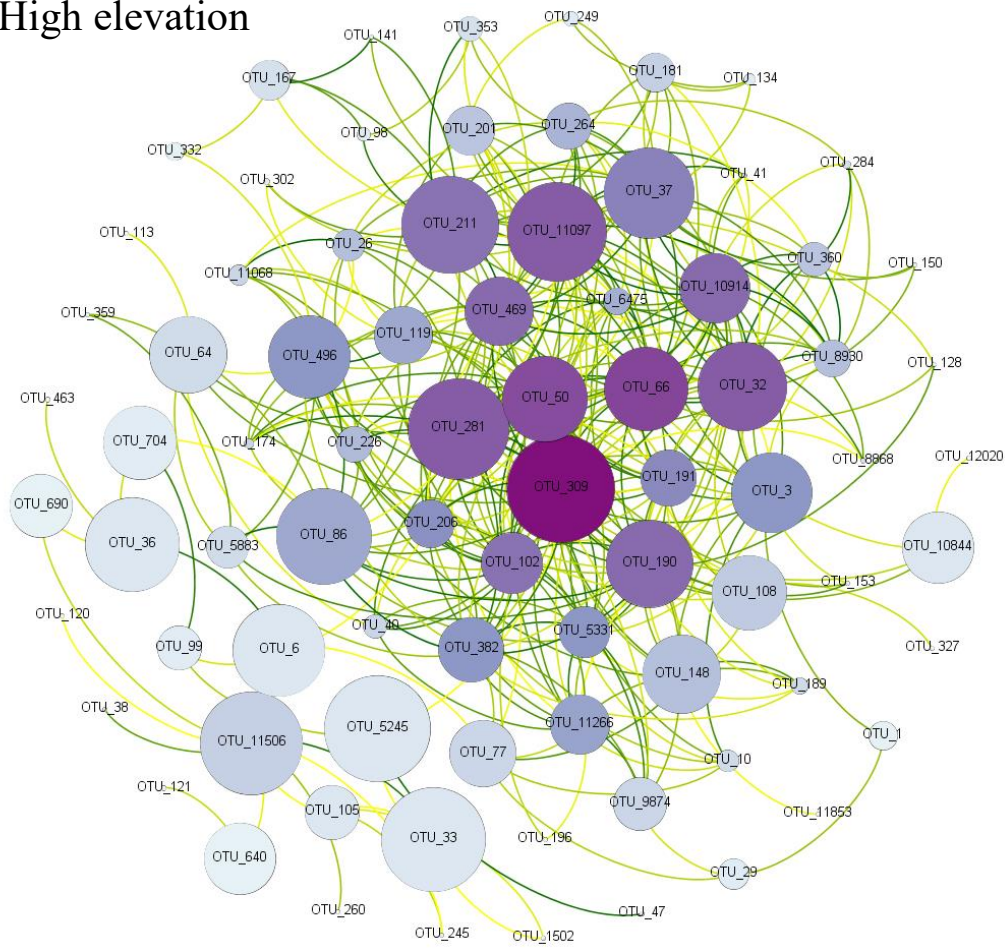
APPENDIX B

Soil fungal communities form closer network associations at lower elevation

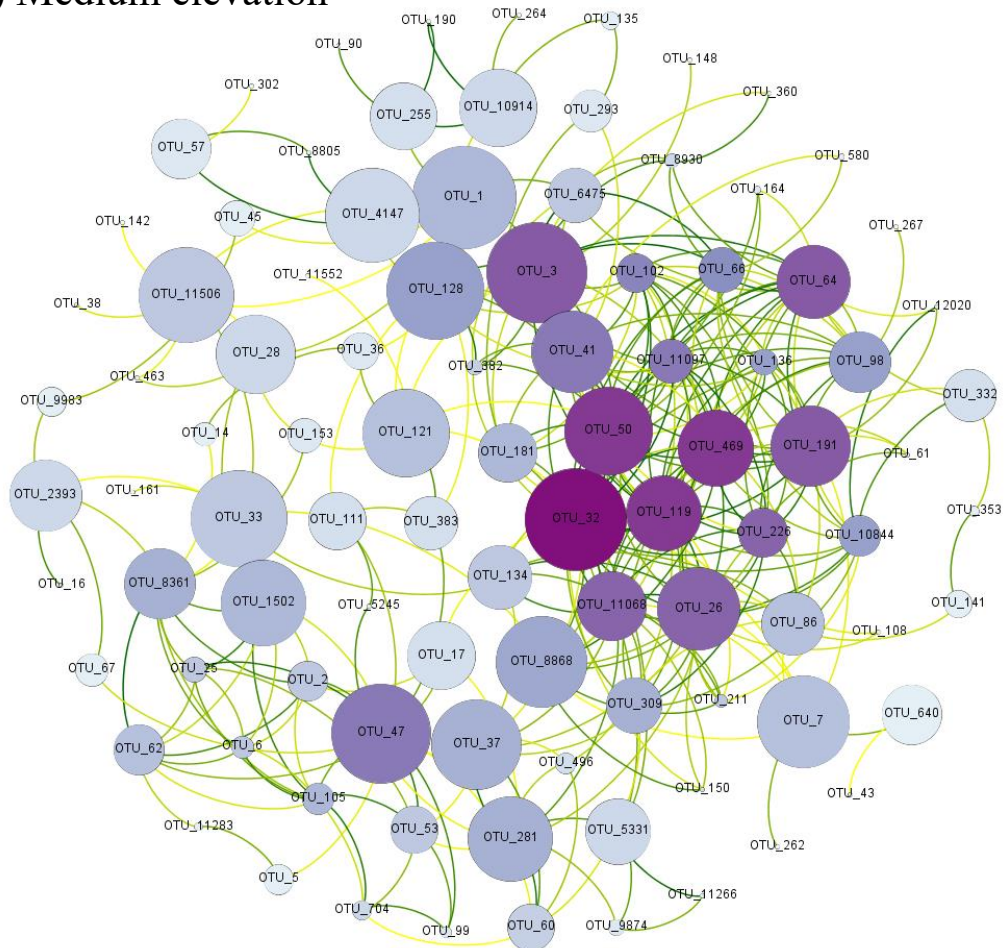
Figure B1. Methods used to characterise fungal community interactions across an elevation gradient. Fungal community data (i.e., 97% fungal ITS1 OTUs) were separated into three elevational groups/datasets (i.e., high-, medium-, and low-elevation). The OTUs present in > 50% of all samples within each dataset were identified to be core communities present at high medium or low elevation. Fungal OTUs present in any of the three core communities were then used to construct the network diagrams, calculated using all possible Spearman's correlations between each pair of OTUs. After filtering fungal OTUs without robust correlation ($\rho > 0.5$, p -value ≤ 0.05), we visualised co-occurrence in network diagrams and calculated networks statistics.



(b) High elevation



(c) Medium elevation



(d) Low elevation

(e) Lower elevation

(f) Bar charts comparing parameters of networks

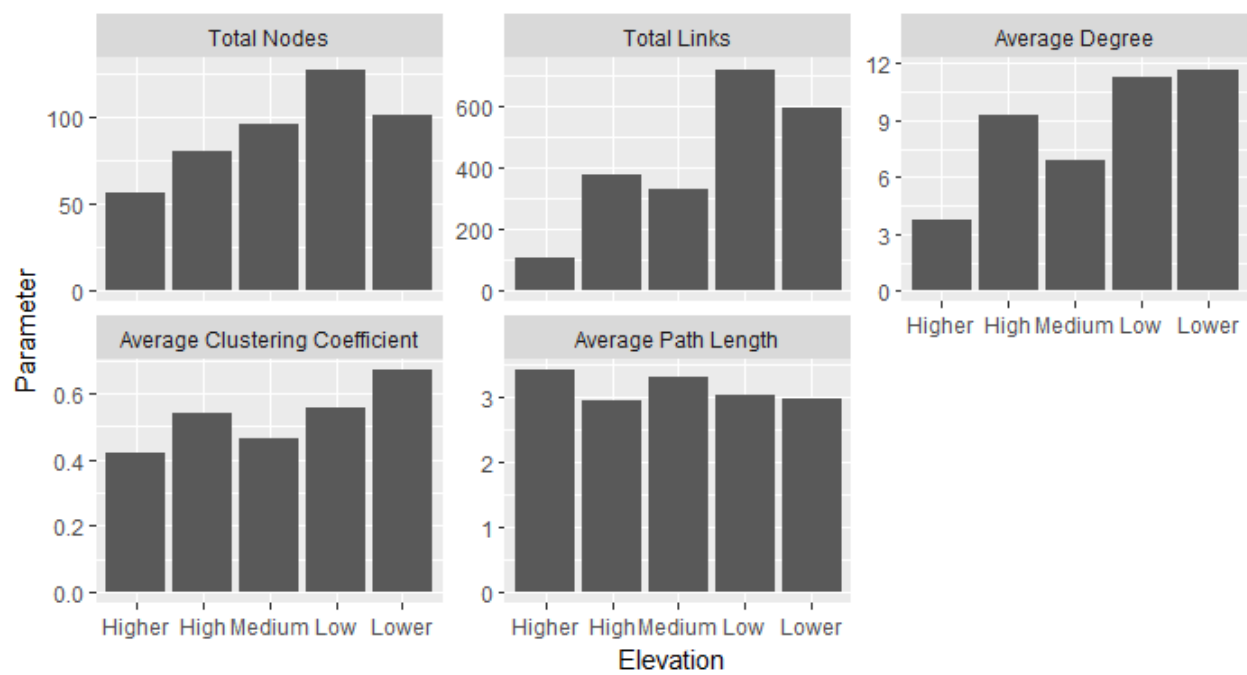


Figure B3. Boxplots of the concentration of ammonium by three elevation groups. The horizontal line within the box corresponds to the median value, with 25th – 75th percentiles as the box limits. The whiskers extend to the minimum and maximum data point no more than 1.5-fold interquartile range. Statistical differences in the concentration of ammonium between each elevational group were analysed by one-way ANOVA ($P < 0.05$) followed by Tukey's post-hoc tests ($P < 0.05$). Within the graph, clusters that do not share the same letter and colour have significantly different means.

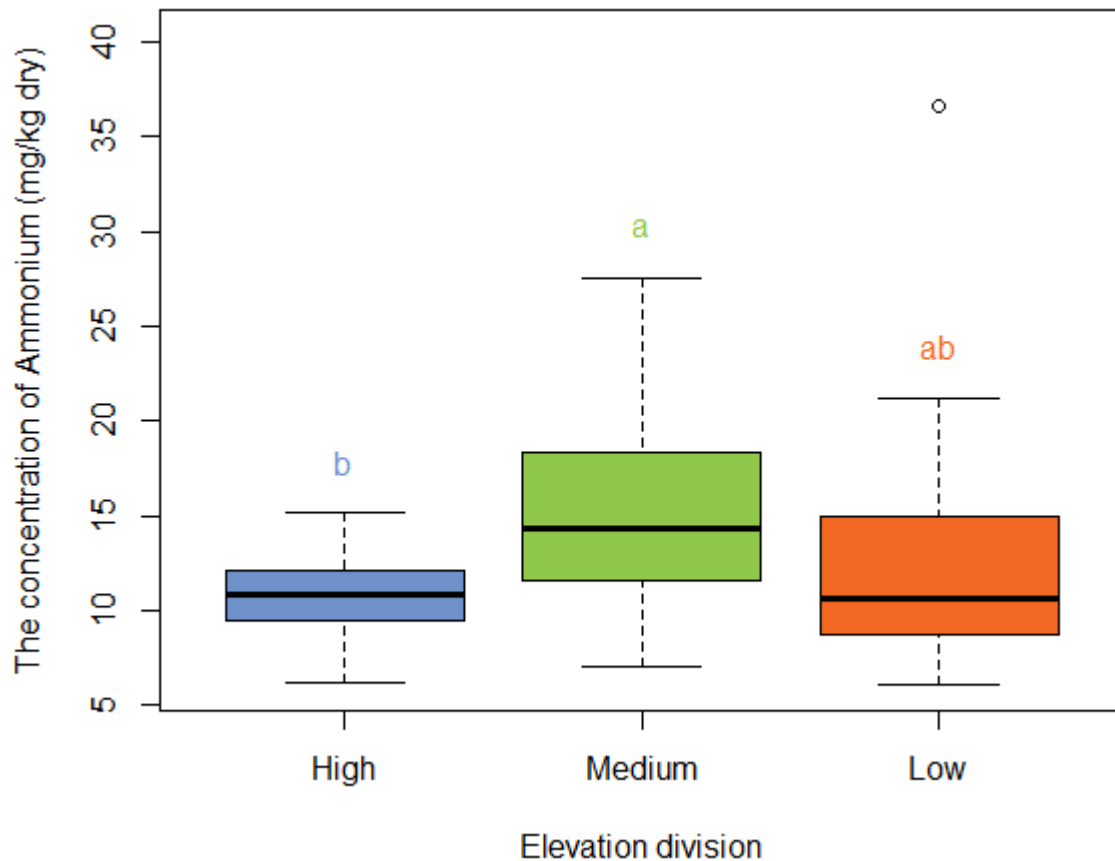


Table B1. Shared generalist OTUs present in all three networks across the whole elevation gradient.

OTU ID	Taxonomy	Trophic states
OTU_99	<i>Basidiomycota; Agaricomycetes; Agaricales; Clavariaceae; Clavaria; Clavaria</i> sp.	Saprotroph
OTU_190	<i>Basidiomycetes; Agaricomycetes; Cantharellales; Clavulinaceae; Clavulina</i>	Saprotroph
OTU_277	<i>Ascomycota; Eurotiomycetes; Chaetothyriales; Herpotrichiellaceae; Cladophialophora; Cladophialophora haetospira</i>	Saprotroph
OTU_50	<i>Ascomycota; Eurotiomycetes; Chaetothyriales; Herpotrichiellaceae; Exophiala</i>	Saprotroph
OTU_98	<i>Ascomycota; Eurotiomycetes; Eurotiales; Thermoascaceae; Paecilomyces</i>	Saprotroph
OTU_148	<i>Ascomycota; Incertae sedis; Incertae sedis; Incertae sedis; Collembolispora; Collembolispora barbata</i>	
OTU_191	<i>Ascomycota; Incertae sedis; Incertae sedis; Incertae sedis; Collembolispora; Collembolispora barbata</i>	
OTU_206	<i>Zygomycota; Incertae sedis; Mortierellales; unidentified; unidentified; Mortierellales</i> sp.	Saprotroph
OTU_7	<i>Zygomycota; Incertae sedis; Mortierellales; unidentified; unidentified; Mortierellales</i> sp.	Saprotroph
OTU_86	<i>Zygomycota; Incertae sedis; Mortierellales; Mortierellaceae; Mortierella; Mortierella amoeboides</i>	Saprotroph
OTU_507	<i>Ascomycota; Leotiomyces; Helotiales; Incertae sedis; Tetracladium; Tetracladium</i> sp.	Saprotroph
OTU_64	<i>Ascomycota; Leotiomyces; Helotiales</i>	Saprotroph
OTU_6475	<i>Ascomycota; Leotiomyces; Thelebolales</i>	Saprotroph
OTU_749	<i>Ascomycota; Leotiomyces; Helotiales</i>	Saprotroph
OTU_382	<i>Ascomycota; Orbiliomyces; Orbiliales; unidentified; unidentified; Orbiliales</i> sp.	Saprotroph
OTU_102	<i>Ascomycota; Sordariomyces; Coniochaetales; Coniochaetaceae</i>	Saprotroph
OTU_309	<i>Ascomycota; Sordariomyces; Coniochaetales; Coniochaetaceae; Lecythophora; Lecythophora</i> sp.	Saprotroph
OTU_128	<i>Ascomycota; Sordariomyces; Hypocreales; Nectriaceae; Neonectria</i>	Biotroph
OTU_1	<i>Basidiomycota; Tremellomyces; Filobasidiales; Filobasidiaceae; Cryptococcus; Cryptococcus terricola</i>	Saprotroph
OTU_32	<i>Basidiomycota; Tremellomyces; Filobasidiales; Filobasidiaceae; Cryptococcus; Cryptococcus terreus</i>	Saprotroph
OTU_119	<i>Basidiomycota; Tremellomyces; Filobasidiales; Filobasidiaceae; Cryptococcus</i>	Saprotroph
OTU_10894	<i>Ascomycota; Pezizomycotina</i>	

OTU_9146	<i>Ascomycota; Pezizomycotina</i>	
OTU_9983	<i>Ascomycota; Pezizomycotina</i>	
OTU_16	<i>Ascomycota; Pezizomycotina</i>	
OTU_359	<i>Fungi incertae sedis; Mortierellomycotina; Mortierellales; Mortierellaceae; Mortierella globulifera</i>	Saprotroph
OTU_832	<i>Ascomycota</i>	

Table B2. The AIC analysis and multi linear regression analysis for all candidate models for the data of fungal community richness. The models were ordered by their $\Delta AICc$ values.

Model	Parameters	AIC analysis				Multi linear regression analysis		
		<i>K</i>	AICc	$\Delta AICc$	<i>w_i</i>	<i>R</i> ²	<i>P</i>	Coefficient (\pm Standard Error)
Soil (<i>Best model</i>)	pH + C + N + C/N ratio + Nitrate + Ammonium + P	9	779.01	0	1	0.44	< 0.001	
	pH						< 0.001	63.52 \pm 17.81
	C						0.51	-24.78 \pm 37.62
	N						0.61	267.60 \pm 515.31
	C/N ratio						0.43	6.70 \pm 8.46
	Nitrate						0.47	1.71 \pm 2.37
	Ammonium						< 0.001	3.81 \pm 0.97
	P						0.48	-0.17 \pm 0.24
	Intercept						0.26	-181.90 \pm 160.75
Full	Elevation*Location + pH + Temperature + Soil Moisture + C + N + C/N ratio + Nitrate + Ammonium + P + Above ground biomass	21	785.83	6.82	0	0.47	< 0.001	
	Elevation						0.97	-0.00092 \pm 0.027
	Location						0.54	-5.86 \pm 9.59
	pH						0.049	42.04 \pm 21.0
	Temperature						0.75	0.47 \pm 1.45
	Soil moisture						0.24	-1.13 \pm 0.96
	C						0.75	-13.39 \pm 41.61
	N						0.70	211.9 \pm 541.6
	C/N ratio						0.48	6.20 \pm 5.76
	Nitrate						0.24	3.08 \pm 2.65

	Ammonium						0.0012	3.64±1.07
	P						0.41	-0.25±0.30
	Above ground biomass						0.50	0.087±0.13
	Elevation*Location						0.72	0.003±0.008
	Intercept						0.77	-56.61±193.2
Climate	Temperature + Soil Moisture	4	793.66	14.65	0	0.21	< 0.001	
	Temperature						0.68	0.56±1.37
	Soil moisture						0.002	-1.36±0.43
	Intercept						< 0.001	300.40±27.90
Site	Elevation*Location	11	798.43	19.42	0	0.17	0.004	
	Elevation						0.40	-0.021±0.025
	Location						0.85	1.79±9.45
	Elevation*Location						0.48	-0.005±0.007
	Intercept						< 0.001	313.65±31.51
Null	Intercept	2	807.51	28.49	0		< 0.001	

Table B3. AIC analysis and multi linear regression analysis for all candidate models for the data of Bray-Curtis dissimilarity of fungal communities. The models were ordered by their $\Delta AICc$ values.

Model	Parameters	AIC analysis				Multi linear regression analysis		
		K	AICc	$\Delta AICc$	w_i	R^2	P	Coefficient (\pm Standard Error)
Soil (<i>Best model</i>)	pH + C + N + C/N ratio + Nitrate + Ammonium + P	9	-325.12	0	0.99	0.30	< 0.001	
	pH						0.52	-0.010 \pm 0.015
	C						0.29	0.034 \pm 0.032
	N						0.27	-0.48 \pm 0.43
	C/N ratio						0.14	-0.011 \pm 0.0071
	Nitrate						0.87	0.0003 \pm 0.002
	Ammonium						< 0.001	-0.004 \pm 0.008
	P						0.34	0.0002 \pm 0.0002
	Intercept						< 0.001	1.08 \pm 0.14
Null	Intercept	2	-313.62	11.50	0		< 0.001	0.84 \pm 0.004
Full	Elevation*Location + pH + Temperature + Soil Moisture + C + N + C/N ratio + Nitrate + Ammonium + P + Above ground biomass	15	-311.27	13.86	0	0.33	0.01	
	Elevation						0.77	0.000007 \pm 0.00002
	Location						0.61	0.004 \pm 0.008
	pH						0.92	0.002 \pm 0.018
	Temperature						0.68	0.0005 \pm 0.001
	Soil moisture						0.88	0.0001 \pm 0.0008
	C						0.37	0.03 \pm 0.04
	N						0.33	-0.45 \pm 0.46

	C/N ratio						0.17	0.01±0.007
	Nitrate						0.76	-0.0007±0.002
	Ammonium						< 0.001	-0.004±0.0009
	P						0.49	0.0002±0.0003
	Above ground biomass						0.89	0.00002±0.0001
	Elevation*Location						0.97	-0.0000003±0.000006
	Intercept						< 0.001	0.99±0.17
Climate	Temperature + Soil Moisture	4	-309.45	15.67	0	0.003	0.9	
	Temperature						0.75	-0.0004±0.001
	Soil moisture						0.65	-0.0002±0.0004
	Intercept						< 0.001	0.85±0.024
Site	Elevation*Location	5	-308.53	16.59	0	0.02	0.68	
	Elevation						0.83	-0.000004±0.00002
	Location						0.58	-0.004±0.008
	Elevation*Location						0.49	0.000004±0.000006
	Intercept						< 0.001	0.85±0.026

APPENDIX C

**Use of ‘inferred metagenomics’ confirms soil
bacterial communities exhibit functional
biogeography across a mountain elevation gradient**

Figure C1. Variation in bacterial community composition across a mountain elevation and aspect gradient based on the analysis of the total soil 16S rRNA gene data. The non-metric multidimensional scaling (nMDS) plot was derived from a Bray-Curtis matrix of the data. The size of points increases with elevation (legend shows elevation above sea level in metres). The colours of points are assigned based on the aspect difference at each elevation (legend indicates points collected from the ridge (R), sunny (SU) and shady (SH) aspects of the mountain ridge).

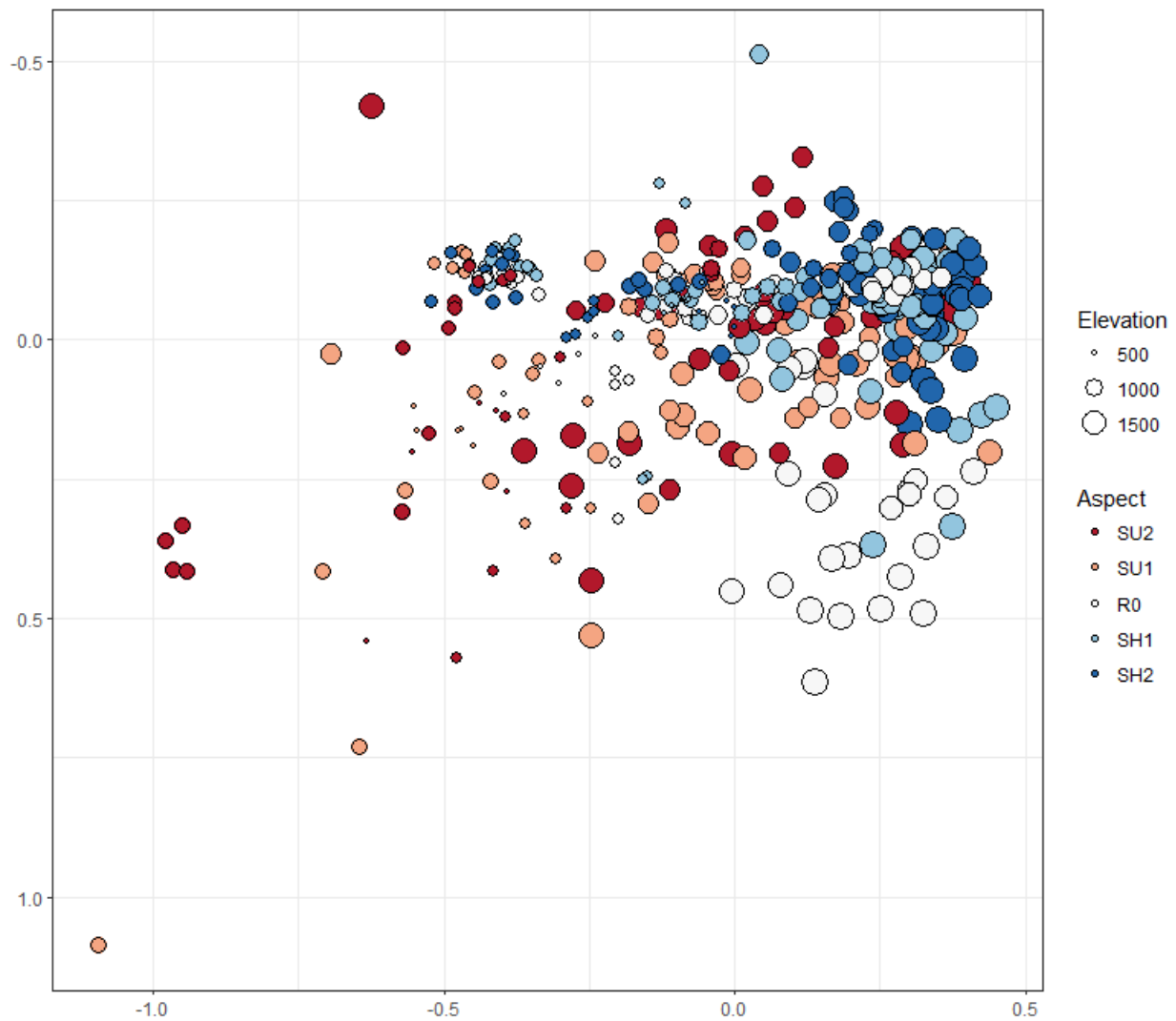


Figure C2. Scatter plot showing the average Bray-Curtis (dis)similarity comparing sample data, for which whole genome sequence is available, separated by different elevational distance. The linear trendline for the data is $y = 0.0002x + 0.1657$ ($R^2 = 0.96$).

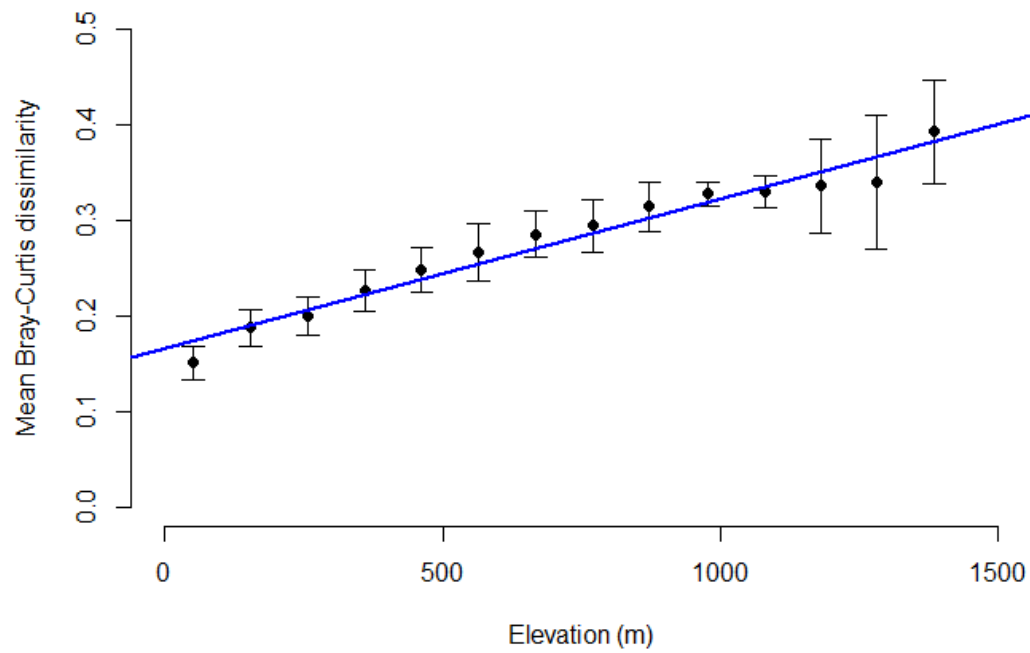


Figure C3. Spearman's correlation heatmap showing the extent of correlation among overall community genome size, elevation gradient and the data of both climatic factors and soil chemistry. The colour gradient from red to blue represents proportionally negative to positive correlation. The Spearman's correlations with non-significant p -value (p -value < 0.05) have been left blank in the heatmap.

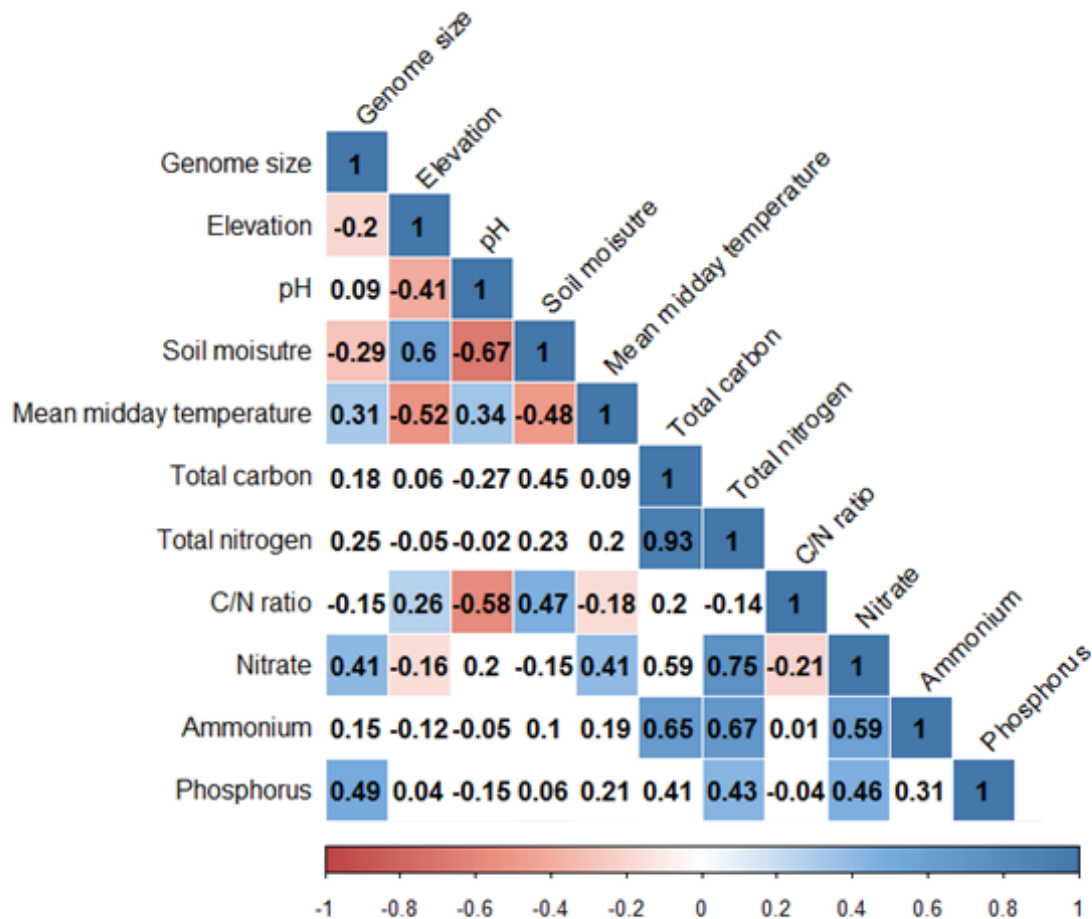


Figure C4. Venn diagram showing the number of inferred genomes shared among different aspects across the whole study site. The ovals with the prefix SU represent sample data from the sunny aspect, with the prefix SH from the shady aspect and with the prefix R, from the ridge. No genome was shared only between aspects R0 and SH2, or between R0 and SH1.

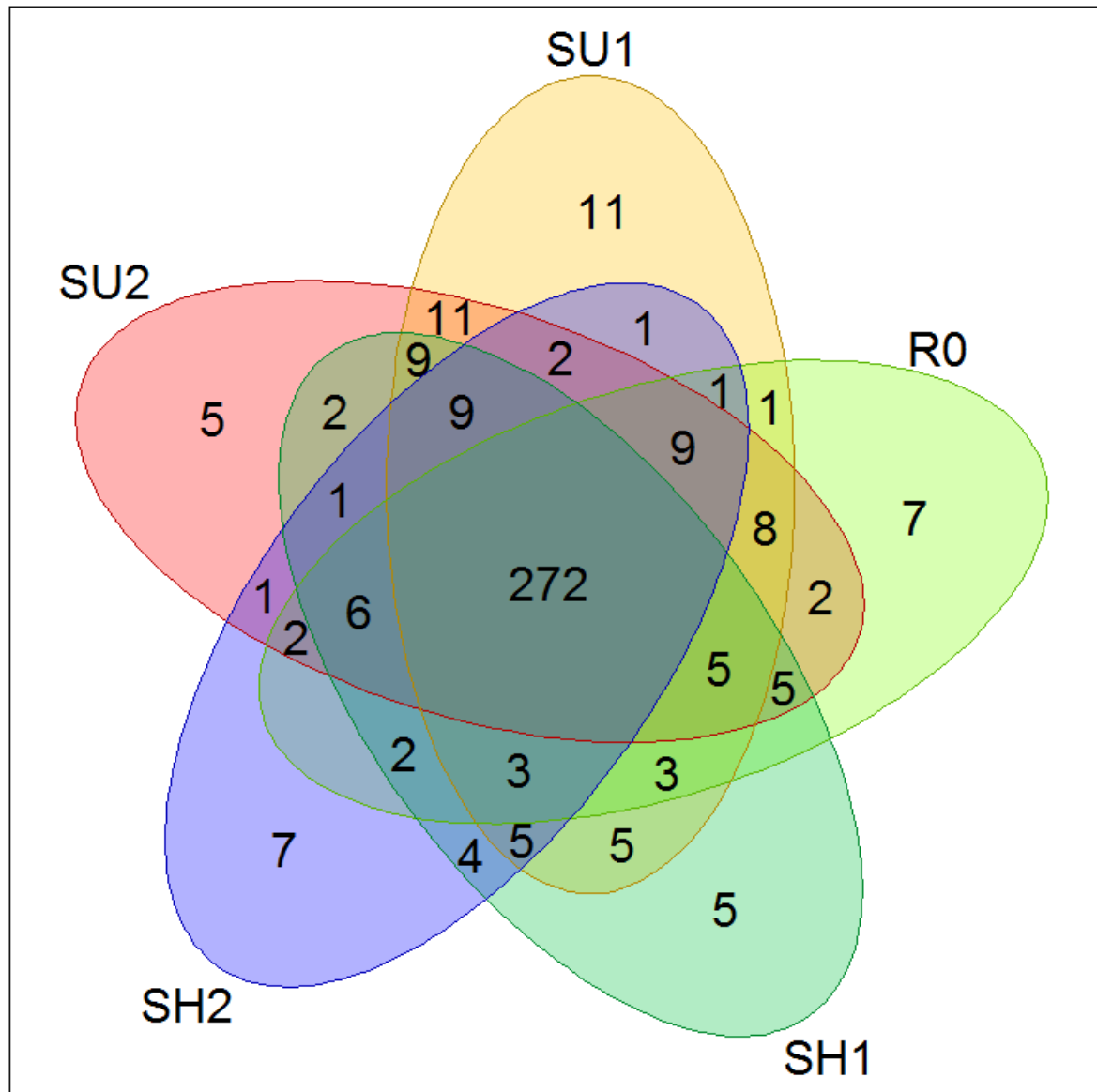
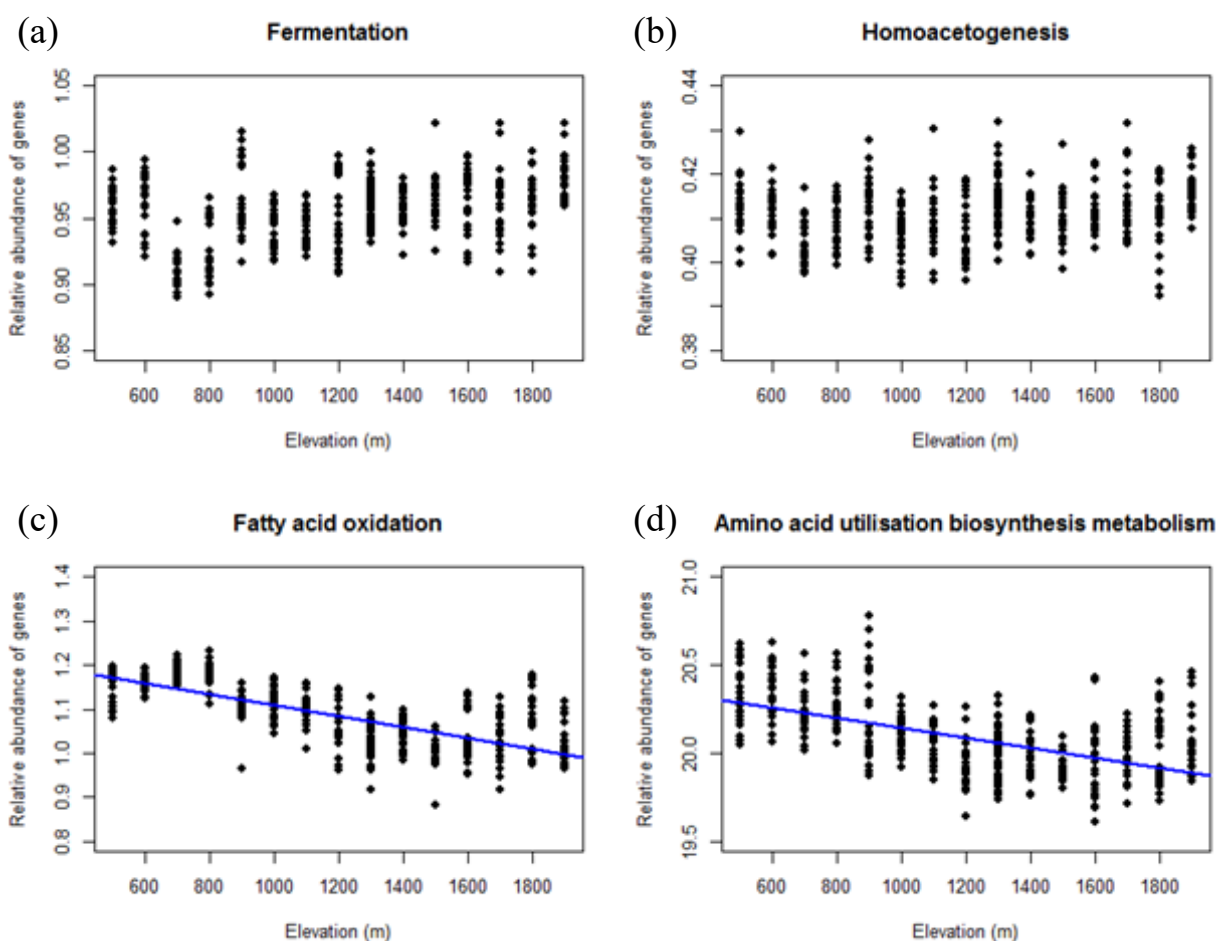
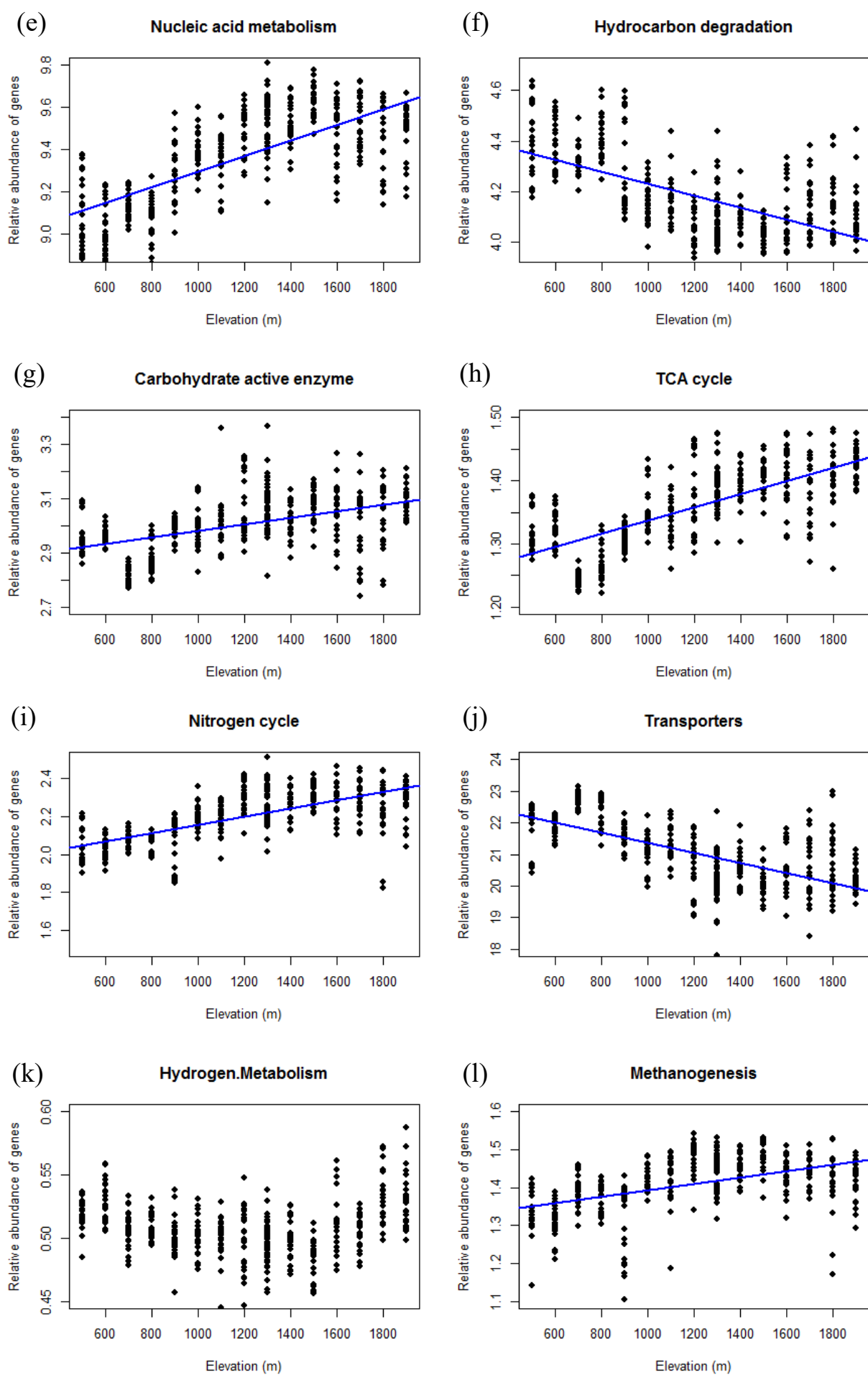


Figure C5. Scatter plots illustrating the relationships between elevation and the relative abundance of functional genes relevant to (a) fermentation, (b) homoacetogenesis, (c) fatty acid oxidation, (d) amino acid utilisation biosynthesis metabolism, (e) nucleic acid metabolism, (f) hydrocarbon degradation, (g) carbohydrate active enzyme, (h) TCA cycle, (i) nitrogen cycle, (j) transporters, (k) hydrogen metabolism, (l) methanogenesis, (m) methylotrophy, (n) Embden Meyerhof-Parnas (EMP), (o) gluconeogenesis, (p) sulfur compounds metabolism, (q) saccharide and derivated synthesis, (r) hydrolysis of polymers, and (s) cellular response to stress. Significant regressions with p -value < 0.001 and adjusted $R^2 > 0.2$ are drawn with a blue line showing the best-fit linear regression. The linear trendlines for specific functional genes are as follows: (c) fatty acid oxidation: $y = -0.0001x + 1.23$, $R^2 = 0.51$, (d) amino acid utilisation biosynthesis metabolism: $y = -0.0003x + 20.43$, $R^2 = 0.31$, (e) nucleic acid metabolism: $y = 0.0004x + 8.93$, $R^2 = 0.45$, (f) hydrocarbon degradation: $y = -0.0002x + 4.47$, $R^2 = 0.37$, (g) carbohydrate active enzyme: $y = 0.0001x + 2.86$, $R^2 = 0.22$, (h) TCA cycle: $y = 0.0001x + 1.23$, $R^2 = 0.46$, (i) nitrogen cycle: $y = 0.0002x + 1.94$, $R^2 = 0.43$, (j) transporters: $y = -0.0016x + 22.98$, $R^2 = 0.43$, (l) methanogenesis: $y = 0.00008x + 1.31$, $R^2 = 0.22$, (m) methylotrophy: $y = 0.00008x + 1.3$, $R^2 = 0.22$, (p) sulfur compounds metabolism: $y = 0.00006x + 1.36$, $R^2 = 0.22$, (q) saccharide and derivated synthesis: $y = 0.0003x + 5.03$, $R^2 = 0.38$, and (s) cellular response to stress: $y = 0.0009x + 1.74$, $R^2 = 0.42$.





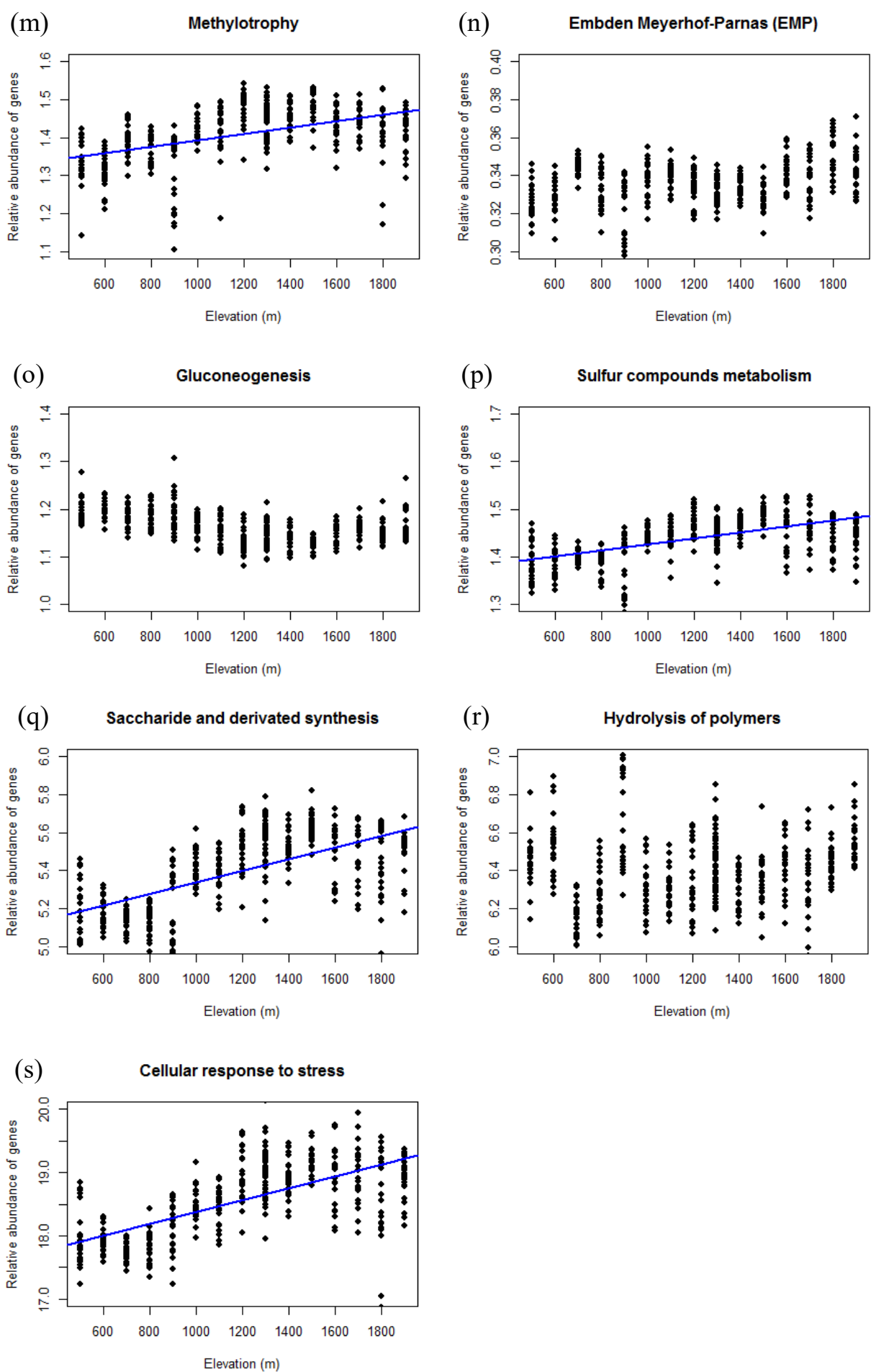


Figure C6. Relative abundances of specific groups of genes categories from three functional categories, ‘nitrogen cycle’, ‘cellular response to stress’ and ‘transporters’, that are more or less abundant in soils collected at low elevation (from 900 m to 500 m) than in soils from higher elevation (> 900 m). Only those gene categories that are significantly different between the two elevational divisions of samples (Bonferroni corrected p -value < 0.05) are shown here. The x axis shows the relative percentage difference in the abundance compared with the samples collected at low elevation (≤ 900 m). The elevation gradient was assigned a range of colours from light blue (1000 m) to violet (1936 m).

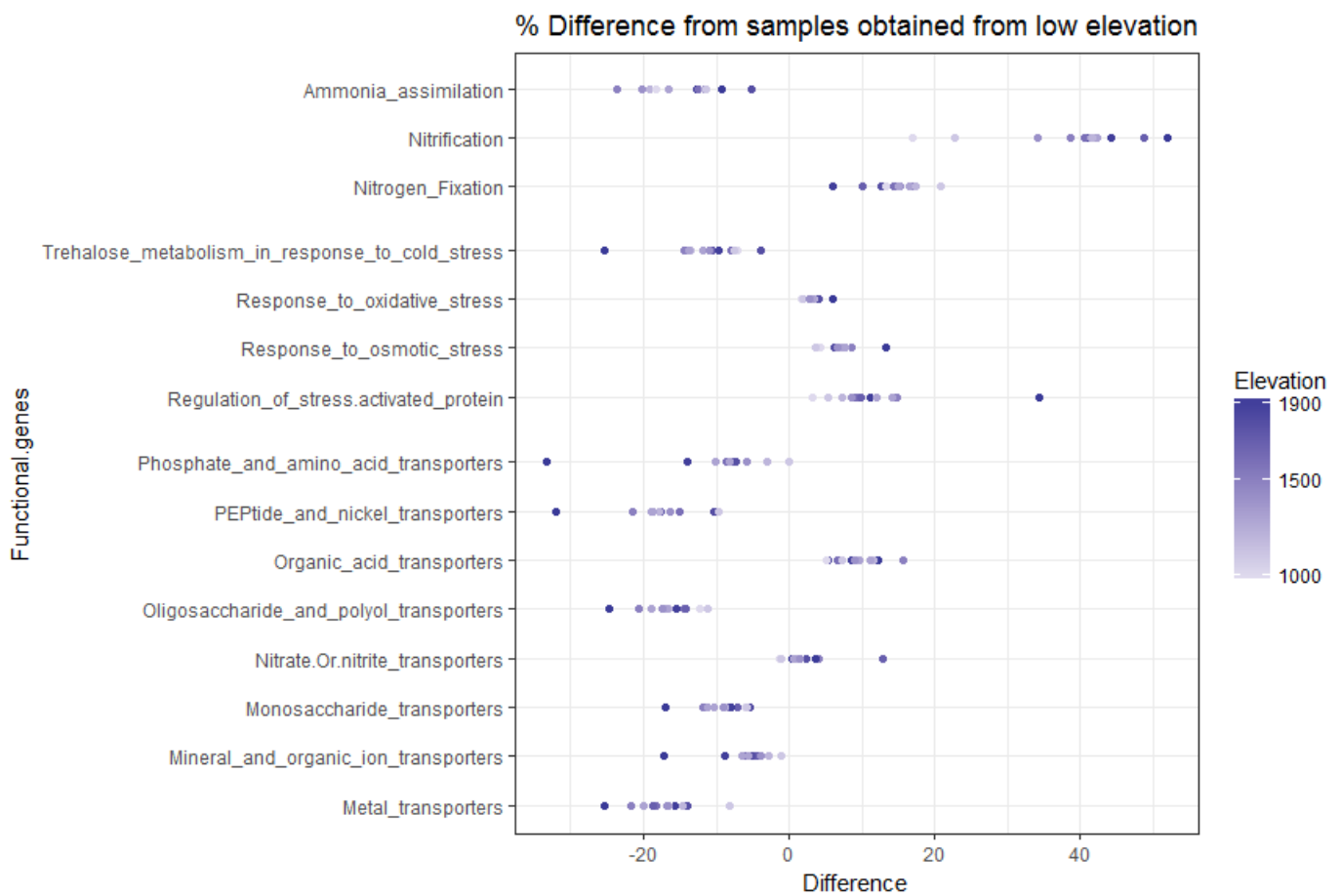


Table C1. AIC and mixed effect model analyses for all candidate models for genome size data observed from 400 taxa for which whole genomic information was available. One variable, the genus information of each OTU, was identified as a random effect in the model. The models were ordered by their ΔAICc values.

Model	Parameters	AIC analysis					Mixed effect model analysis		
		<i>K</i>	AICc	ΔAICc	w_i	Log-likelihood	Coefficient (\pm Standard Error)	<i>P</i>	χ^2
Elevation (<i>Best-fitting model</i>)	Elevation	4	12623.18	0	0.73	-6307.54	61650 (12659.3)	< 0.001	23.84
	Intercept						3981341 (192676.9)	< 0.001	
Both elevation and aspect	Elevation + Aspect	5	12625.22	2.05	0.26	-6307.54	62938 (21665.4)	< 0.001	8.5
	Elevation						-7511 (102507.4)	< 0.001	0.005
	Aspect						3752921 (390969.1)	< 0.001	
Aspect	Intercept								
	Aspect	4	12631.59	8.41	0.01	-6311.74	233923 (60517.4)	< 0.001	15.02
	Intercept						3696939 (284609.1)	< 0.001	

Table C2. Table comparing the number of OTUs and relative abundance of the two most abundant species (*Bradyrhizobium* sp. S23321 and *Candidatus Solibacter usitatus* Ellin6076) and two species with small genome size (*Candidatus Tremblaya princeps* and *Microbacterium testaceum* StLB037) across the whole elevation gradient from 1936 m 500 m.

Elevation (m)	<i>Bradyrhizobium</i> sp. S23321 (7.2 Mb)		<i>Candidatus Solibacter usitatus</i> Ellin6076 (10.0 Mb)		<i>Candidatus Tremblaya princeps</i> (0.1 Mb)		<i>Microbacterium testaceum</i> StLB037 (4.0 Mb)	
	Number of OTUs	% abundance of OTUs	Number of OTUs	% abundance of OTUs	Number of OTUs	% abundance of OTUs	Number of OTUs	% abundance of OTUs
1936	641	5.6	1583	14.1	0	0	4	0.04
1900	5806	10.0	6374	10.9	0	0	33	0.05
1800	6874	11.3	6086	9.9	0	0	11	0.02
1700	6384	11.2	5609	9.8	0	0	17	0.03
1600	6658	11.6	5955	10.5	0	0	3	0.005
1500	6838	13.5	5534	10.9	0	0	1	0.002
1400	7247	13.7	5430	10.3	0	0	10	0.02
1301	6859	12.6	5676	10.4	0	0	9	0.02
1300	7504	13.1	5936	10.4	0	0	17	0.03
1200	8662	16.5	6097	11.4	0	0	15	0.03
1100	9290	16.2	4829	8.5	1	0.002	49	0.08
1000	8429	15.8	4436	8.4	0	0	19	0.03
900	6851	11.1	3371	5.3	6	0.006	271	0.3
800	8352	15.6	2533	4.6	1	0.002	74	0.1
700	9554	18.0	1995	3.8	1	0.002	117	0.2
600	7891	11.5	4295	6.1	1	0.002	52	0.07
500	7762	12.2	4045	6.4	65	0.1	150	0.2

Table C3. PERMANOVA comparison of the average genome size of subsets of bacterial communities derived from 16S rRNA gene data for which genomic information is available, comparing data collected at different elevations and aspects. There were 16 elevational sampling locations along the mountain, and five aspects at each elevation (SU1, SU2, SH1, SH2, and R0).

Source of variation	df	MS	Psuedo-F	R²	P (perm)
Elevation	16	5083	24	0.39	< 0.01
Aspect	4	2893	14	0.06	< 0.01
Elevation x Aspect	60	795	4	0.23	< 0.01
Residual	320	211			

Table C4. Number of total genomes and unique genomes obtained from each elevation (from 1936 m to 500 m).

Elevation (m)	Number of total genomes	Number of unique genomes
1936	218	0
1900	266	6
1800	280	2
1700	280	0
1600	258	1
1500	235	1
1400	244	1
1301	252	0
1300	281	0
1200	259	2
1100	257	0
1000	252	0
900	318	13
800	284	1
700	267	1
600	261	1
500	298	4

Table C5. PERMANOVA results comparing the relative abundance of 19 major functional categories between samples at different elevations and aspects. There were 16 elevational sampling locations along the mountain, and five aspects sampled at each elevation (SU1, SU2, SH1, SH2, and R0).

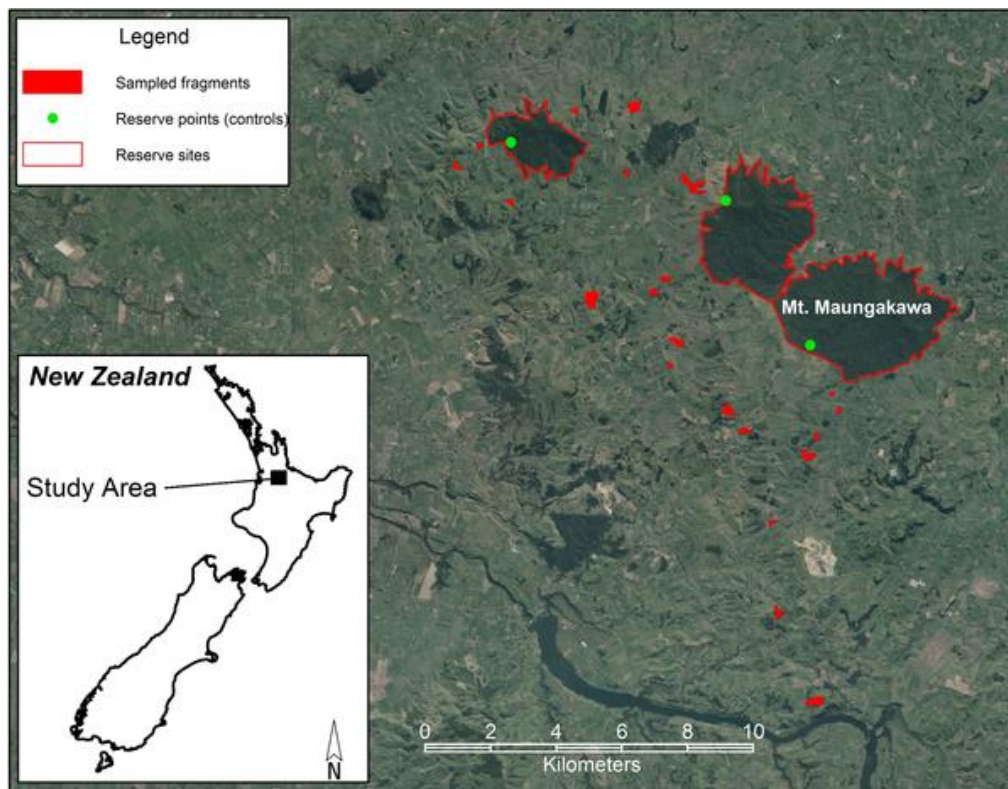
Source of variation	df	MS	Psuedo-F	R ²	<i>P</i> (perm)
Elevation	16	171	27	0.41	< 0.01
Aspect	4	64	10	0.04	< 0.01
Elevation x Aspect	60	27	4	0.25	< 0.01
Residual	320	6			

APPENDIX D

**Livestock exclusion reduces the spillover effects of
pastoral agriculture on soil microbial communities in
adjacent forest fragments**

Figure D1. (a) Locations of the study farms and natural reserves. Sampling sites located in the Waikato region, New Zealand. Reprinted from “Agricultural Intensification Exacerbates Spillover Effects on Soil Biogeochemistry in Adjacent Forest Remnants”, by Didham RK, Barker GM, Bartlam S, Deakin EL, Denmead LH, et al. (2015). PLOS ONE 10(1): e0116474. <https://doi.org/10.1371/journal.pone.0116474>. (b) Samples were collected along a transect line from the interior of grazed pasture (-46.5 m) to the interior of forest (46.5 m) in each farm with or without a fence on the boundary between different land use types.

(a)



(b)

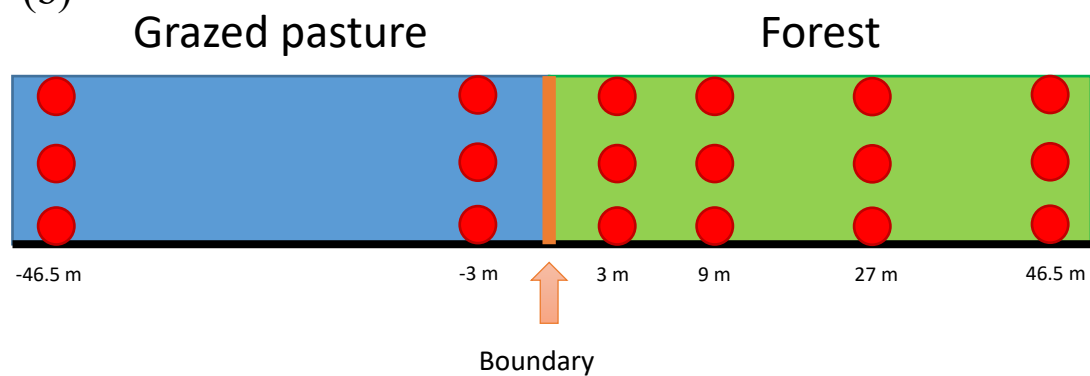


Figure D2. Boxplot showing variation in average sample-level richness derived from different land types (i.e., pasture, forest and native forest reserve) and different fence categories (i.e., fenced versus unfenced) based on the richness of OTUs, rarefied to 5100 sequences per sample. The horizontal line within the box corresponds to the median value with 25th – 75th percentiles as the box limits. The whiskers extend to the minimum and maximum data point, but to no more than 1.5-fold interquartile range. Statistical differences in the data between pasture and forest were analysed by Student's *t*-test (fenced farms, $p = 0.008$, average richness in forest = 1053.5 ± 31.9 (mean \pm standard error), in pasture = 1178.3 ± 32.1 ; unfenced farms, $p = 0.49$, average richness in forest = 1118.3 ± 25 , in pasture = 1145.9 ± 30.3 ; and average richness in reserve = 1072.2 ± 29.2).

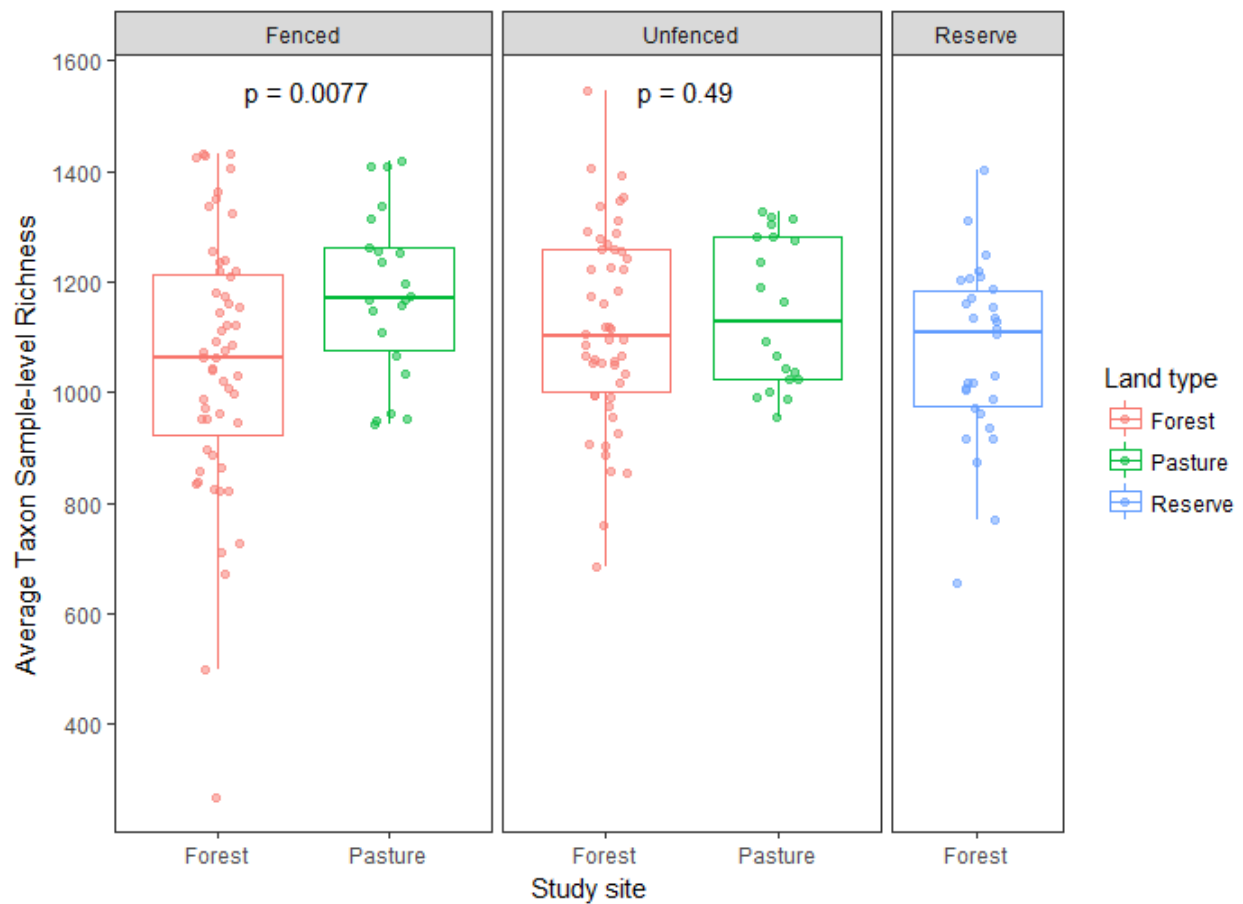


Figure D3. Heatmap showing variation in relative bacterial taxon richness per sample across farm transects based on the richness of OTUs. Different columns represent data collected from different farms; different rows represent data from different sites along the transect line. Sample data are assigned colours across a gradient from red (highest average richness) to blue (lowest average richness). The sites with no data are shown in grey. The black dashed line represents where the fence is along the transect line. The colour bar at the top of the heatmap shows the land type, that is, fenced farm (black), unfenced farm (red) and forest reserve (green). The farms within each fence category (i.e., fenced or unfenced) are ordered (left to right) by increasing mean richness across the study site.

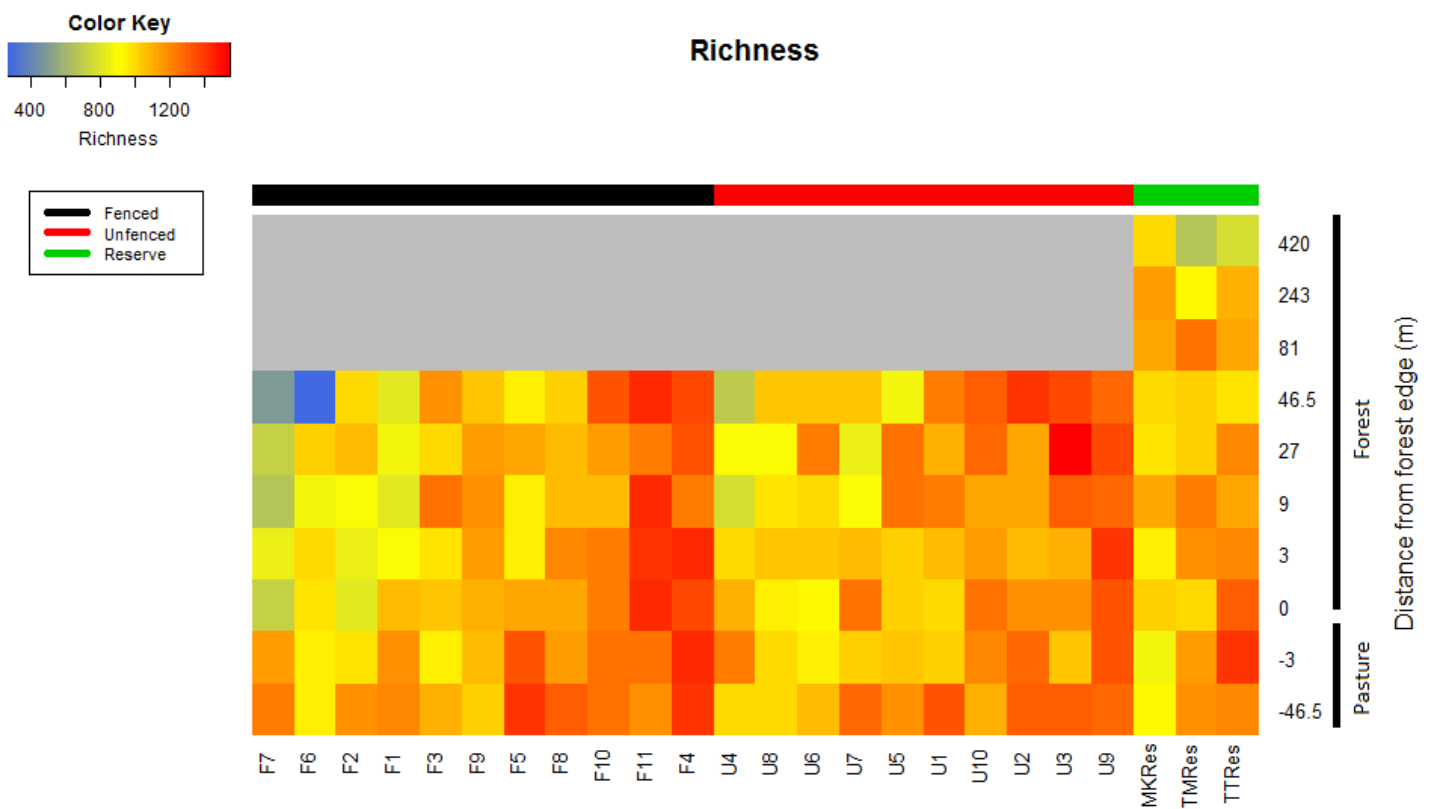


Figure D4. Variation in the composition of bacterial sequence data and their correlation with various environmental factors (represented by continuous arrows). Plot is a metric multidimensional scaling (nMDS) plot constructed using Bray-Curtis dissimilarity matrix derived from 16S rRNA gene data grouped into operational taxonomic groups at 97% DNA sequence similarity. The colour of points is assigned based on their land types (i.e., forest, pasture or natural forest reserve). The shape of points represents the presence of a fence (i.e., (●) fenced farm, (■) unfenced farm or (▲) forest reserve). Only significant environmental factors were overlaid onto nMDS coordinates. Significant environmental factors were selected using a *stepwise* model with AIC criterion (for results see Appendix D Table D2, in all cases, p -value < 0.05). We eliminated four data outliers from the MDS plot. These were samples collected at 46.5 m from the fenced farm F6 ($n = 3$) and F7 ($n = 1$) using the farm ID codes from Didham et al. (2015).

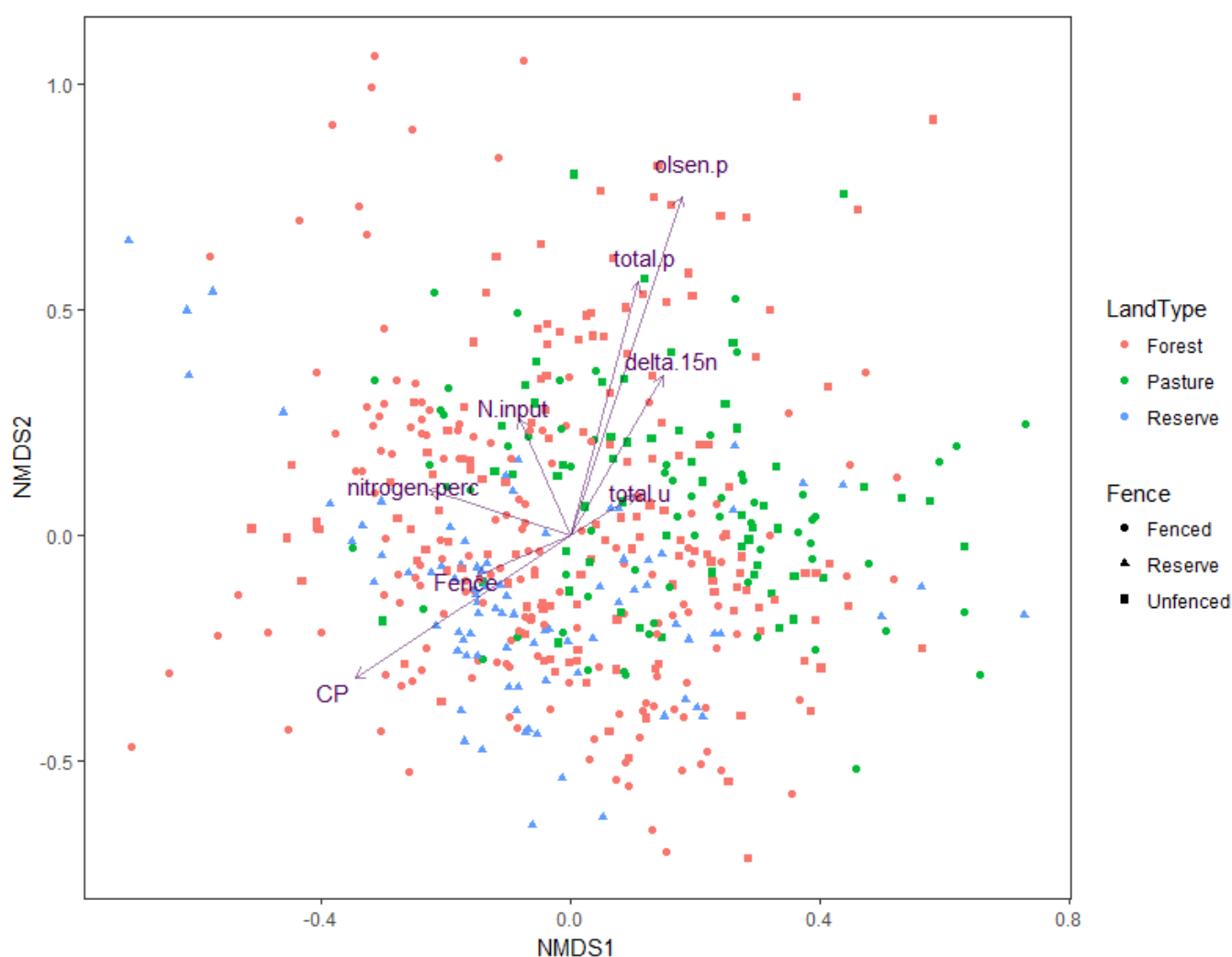
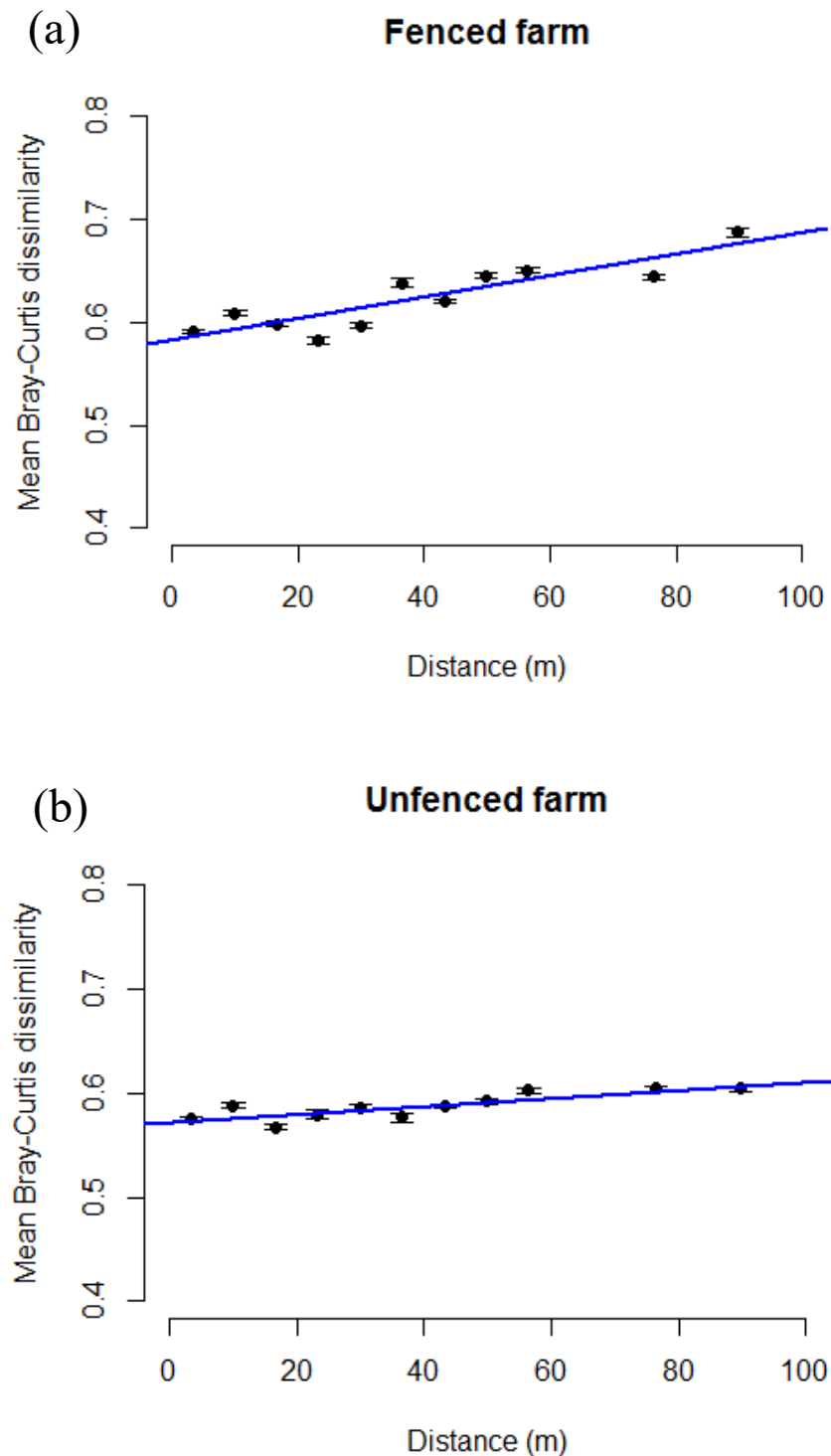


Figure D5. Scatter plots showing the average decline in bacterial community similarity, measured from Bray-Curtis dissimilarity scores for sample data collected from (a) fenced sites, (b) unfenced sites and (d) natural reserves. Error bars for each point indicate standard deviations in Bray-Curtis dissimilarity across different distances. Linear trend lines for the data are (a) fenced farm: $y = 0.001x + 0.583$ ($R^2 = 0.79$), (b) unfenced farm: $y = 0.00038x + 0.573$ ($R^2 = 0.70$), and (c) natural reserve: $y = 0.00032x + 0.491$ ($R^2 = 0.81$).



(c)

Natural reserve

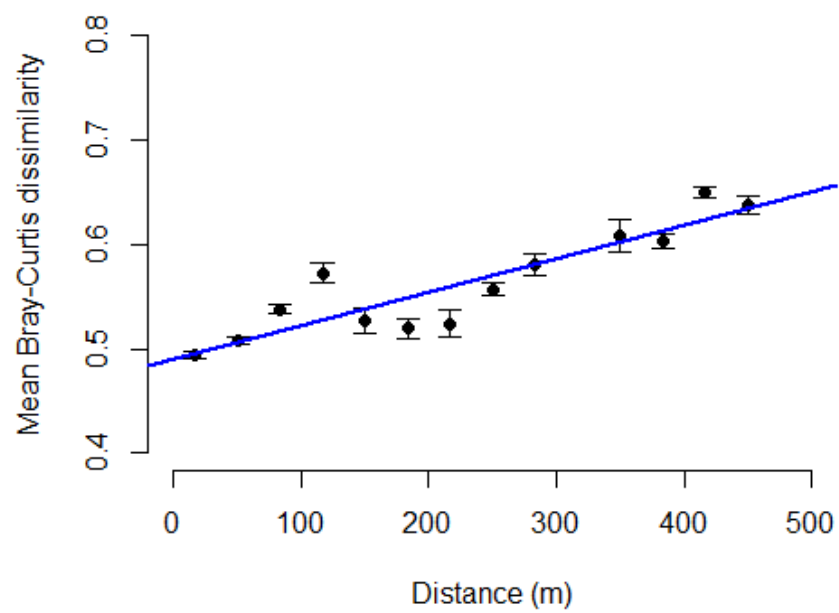


Figure D6. Boxplot showing the number of unique or shared OTUs obtained from different land uses (i.e., pasture and forest) across the sampling line within each individual farm bounded to the adjacent forest with or without a fence. The data were rarefied to 5100 sequences per sample based on OTUs. The horizontal line within the box corresponds to the median value, with 25th – 75th percentiles as the box limits. The whiskers extend to the minimum and maximum data point no more than 1.5-fold interquartile range. The colour of the boxes is assigned based on the presence of a fence on the land use boundary of each farm. Statistical differences in the data between pasture and forest were analysed by Student's *t*-test ($p < 0.05$). Data groups with the significantly different *p*-values ($p < 0.05$) have significantly different means.

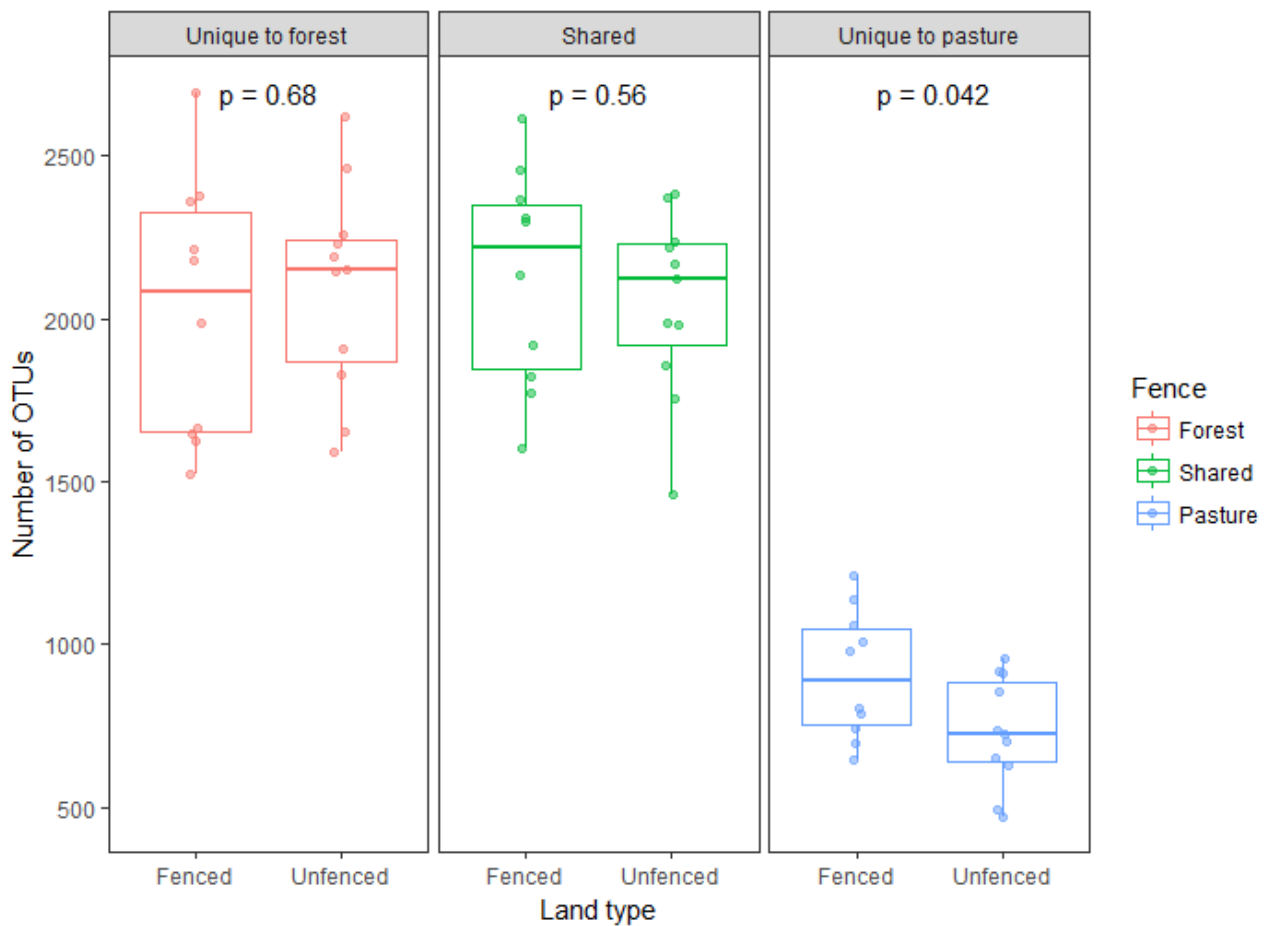


Figure D7. Stacked bar chart showing the average relative abundance (%) of the top 20 dominant phyla in samples along the transect line (from -46.5 m to 46.5 m) obtained from three different land types (i.e., unfenced farm, fenced farm, and natural forest reserve). Other phyla or DNA sequences from unidentified taxa are annotated 'other'. Daggers (†) indicate phyla which were best predictors of soil bacterial composition as suggested by the results of a distance-based linear model (distLM) in Figure 5.3.

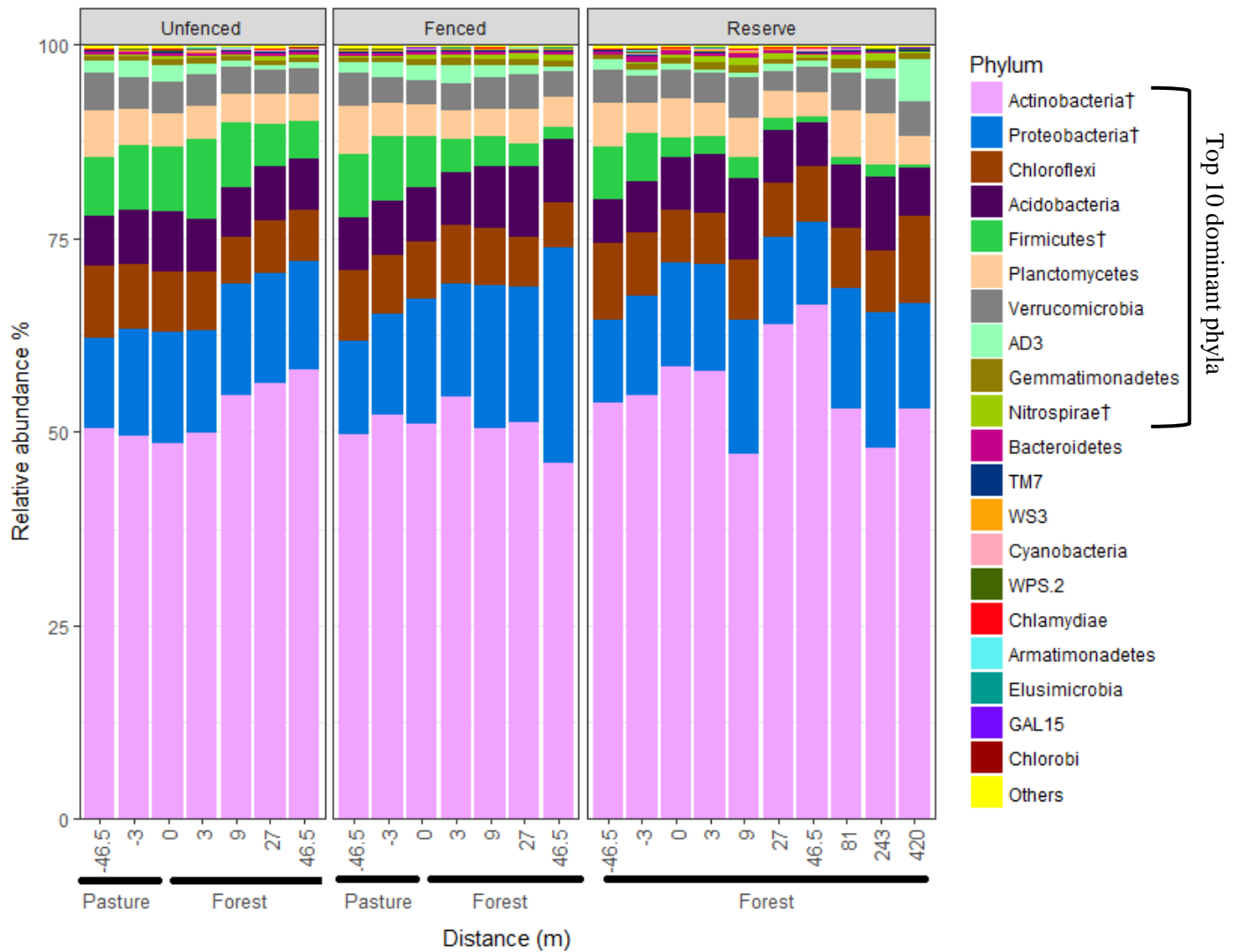
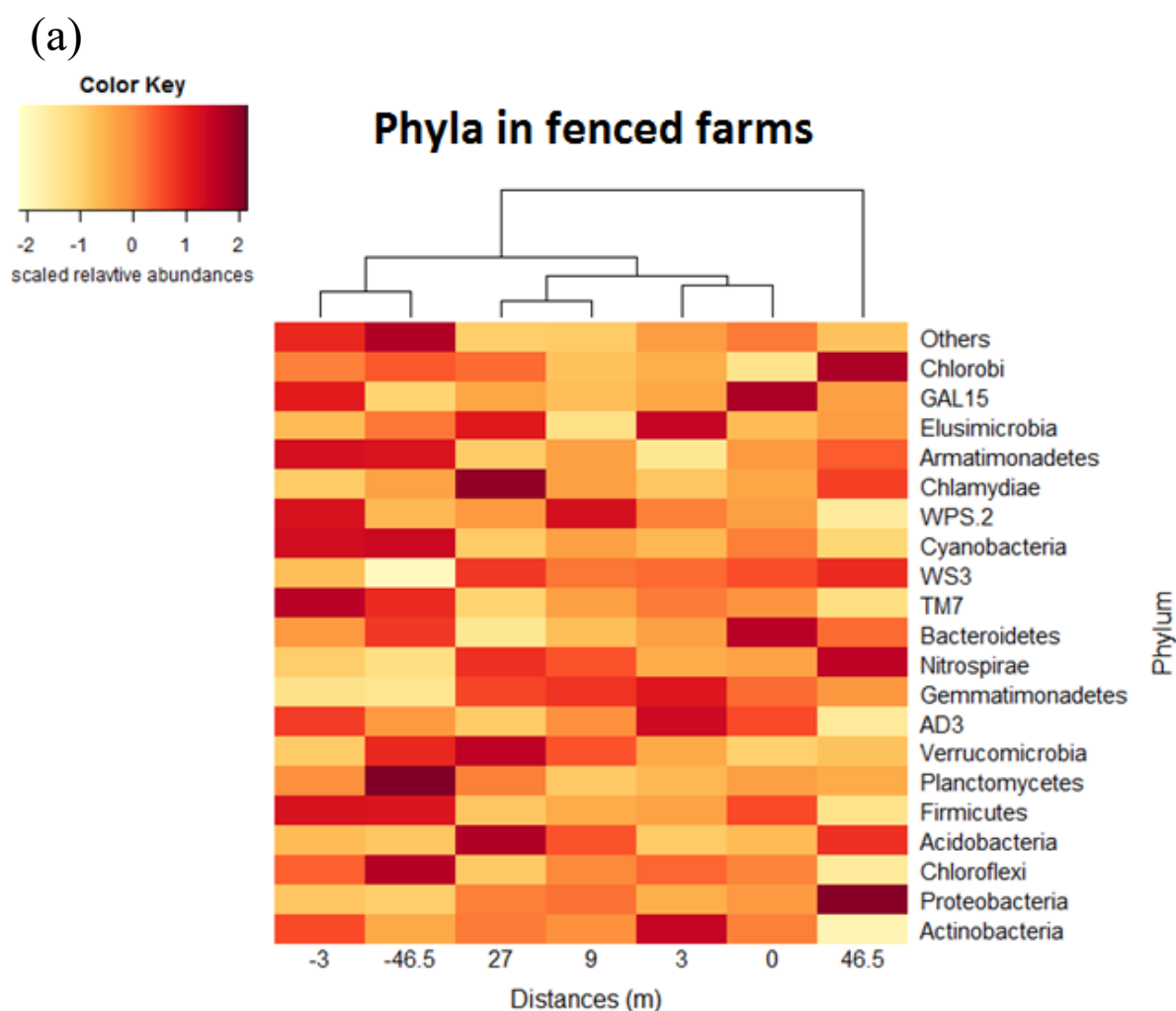
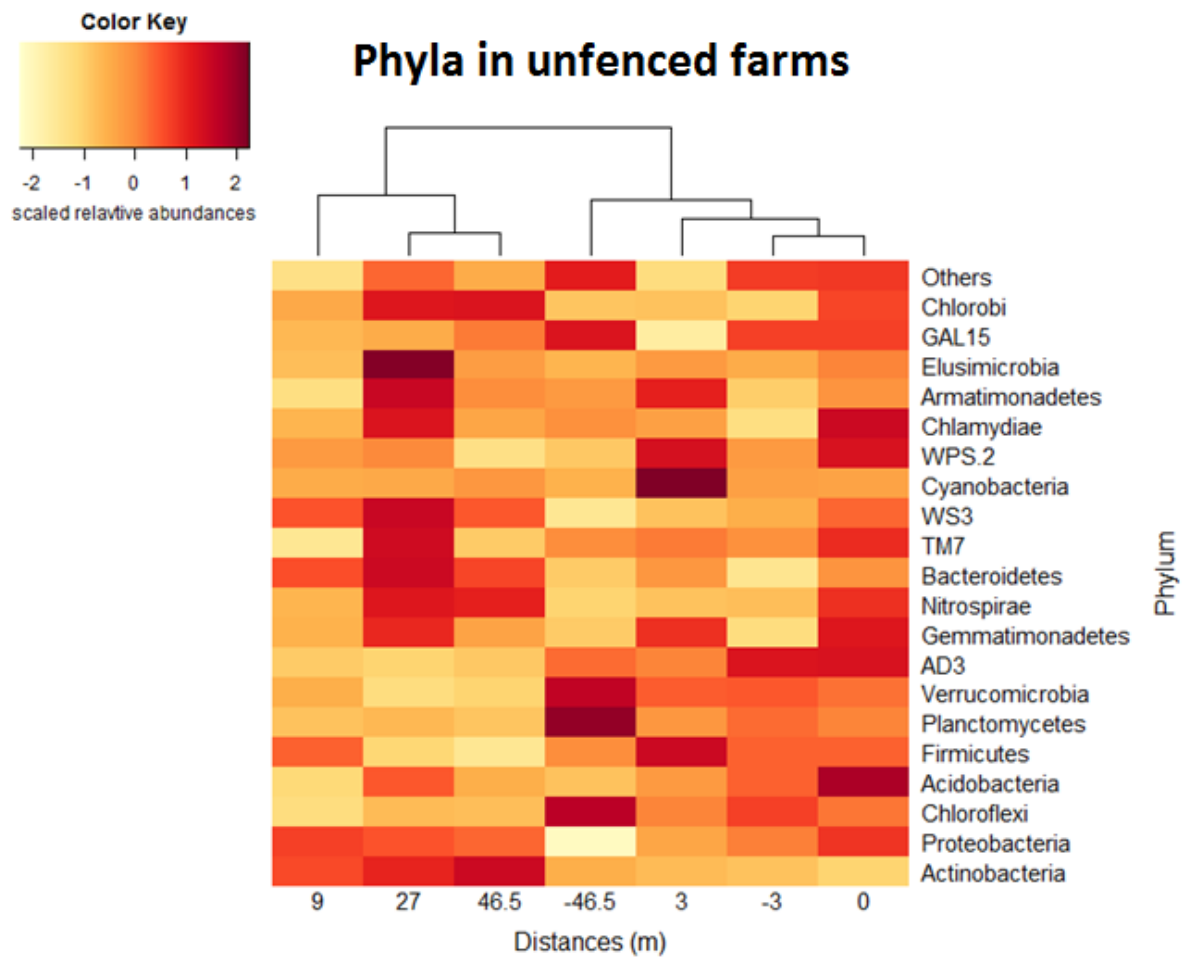


Figure D8. Variation in the average relative abundance of the top 20 phyla obtained across sampling transects from (a) fenced farms, (b) unfenced farms, and (c) natural reserves. Different columns represent data collected from different distances from land use boundary (0 m); different rows represent data from different phyla. To reduce the impact of extreme values, the data was scaled to have mean zero and standard deviation, while assigning the range of colours in the heatmap. Therefore, the data were assigned colours across a gradient from yellow (relative lowest abundance) to red (relative highest abundance) based on their scaled relative abundance. The dendrogram was computed and reordered based on the mean of relative abundance at each distance.



(b)



(c)

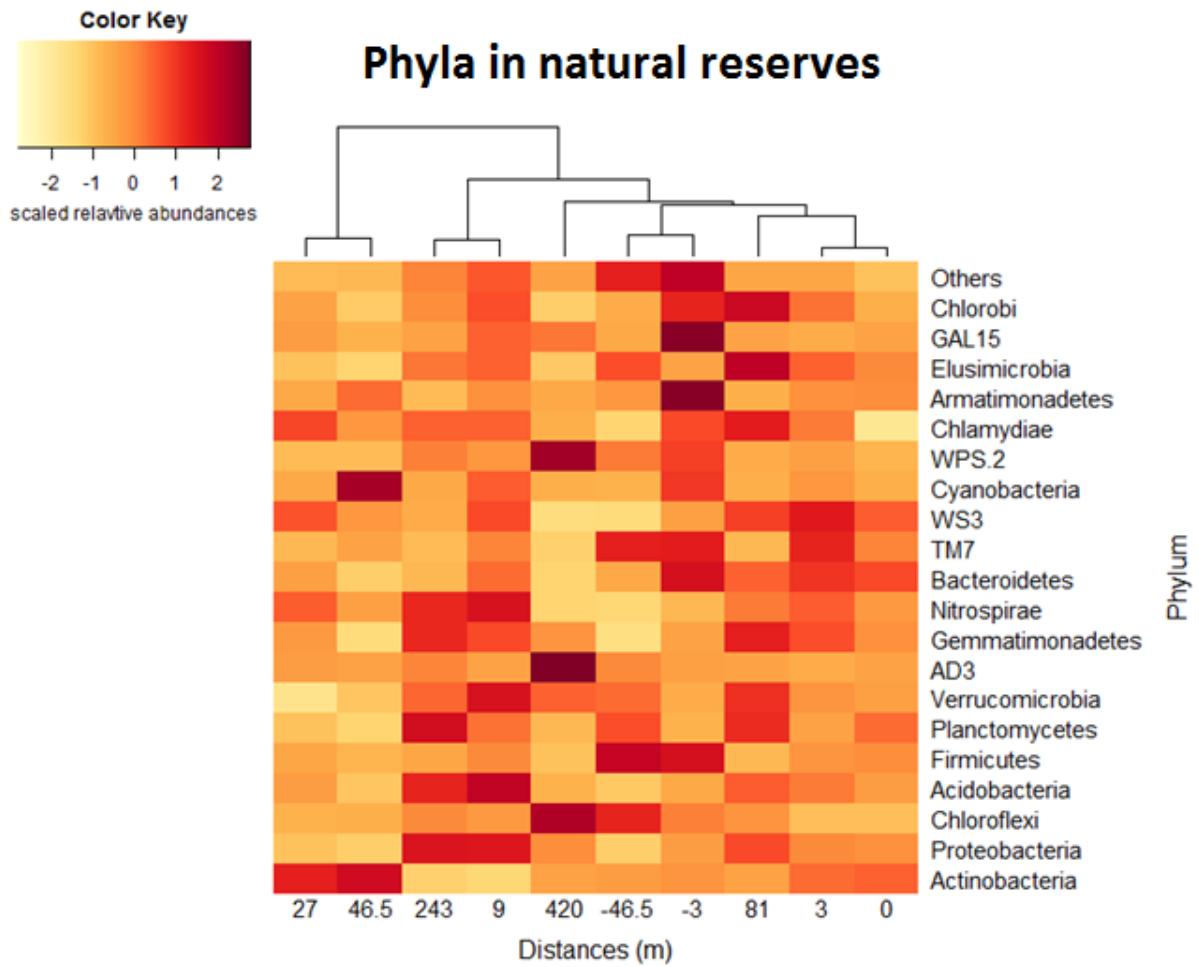


Table D1. Results of (a) linear regression and (b) ANOVA to examine changes in bacterial community richness along sampling lines in different farms, transecting across pasture and into adjacent forest land uses and with land use boundaries either separated with or without a fence. The coefficient for distance indicates that for every additional meter into forest, we could expect the OTU richness to decrease by an average of 2 units.

(a) Linear regression

Data	Parameters	Linear model analysis			
		R ²	P	Coefficient (± Standard Error)	P
Richness (Fenced farm)	~ Distance + Farm	0.536	< 0.001*		
	Intercept			976.72 (37.48)	< 0.001*
	Distance			-2.04 (0.43)	< 0.001*
	FarmF1†			8.81 (52.92)	0.868
	FarmF3			106.91 (52.92)	0.045*
	FarmF4			395.48 (52.92)	< 0.001*
	FarmF5			154.05 (52.92)	0.004*
	FarmF6			-109.05 (52.92)	0.041*
	FarmF7			-164.74 (54.34)	0.003*
	FarmF8			166.78 (53.57)	0.002*
	FarmF9			135.76 (52.92)	0.044*
	FarmF10			252.29 (52.92)	< 0.001*
	FarmF11			371.24 (52.92)	< 0.001*

- **Asterisks (*):** The *p*-value is significant (*p*-value < 0.05).
- **Dagger (†):** The numeric identifier for each farm (e.g. FarmF7) was taken from Didham et al (2015)

(b) ANOVA

Data	Parameter	Df	Sum Sq	Mean Sq	F	P
Richness (Fenced farm)	~ Distance + Farm					
	Distance	1	784653	784653	26.69	< 0.001*
	Farm	10	6561274	656127	22.32	< 0.001*
	Residuals	216	6350462	29400		
Richness (Unfenced farm)	~ Distance + Farm					
	Distance	1	32792	32792	1.44	0.232
	Farm	9	2195447	243939	10.69	< 0.001*
	Residuals	183	4176934	22825		
Richness (Overall dataset)	~ Distance + Fence / Farm					
	Distance	1	596626	596626	22.29	< 0.001*
	Fence	1	60152	60152	2.25	0.13
	Fence:Farm	19	8802198	463274	17.31	< 0.001*
	Residuals	400	10705905	26765		

Table D2. Results of stepwise regression and mixed effect model analyses assessing relationships between bacterial community composition and various soil chemical parameters. Two variables, the distance along the transect line (i.e., the distance from the farm fence) and triplicates collected at each site, were identified as the random effects in the model. As AIC comparison analysis indicate no improvement on null model from all of our candidate models, the *stepwise* stepwise regression was used to select a subset of environmental variables to comprise mixed effect model.

Data		Parameters		Mixed effect model analysis			
				AIC	Log-likelihood	Coefficient (± Standard Error)	<i>P</i>
Composition	Fence + N input + Lime input + Nitrogen + Olsen P + Total P + Total U + Delta ¹⁵ N + C/P ratio		-1647.549	836.7745			
	Fence				0.023 (0.008)	0.003*	8.79
	N input				-0.0002 (0.00009)	0.020*	5.44
	Lime input				-0.019 (0.007)	0.003*	8.77
	Nitrogen				-0.06 (0.023)	0.010*	6.68
	Olsen P				0.0004 (0.0002)	0.023*	5.19
	Total P				0.00006 (0.00002)	0.003*	8.62
	Total U				-0.029 (0.011)	0.008*	6.96
	Delta ¹⁵ N				0.007 (0.004)	0.049*	3.70
	C/P ratio				0.0003 (0.0001)	0.002*	9.36
	Intercept				0.58 (0.026)	< 0.001*	

Asterisks (*): The p -value is significant (p -value < 0.05).

Table D3. Results of PERMDISP analysis examining differences in data dispersion based on Bray-Curtis dissimilarities using the overall dataset of bacterial communities grouped according to different land types (i.e., pasture, forest and natural reserve), and the presence or absence of a fence on the land use boundaries (i.e., fenced versus unfenced). A p -value ($F = 5.86$, p -value < 0.001) was obtained using 9999 permutations of the data.

Parameters	n	Average	SE
Reserve	86	38.189	0.603
Forest + Fenced	164	41.552	0.572
Forest + Unfenced	139	40.248	0.407
Pasture + Fenced	64	39.231	0.551
Pasture + Unfenced	55	38.965	0.569

Table D4. Error matrix in the comparison of predicted fence categories (i.e., fenced or unfenced) and actual fence categories for 100 test sites collected from 21 forest soils bounded with or without a fence between land uses (10 unfenced farms and 11 fenced farms).

	Number of actual sites		
	Actual fenced	Actual unfenced	
Number of predicted sites			
Predicted fenced	49	6	
Predicted unfenced	6	39	
	Sensitivity (True positive rate)	Specificity (True negative rate)	Accuracy
	89.09%	86.67%	88.0%
	(n = 49)	(n = 39)	(n = 88)

Table D5. AIC and mixed effects analysis for all candidate models for a subset of bacterial richness data observed from adjacent forests. Random intercepts were specified for the distance along the transect line (i.e., the distance from the farm fence) and triplicates collected at each site. The models were ordered by their ΔAICc values. We only show the results of mixed effect model analysis derived from the best-fitting candidate model.

Model	Parameters	AIC analysis					Mixed effect model analysis		
		<i>K</i>	AICc	ΔAICc	w_i	Log-likelihood	Coefficient (\pm Standard Error)	<i>P</i>	χ^2
Soil (<i>Best-fitting model</i>)	Bulk density + Carbon + Nitrogen + Olsen P + Total P + Total Cd + Total U + pH + Soil moisture + C/N ratio + Delta ^{15}N + CP + NP	17	4987.58	0	0.96	-2472.86			
	Bulk density						87.15 (74.53)	0.233	1.42
	Carbon						-2.61 (29.24)	0.927	0.008
	Nitrogen						-86.15 (331.41)	0.791	0.07
	Olsen P						-0.21 (0.88)	0.806	0.06
	Total P						-0.05 (0.10)	0.616	0.25
	Total Cd						281.72 (118.30)	0.015*	5.90
	Total U						-86.40 (52.12)	0.091	2.86
	pH						133.42 (30.10)	< 0.001*	20.43
	Soil moisture						-275.60 (577.45)	0.626	0.24
	C/N ratio						-3919.80 (2283.80)	0.080	3.06
	Delta ^{15}N						-23.31 (15.67)	0.129	2.30
	CP						-0.36 (1.42)	0.798	0.07
	NP						-6.96 (17.65)	0.688	0.16
	Intercept						686.52 (321.69)	0.034*	

Full	Bulk density + Carbon + Nitrogen + Olsen P + Total P + Total Cd + Total U + pH + Soil moisture + C/N ratio + Delta ¹⁵ N + CP + NP + Fencing category (fenced or unfenced) + N input + P input + lime input + stocking rate + Land use intensity + Patch area	24	4993.91	6.33	0.04	-2470.16	
Null	Intercept	4	5024.20	36.62	0	-2508.05	
Land use intensity†	Land use intensity	5	5024.23	36.66	0	-2507.04	
Fence effect	Fencing category (fenced or unfenced)	5	5025.62	38.04	0	-2507.73	
Farming input	N input + P input + Lime input + Stocking rate + Patch area	9	5028.40	40.82	0	-2504.96	

Asterisks (*): The *p*-value is significant (*p*-value < 0.05).

Dagger (†): The land use intensity of each farm was identified from Didham et al. (2015).

Table D6. AIC and mixed effect model analyses for all candidate models for a subset of bacterial composition data observed from adjacent forests. Two variables, the distance along the transect line (i.e., the distance from the farm fence) and triplicates collected at each site, were identified as the random effects in the model. The models were ordered by their ΔAICc values. We only show the results of mixed effect model analysis derived from the best-fitting candidate model.

Model	Parameters	AIC analysis					Mixed effect model analysis		
		<i>K</i>	AICc	ΔAICc	w_i	Log-likelihood	Coefficient (\pm Standard Error)	<i>P</i>	χ^2
Soil (<i>Best-fitting model</i>)	Bulk density + Carbon + Nitrogen + Olsen P + Total P + Total Cd + Total U + pH + Soil moisture + C/N ratio + Delta ^{15}N + CP + NP	17	-1237.73	0	0.89	637.79			
	Bulk density						-0.01 (0.02)	0.658	0.20
	Carbon						0.001 (0.01)	0.895	0.02
	Nitrogen						-0.11 (0.11)	0.313	1.02
	Olsen P						0.0004 (0.0003)	0.208	1.59
	Total P						0.00007 (0.00003)	0.041*	4.18
	Total Cd						-0.024 (0.039)	0.539	0.38
	Total U						-0.024 (0.039)	0.031*	4.67
	pH						0.003 (0.01)	0.730	0.12
	Soil moisture						0.10 (0.19)	0.583	0.30
	C/N ratio						0.44 (0.78)	0.563	0.34
	Delta ^{15}N						0.003 (0.005)	0.593	0.29
	CP						0.0004 (0.0005)	0.372	0.80
	NP						-0.0009 (0.006)	0.873	0.03
	Intercept						0.63 (0.11)	< 0.001*	

Full	Bulk density + Carbon + Nitrogen + Olsen P + Total P + Total Cd + Total U + pH + Soil moisture + C/N ratio + Delta ¹⁵ N + CP + NP + Fencing category (fenced or unfenced) + N input + P input + Lime input + stocking rate + Land use intensity + Patch area	24	-1233.55	4.18	0.11	643.57	
Null	Intercept	4	-1225.44	12.29	0	616.77	
Farming input	N input + P input + Lime input + Stocking rate + Patch area	9	-1224.09	13.65	0	621.28	
Fence effect	Fencing category (fenced or unfenced)	5	-1224.06	13.67	0	617.11	
Land use intensity†	Land use intensity	5	-1223.40	14.34	0	616.78	

Asterisks (*): The *p*-value is significant (*p*-value < 0.05).

Dagger (†): The land use intensity of each farm was identified from Didham et al. (2015).

Table D7. The results of stepwise regression analysis and mixed effect model analysis for the data of bacterial richness and composition collected from farm pastures. Random intercepts were specified for the distance along the transect line (i.e., the distance from the farm fence) and triplicates collected at each site. As AIC comparison analysis indicate no improvement on null model from all of our candidate models, the *stepwise* stepwise regression was used to select a subset of environmental variables to comprise mixed effect model.

Data	Parameters	Mixed effect model analysis				
		AIC	Log-likelihood	Coefficient (\pm Standard Error)	P	χ^2
Richness	Carbon + Nitrogen + Olsen P + Total P + pH + Soil moisture + C/N ratio + NP	1537.61	-756.81			
	Carbon			182.92 (74.99)	0.011*	6.44
	Nitrogen			-2161.62 (780.47)	0.004*	8.30
	Olsen P			-5.66 (2.01)	0.003*	8.58
	Total P			0.36 (0.11)	< 0.001*	12.79
	pH			69.16 (56.80)	0.205	1.60
	Soil moisture			-2115.99 (1393.21)	0.114	2.50
	C/N ratio			-90.92 (61.52)	0.124	2.36
	NP			29.15 (12.53)	0.016*	5.86
	Intercept			1665.67 (689.04)	0.018*	
Composition	Nitrogen + Olsen P + Soil moisture	-398.53	206.26			
	Nitrogen			0.064 (0.033)	0.046*	3.98
	Olsen P			0.0008 (0.0003)	0.008*	7.11
	Soil moisture			-0.96 (0.34)	0.004*	8.33
	Intercept			0.63 (0.021)	< 0.001*	

Asterisks (*): The *p*-value is significant (*p*-value < 0.05).

Bibliography

- Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., et al. (2010). The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytologist* 186(2), 281-285.
- Acharya, B.K., Chettri, B., and Vijayan, L. (2011). Distribution pattern of trees along an elevation gradient of Eastern Himalaya, India. *Acta Oecologica* 37(4), 329-336.
- Achtman, M., and Wagner, M. (2008). Microbial diversity and the genetic nature of microbial species. *Nature Reviews Microbiology* 6(6), 431-440.
- Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6), 716-723.
- Allen, A.P., Brown, J.H., and Gillooly, J.F. (2002). Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* 297(5586), 1545-1548.
- Allison, S.D., Hanson, C.A., and Treseder, K.K. (2007). Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry* 39(8), 1878-1887.
- Amann, R.L., Ludwig, W., and Schleifer, K.H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* 59(1), 143-169.
- Anderson, C., Beare, M., Buckley, H.L., and Lear, G. (2017). Bacterial and fungal communities respond differently to varying tillage depth in agricultural soils. *PeerJ* 5, e3930.
- Anderson, D., Burnham, K., and White, G. (1998). Comparison of Akaike information criterion and consistent Akaike information criterion for model selection and statistical inference from capture-recapture studies. *Journal of Applied Statistics* 25(2), 263-282.
- Anderson, D.R. (2007). *Model based inference in the life sciences: a primer on*

- evidence. Springer Science & Business Media.
- Anderson, M., Gorley, R.N., and Clarke, R.K. (2008). *Permanova+ for Primer: Guide to software and statistical methods*. Primer-E Limited.
- Anderson, M.J. (2004). DISTLM v. 5: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model. *Department of Statistics, University of Auckland, New Zealand* 10, 2016.
- Andrew, C., Heegaard, E., Halvorsen, R., Martinez-Peña, F., Egli, S., Kirk, P.M., et al. (2016). Climate impacts on fungal community and trait dynamics. *Fungal Ecology* 22, 17-25. doi: 10.1016/j.funeco.2016.03.005.
- Aragón, P., Lobo, J.M., Olalla-Tárraga, M.Á., and Rodríguez, M.Á. (2010). The contribution of contemporary climate to ectothermic and endothermic vertebrate distributions in a glacial refuge. *Global Ecology and Biogeography* 19(1), 40-49.
- Ashton, K.G., Tracy, M.C., and Queiroz, A.d. (2000). Is Bergmann's rule valid for mammals? *The American Naturalist* 156(4), 390-415.
- Asner, G.P., and Martin, R.E. (2015). Convergent elevation trends in canopy chemical traits of tropical forests. *Global Change Biology* 22(6), 2216-2227.
- Astorga, A., Oksanen, J., Luoto, M., Soininen, J., Virtanen, R., and Muotka, T. (2012). Distance decay of similarity in freshwater communities: do macro-and microorganisms follow the same rules? *Global Ecology and Biogeography* 21(3), 365-375.
- Aubry, S., Magnin, F., Bonnet, V., and Preece, R.C. (2005). Multi-scale altitudinal patterns in species richness of land snail communities in south-eastern France. *Journal of Biogeography* 32(6), 985-998. doi: 10.1111/j.1365-2699.2005.01275.x.
- Averill, C., Waring, B.G., and Hawkes, C.V. (2016). Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture. *Global Change Biology* 22(5), 1957-1964.

- Axmacher, J.C., Holtmann, G., Scheuermann, L., Brehm, G., Müller-Hohenstein, K., and Fiedler, K. (2004). Diversity of geometrid moths (Lepidoptera: Geometridae) along an Afrotropical elevational rainforest transect. *Diversity and Distributions* 10(4), 293-302.
- Baar, J., and Stanton, N.L. (2000). Ectomycorrhizal fungi challenged by saprotrophic basidiomycetes and soil microfungi under different ammonium regimes *in vitro*. *Mycological Research* 104(6), 691-697. doi: 10.1017/s0953756299002257.
- Baas Becking, L.G.M. (1934). Geobiologie of inleiding tot de milieukunde. *The Hague, the Netherlands: W.P. Van Stockum & Zoon*.
- Bahram, M., Pölme, S., Kõljalg, U., Zarre, S., and Tedersoo, L. (2012). Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist* 193(2), 465-473.
- Bailey, V.L., Fansler, S.J., Stegen, J.C., and McCue, L.A. (2013). Linking microbial community structure to beta-glucosidic function in soil aggregates. *The ISME Journal* 7(10), 2044-2053. doi: 10.1038/ismej.2013.87.
- Bakelaar, R.G., and Odum, E.P. (1978). Community and population level responses to fertilization in an old-field ecosystem. *Ecology* 59(4), 660-665.
- Baker, K.L., Langenheder, S., Nicol, G.W., Ricketts, D., Killham, K., Campbell, C.D., et al. (2009). Environmental and spatial characterisation of bacterial community composition in soil to inform sampling strategies. *Soil Biology and Biochemistry* 41(11), 2292-2298.
- Baldrian, P., and Lindahl, B. (2011). Decomposition in forest ecosystems: after decades of research still novel findings. *Fungal Ecology* 4(6), 359-361.
- Baldy, V., Gobert, V., Guérol, F., Chauvet, E., Lambrigot, D., and Charcosset, J.Y. (2007). Leaf litter breakdown budgets in streams of various trophic status: effects of dissolved inorganic nutrients on microorganisms and invertebrates. *Freshwater Biology* 52(7), 1322-1335.

- Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A., and Richardson, A.E. (2016). Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biology and Biochemistry* 97, 188-198. doi: 10.1016/j.soilbio.2016.03.017.
- Barberán, A., Bates, S.T., Casamayor, E.O., and Fierer, N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *The ISME Journal* 6(2), 343-351.
- Barberan, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H., and Fierer, N. (2014). Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. *Ecology Letters* 17(7), 794-802. doi: 10.1111/ele.12282.
- Bardgett, R. (2005). *The biology of soil: a community and ecosystem approach*. Oxford University Press.
- Bardgett, R.D., and van der Putten, W.H. (2014). Belowground biodiversity and ecosystem functioning. *Nature* 515(7528), 505-511.
- Barreto, D.P., Conrad, R., Klose, M., Claus, P., and Enrich-Prast, A. (2014). Distance-decay and taxa-area relationships for bacteria, archaea and methanogenic archaea in a tropical lake sediment. *PLOS One* 9(10), e110128.
- Bascompte, J., and Jordano, P. (2007). Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 38, 567-593.
- Bastian, M., Heymann, S., and Jacomy, M. (2009). Gephi: an open source software for exploring and manipulating networks. *Icwsn* 8, 361-362.
- Beales, N. (2004). Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: A review. *Comprehensive Reviews in Food Science and Food Safety* 3(1), 1-20. doi: 10.1111/j.1541-4337.2004.tb00057.x.

- Behera, M.D., and Kushwaha, S.P.S. (2006). "An analysis of altitudinal behavior of tree species in Subansiri district, Eastern Himalaya," in *Plant Conservation and Biodiversity*, 277-291.
- Bell, T. (2010). Experimental tests of the bacterial distance–decay relationship. *The ISME Journal* 4(11), 1357-1365.
- Bell, T., Ager, D., Song, J.I., Newman, J.A., Thompson, I.P., Lilley, A.K., et al. (2005). Larger islands house more bacterial taxa. *Science* 308(5730), 1884-1884.
- Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., and Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases. *BMC Microbiology* 10, 189. doi: 10.1186/1471-2180-10-189.
- Bellingham, P.J., and Sparrow, A.D. (2009). Multi-stemmed trees in montane rain forests: their frequency and demography in relation to elevation, soil nutrients and disturbance. *Journal of Ecology* 97(3), 472-483.
- Bennie, J., Huntley, B., Wiltshire, A., Hill, M.O., and Baxter, R. (2008). Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecological Modelling* 216(1), 47-59. doi: 10.1016/j.ecolmodel.2008.04.010.
- Bentkowski, P., Van Oosterhout, C., and Mock, T. (2015). A model of genome size evolution for prokaryotes in stable and fluctuating environments. *Genome Biology and Evolution* 7(8), 2344-2351.
- Bergkemper, F., Scholer, A., Engel, M., Lang, F., Kruger, J., Schlöter, M., et al. (2016). Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environmental Microbiology* 18(6), 1988-2000. doi: 10.1111/1462-2920.13188.
- Berlemont, R., Allison, S.D., Weihe, C., Lu, Y., Brodie, E.L., Martiny, J.B., et al. (2014). Cellulolytic potential under environmental changes in microbial communities from grassland litter. *Frontiers in Microbiology* 5, 639.

- Berthrong, S.T., Jobbagy, E.G., and Jackson, R.B. (2009). A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. *Ecological Applications* 19(8), 2228-2241.
- Bhatt, J.P., Manish, K., and Pandit, M.K. (2012). Elevational gradients in fish diversity in the Himalaya: water discharge is the key driver of distribution patterns. *PLOS One* 7(9), e46237.
- Bierregaard, R.O. (2001). *Lessons from Amazonia: the ecology and conservation of a fragmented forest*. Yale University Press.
- Binnewies, T.T., Motro, Y., Hallin, P.F., Lund, O., Dunn, D., Lea, T., et al. (2006). Ten years of bacterial genome sequencing: comparative-genomics-based discoveries. *Functional and Integrative Genomics* 6, 165-185.
- Blackburn, T.M., Gaston, K.J., and Loder, N. (1999). Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions* 5(4), 165-174.
- Blackburn, T.M., and Hawkins, B.A. (2004). Bergmann's rule and the mammal fauna of northern North America. *Ecography* 27(6), 715-724.
- Blakemore, L.C. (1987). Methods for chemical analysis of soils. *NZ Soil Bureau Scientific Report* 80.
- Blitzer, E.J., Dormann, C.F., Holzschuh, A., Klein, A.-M., Rand, T.A., and Tschardtke, T. (2012). Spillover of functionally important organisms between managed and natural habitats. *Agriculture, Ecosystems & Environment* 146(1), 34-43. doi: 10.1016/j.agee.2011.09.005.
- Boberg, J., Finlay, R., Stenlid, J., Nasholm, T., and Lindahl, B. (2008). Glucose and ammonium additions affect needle decomposition and carbon allocation by the litter degrading fungus *Mycena epipterygia*. *Soil Biology and Biochemistry* 40(4), 995-999. doi: 10.1016/j.soilbio.2007.11.005.
- Boddy, L., and Watkinson, S.C. (1995). Wood decomposition, higher fungi, and their role in nutrient redistribution. *Canadian Journal of Botany* 73(S1), 1377-1383.
- Bokdam, J., and Gleichman, J.M. (2000). Effects of grazing by free-ranging cattle on

- vegetation dynamics in a continental north-west European heathland. *Journal of Applied Ecology* 37(3), 415-431.
- Bossio, D.A., Scow, K.M., Gunapala, N., and Graham, K. (1998). Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* 36(1), 1-12.
- Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., and Paustian, K. (2001). Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology* 16(3), 195-208.
- Brehm, G., Colwell, R.K., and Kluge, J. (2007). The role of environment and mid-domain effect on moth species richness along a tropical elevational gradient. *Global Ecology and Biogeography* 16(2), 205-219.
- Brehm, G., Homeier, J., and Fiedler, K. (2003). Beta diversity of geometrid moths (*Lepidoptera: Geometridae*) in an Andean montane rainforest. *Diversity and Distributions* 9(5), 351-366.
- Brown, J.H. (2001). Mammals on mountainsides: elevational patterns of diversity. *Global Ecology and Biogeography* 10(1), 101-109.
- Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., and Green, J.L. (2008). Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences* 105, 11505-11511.
- Burnham, K.P., and Anderson, D.R. (2003). *Model selection and multimodel inference: a practical information-theoretic approach*. Springer Science & Business Media.
- Campbell, B.J., and Kirchman, D.L. (2013). Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *The ISME Journal* 7(1), 210-220.
- Campbell, B.J., Polson, S.W., Hanson, T.E., Mack, M.C., and Schuur, E.A. (2010). The effect of nutrient deposition on bacterial communities in Arctic tundra soil.

Environmental Microbiology 12(7), 1842-1854.

- Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. (2009). The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. *Nucleic Acids Research* 37, D233-D238. doi: 10.1093/nar/gkn663.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5), 335-336.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* 6(8), 1621-1624.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* 108, 4516-4522.
- Carbonero, F., Oakley, B.B., and Purdy, K.J. (2014). Metabolic flexibility as a major predictor of spatial distribution in microbial communities. *PLOS One* 9(1), e85105. doi: 10.1371/journal.pone.0085105.
- Cardenas, E., Wu, W.-M., Leigh, M.B., Carley, J., Carroll, S., Gentry, T., et al. (2008). Microbial communities in contaminated sediments, associated with bioremediation of uranium to submicromolar levels. *Applied and Environmental Microbiology* 74(12), 3718-3729.
- Carletti, P., Vendramin, E., Pizzeghello, D., Concheri, G., Zanella, A., Nardi, S., et al. (2009). Soil humic compounds and microbial communities in six spruce forests as function of parent material, slope aspect and stand age. *Plant and Soil* 315(1-2), 47-65.
- Carneiro, E., Mielke, O.H.H., Casagrande, M.M., and Fiedler, K. (2013). Skipper richness (Hesperiidae) along elevational gradients in Brazilian Atlantic Forest. *Neotropical Entomology* 43(1), 27-38. doi: 10.1007/s13744-013-0175-8.

- Carpenter, C. (2005). The environmental control of plant species density on a Himalayan elevation gradient. *Journal of Biogeography* 32(6), 999-1018.
- Carter, B.J., and Ciolkosz, E.J. (1991). Slope gradient and aspect effects on soils developed from sandstone in Pennsylvania. *Geoderma* 49(3), 199-213.
- Casamayor, E.O., Massana, R., Benlloch, S., Øvreås, L., Díez, B., Goddard, V.J., et al. (2002). Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environmental Microbiology* 4(6), 338-348.
- Casteleyn, G., Leliaert, F., Backeljau, T., Debeer, A.E., Kotaki, Y., Rhodes, L., et al. (2010). Limits to gene flow in a cosmopolitan marine planktonic diatom. *Proceedings of the National Academy of Sciences* 107(29), 12952-12957.
- Cerniglia, C.E. (1997). Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. *Journal of Industrial Microbiology & Biotechnology* 19(5), 324-333.
- Chaffron, S., Rehrauer, H., Pernthaler, J., and von Mering, C. (2010). A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome Research* 20(7), 947-959.
- Chalot, M., and Plassard, C. (2011). Ectomycorrhiza and nitrogen provision to the host tree. *Ecological Aspects of Nitrogen Metabolism in Plants*, 69-94.
- Chambers, J.M. (1992). Linear models. *Statistical models in S*.
- Champagne, C., Gardner, N., Soullignac, L., and Innocent, J. (2000). The production of freeze-dried immobilized cultures of *Streptococcus thermophilus* and their acidification properties in milk. *Journal of Applied Microbiology* 88(1), 124-131.
- Chang, Y.J., Land, M., Hauser, L., Chertkov, O., Del Rio, T.G., Nolan, M., et al. (2011). Non-contiguous finished genome sequence and contextual data of the filamentous soil bacterium *Ktedonobacter racemifer* type strain (SOSP1-21 T). *Standards in Genomic Sciences* 5(1), 97.

- Chatzaki, M., Lymberakis, P., Markakis, G., and Mylonas, M. (2005). The distribution of ground spiders (Araneae, Gnaphosidae) along the altitudinal gradient of Crete, Greece: species richness, activity and altitudinal range. *Journal of Biogeography* 32(5), 813-831.
- Chettri, B., Bhupathy, S., and Acharya, B.K. (2010). Distribution pattern of reptiles along an eastern Himalayan elevation gradient, India. *Acta Oecologica* 36(1), 16-22.
- Cho, J.C., and Kim, S.J. (2000). Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input. *Applied and Environmental Microbiology* 66(3), 956-965.
- Cho, J.C., and Tiedje, J.M. (2000). Biogeography and degree of endemism of fluorescent *Pseudomonas* strains in soil. *Applied and Environmental Microbiology* 66(12), 5448-5456.
- Chu, H., Fierer, N., Lauber, C.L., Caporaso, J.G., Knight, R., and Grogan, P. (2010). Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environmental Microbiology* 12(11), 2998-3006.
- Clark, C.M., and Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451(7179), 712-715.
- Clarke, K., and Gorley, R. (2005). PRIMER: Getting started with v6. *PRIMER-E Ltd: Plymouth, UK*.
- Clemmensen, K.E., Sorensen, P.L., Michelsen, A., Jonasson, S., and Ström, L. (2008). Site-dependent N uptake from N-form mixtures by arctic plants, soil microbes and ectomycorrhizal fungi. *Oecologia* 155(4), 771-783.
- Cobo-Simon, M., and Tamames, J. (2017). Relating genomic characteristics to environmental preferences and ubiquity in different microbial taxa. *BMC Genomics* 18(1), 499. doi: 10.1186/s12864-017-3888-y.
- Coince, A., Cordier, T., Lengelle, J., Defosse, E., Vacher, C., Robin, C., et al. (2014). Leaf and root-associated fungal assemblages do not follow similar elevational

- diversity patterns. *PLOS One* 9(6), e100668. doi: 10.1371/journal.pone.0100668.
- Collins, C.G., Stajich, J.E., Weber, S.E., Pombubpa, N., and Diez, J.M. (2018). Shrub range expansion alters diversity and distribution of soil fungal communities across an alpine elevation gradient. *Molecular Ecology* 27(10), 2461-2476.
- Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C., and Longino, J.T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* 322(5899), 258-261.
- Condit, R., Pitman, N., Leigh, E.G., Chave, J., Terborgh, J., Foster, R.B., et al. (2002). Beta-diversity in tropical forest trees. *Science* 295(5555), 666-669.
- Connor, E.F., and McCoy, E.D. (1979). The statistics and biology of the species-area relationship. *The American Naturalist* 113(6), 791-833.
- Cordier, T., Robin, C., Capdevielle, X., Fabreguettes, O., Desprez-Loustau, M.L., and Vacher, C. (2012). The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. *New Phytologist* 196(2), 510-519.
- Corneo, P.E., Pellegrini, A., Cappellin, L., Roncador, M., Chierici, M., Gessler, C., et al. (2013). Microbial community structure in vineyard soils across altitudinal gradients and in different seasons. *FEMS Microbiology Ecology* 84(3), 588-602.
- Costello, E.K., Stagaman, K., Dethlefsen, L., Bohannan, B.J., and Relman, D.A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science* 336(6086), 1255-1262.
- Crowther, T.W., and Bradford, M.A. (2013). Thermal acclimation in widespread heterotrophic soil microbes. *Ecology Letters* 16(4), 469-477.
- Crump, B.C., Hopkinson, C.S., Sogin, M.L., and Hobbie, J.E. (2004). Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. *Applied and Environmental Microbiology* 70(3), 1494-1505.

- Currie, D.J. (1991). Energy and large-scale patterns of animal-and plant-species richness. *The American Naturalist* 137(1), 27-49.
- Currie, D.J., and Kerr, J.T. (2008). Tests of the mid-domain hypothesis: a review of the evidence. *Ecological Monoographs* 7, 83-18.
- Daniell, T., Husband, R., Fitter, A., and Young, J. (2001). Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *FEMS Microbiology Ecology* 36(2-3), 203-209.
- Darwin, C. (1859). On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. *London* 1, 859.
- Davies, Z.G., Wilson, R.J., Coles, S., and Thomas, C.D. (2006). Changing habitat associations of a thermally constrained species, the silver-spotted skipper butterfly, in response to climate warming. *Journal of Animal Ecology* 75(1), 247-256.
- DeAngelis, K.M., Pold, G., Topçuoğlu, B.D., van Diepen, L.T., Varney, R.M., Blanchard, J.L., et al. (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology* 6, 104.
- Degnan, P.H., and Ochman, H. (2012). Illumina-based analysis of microbial community diversity. *The ISME Journal* 6(1), 183-194.
- Delmont, T.O., Prestat, E., Keegan, K.P., Faubladier, M., Robe, P., Clark, I.M., et al. (2012). Structure, fluctuation and magnitude of a natural grassland soil metagenome. *The ISME Journal* 6(9), 1677-1687. doi: 10.1038/ismej.2011.197.
- Deng, Y., Jiang, Y.-H., Yang, Y., He, Z., Luo, F., and Zhou, J. (2012). Molecular ecological network analyses. *BMC Bioinformatics* 13(1), 1.
- Dennis, P.G., Rushton, S.P., Newsham, K.K., Lauducina, V.A., Ord, V.J., Daniell, T.J., et al. (2012). Soil fungal community composition does not alter along a latitudinal gradient through the maritime and sub-Antarctic. *Fungal Ecology* 5(4), 403-408. doi: 10.1016/j.funeco.2011.12.002.
- Descombes, P., Marchon, J., Pradervand, J.-N., Bilat, J., Guisan, A., Rasmann, S., et al.

- (2017). Community-level plant palatability increases with elevation as insect herbivore abundance declines. *Journal of Ecology* 105(1), 142-151.
- Di, H., Cameron, K., Shen, J.P., Winefield, C., O'callaghan, M., Bowatte, S., et al. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience* 2(9), 621-624.
- Didham, R.K., Barker, G.M., Bartlam, S., Deakin, E.L., Denmead, L.H., Fisk, L.M., et al. (2015). Agricultural intensification exacerbates spillover effects on soil biogeochemistry in adjacent forest remnants. *PLOS One* 10(1), e0116474. doi: 10.1371/journal.pone.0116474.
- Diniz Filho, J.A.F., Bini, L.M., Vieira, C.M., Blamires, D., Terribile, L.C., Bastos, R.P., et al. (2008). Spatial patterns of terrestrial vertebrate species richness in the Brazilian Cerrado.
- Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14(6), 927-930.
- Doran, J.W., and Zeiss, M.R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology* 15(1), 3-11.
- Drakare, S., Lennon, J.J., and Hillebrand, H. (2006). The imprint of the geographical, evolutionary and ecological context on species–area relationships. *Ecology Letters* 9(2), 215-227.
- Duarte, S., Pascoal, C., Garabetian, F., Cassio, F., and Charcosset, J.Y. (2009). Microbial decomposer communities are mainly structured by trophic status in circumneutral and alkaline streams. *Applied and Environmental Microbiology* 75(19), 6211-6221. doi: 10.1128/AEM.00971-09.
- Dubuis, A., Giovanettina, S., Pellissier, L., Pottier, J., Vittoz, P., and Guisan, A. (2013). Improving the prediction of plant species distribution and community composition by adding edaphic to topo-climatic variables. *Journal of Vegetation Science* 24(4), 593-606.
- Eddy, R.S., Wheeler, J.T., and the HMMER development team (2015). HMMER user's

guide: biological sequence analysis using profile hidden Markov models.

- Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10(10), 996-998. doi: 10.1038/nmeth.2604.
- Edwards, D., Larson, B., and Lim, T. (2000). Runoff nutrient and fecal coliform content from cattle manure application to fescue plots. *Journal of the American Water Resources Association* 36(4), 711-721.
- Edwards, K.F., Litchman, E., and Klausmeier, C.A. (2013). Functional traits explain phytoplankton responses to environmental gradients across lakes of the United States. *Ecology* 94(7), 1626-1635. doi: 10.1890/12-1459.1.
- Eiler, A., Heinrich, F., and Bertilsson, S. (2012). Coherent dynamics and association networks among lake bacterioplankton taxa. *The ISME Journal* 6(2), 330-342.
- Eisen, J.A. (2007). Environmental shotgun sequencing: Its potential and challenges for studying the hidden world of microbes. *PLOS Biology* 5(3), 384-388. doi: 10.1371/journal.pbio.0050082.
- Falkowski, P.G., Fenchel, T., and Delong, E.F. (2008). The microbial engines that drive Earth's biogeochemical cycles. *Science* 320(5879), 1034-1039.
- Faust, K., and Raes, J. (2012). Microbial interactions: from networks to models. *Nature Reviews Microbiology* 10(8), 538-550.
- Fenchel, T. (2003). Biogeography for bacteria. *Science* 301(5635), 925-926.
- Fenchel, T., Esteban, G.F., and Finlay, B.J. (1997). Local versus global diversity of microorganisms: cryptic diversity of ciliated protozoa. *Oikos*, 220-225.
- Ferrari, B.C., Binnerup, S.J., and Gillings, M. (2005). Microcolony cultivation on a soil substrate membrane system selects for previously uncultured soil bacteria. *Applied and Environmental Microbiology* 71(12), 8714-8720. doi: 10.1128/aem.71.12.8714-8720.2005.
- Field, R., O'Brien, E.M., and Whittaker, R.J. (2005). Global models for predicting woody plant richness from climate: development and evaluation. *Ecology* 86(9),

2263-2277.

- Fierer, N. (2008). "Microbial biogeography: patterns in microbial diversity across space and time," in *Accessing Uncultivated Microorganisms*. American Society of Microbiology, 95-115.
- Fierer, N., and Jackson, R.B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences* 103(3), 626-631. doi: 10.1073/pnas.0507535103.
- Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., and Knight, R. (2012a). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* 6(5), 1007-1017. doi: 10.1038/ismej.2011.159.
- Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., et al. (2012b). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences* 109(52), 21390-21395. doi: 10.1073/pnas.1215210110.
- Fierer, N., McCain, C.M., Meir, P., Zimmermann, M., Rapp, J.M., Silman, M.R., et al. (2010). Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 92(4), 797-804. doi: 10.1890/10-1170.1.
- Fierer, N., McCain, C.M., Meir, P., Zimmermann, M., Rapp, J.M., Silman, M.R., et al. (2011). Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 92(4), 797-804.
- Fierer, N., Morse, J.L., Berthrong, S.T., Bernhardt, E.S., and Jackson, R.B. (2007). Environmental controls on the landscape-scale biogeography of stream bacterial communities. *Ecology* 88(9), 2162-2173.
- Fierer, N., Schimel, J.P., and Holden, P.A. (2003). Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial Ecology* 45(1), 63-71. doi: 10.1007/s00248-002-1007-2.
- Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., and Cleveland, C.C. (2009).

- Global patterns in belowground communities. *Ecology Letters* 12(11), 1238-1249.
- Finlay, B.J. (2002). Global dispersal of free-living microbial eukaryote species. *Science* 296(5570), 1061-1063.
- Finn, R.D., Clements, J., Arndt, W., Miller, B.L., Wheeler, T.J., Schreiber, F., et al. (2015). HMMER web server: 2015 update. *Nucleic Acids Research* 43(W1), W30-W38. doi: 10.1093/nar/gkv397.
- Fleishman, E., Austin, G.T., and Weiss, A.D. (1998). An empirical test of Rapoport's rule: elevational gradients in montane butterfly communities. *Ecology* 79(7), 2482-2493.
- Fondi, M., Karkman, A., Tamminen, M.V., Bosi, E., Virta, M., Fani, R., et al. (2016). "Every gene is everywhere but the environment selects": Global geolocalization of gene sharing in environmental samples through network analysis. *Genome Biology and Evolution* 8(5), 1388-1400. doi: 10.1093/gbe/evw077.
- Fortunato, C.S., Herfort, L., Zuber, P., Baptista, A.M., and Crump, B.C. (2012). Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient. *The ISME Journal* 6(3), 554-563.
- Fox, R., and Hoffman, L. (1981). The effect of N fertilizer source on grain yield, N uptake, soil pH, and lime requirement in no-till corn 1. *Agronomy Journal* 73(5), 891-895.
- Freilich, S., Kreimer, A., Meilijson, I., Gophna, U., Sharan, R., and Ruppin, E. (2010). The large-scale organization of the bacterial network of ecological co-occurrence interactions. *Nucleic Acids Research* 38(12), 3857-3868. doi: 10.1093/nar/gkq118.
- Frey, S.D., Knorr, M., Parrent, J.L., and Simpson, R.T. (2004). Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* 196(1), 159-171.

- Fu, B., Liu, S., Ma, K., and Zhu, Y. (2004). Relationships between soil characteristics, topography and plant diversity in a heterogeneous deciduous broad-leaved forest near Beijing, China. *Plant and Soil* 261(1-2), 47-54.
- Fuhrman, J.A. (2009). Microbial community structure and its functional implications. *Nature* 459(7244), 193-199.
- Fuhrman, J.A., and Steele, J.A. (2008). Community structure of marine bacterioplankton: patterns, networks, and relationships to function. *Aquatic Microbial Ecology* 53(1), 69-81.
- Fuhrman, J.A., Steele, J.A., Hewson, I., Schwalbach, M.S., Brown, M.V., Green, J.L., et al. (2008). A latitudinal diversity gradient in planktonic marine bacteria. *Proceedings of the National Academy of Sciences* 105(22), 7774-7778.
- Gange, A.C., Mohammad, A.B., Damialis, A., and Gange, E.G. (2013). Mushroom phenological changes: A role for resource availability? *Proceedings of the National Academy of Sciences* 110(5), E333-E334.
- Garbeva, P.v., Van Veen, J., and Van Elsas, J. (2004). Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* 42, 243-270.
- Garcia-Pichel, F., Prufert-Bebout, L., and Muyzer, G. (1996). Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. *Applied and Environmental Microbiology* 62(9), 3284-3291.
- Gaston, K.J. (2000). Global patterns in biodiversity. *Nature* 405(6783), 220-227.
- Giladi, I., Ziv, Y., May, F., and Jeltsch, F. (2011). Scale-dependent determinants of plant species richness in a semi-arid fragmented agro-ecosystem. *Journal of Vegetation Science* 22(6), 983-996.
- Gilbert, J.A., Field, D., Swift, P., Thomas, S., Cummings, D., Temperton, B., et al. (2010). The taxonomic and functional diversity of microbes at a temperate coastal site: a 'multi-omic' study of seasonal and diel temporal variation. *PLOS One* 5(11), e15545. doi: 10.1371/journal.pone.0015545.

- Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbrück, L., Reeder, J., Temperton, B., et al. (2012). Defining seasonal marine microbial community dynamics. *The ISME Journal* 6(2), 298-308.
- Gloor, G.B., Hummelen, R., Macklaim, J.M., Dickson, R.J., Fernandes, A.D., MacPhee, R., et al. (2010). Microbiome profiling by illumina sequencing of combinatorial sequence-tagged PCR products. *PLOS One* 5(10), e15406.
- Godwin, C.M., and Cotner, J.B. (2015). Stoichiometric flexibility in diverse aquatic heterotrophic bacteria is coupled to differences in cellular phosphorus quotas. *Frontiers in Microbiology* 6, 159.
- Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., et al. (2011). Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Frontiers in Microbiology* 2, 94.
- Gonzalez, A.M.M., Dalsgaard, B., and Olesen, J.M. (2010). Centrality measures and the importance of generalist species in pollination networks. *Ecological Complexity* 7(1), 36-43. doi: 10.1016/j.ecocom.2009.03.008.
- Goodman, S.M., and Rasolonandrasana, B.P. (2001). Elevational zonation of birds, insectivores, rodents and primates on the slopes of the Andringitra Massif, Madagascar. *Journal of Natural History* 35(2), 285-305.
- Gossner, M.M., Lewinsohn, T.M., Kahl, T., Grassein, F., Boch, S., Prati, D., et al. (2016). Land-use intensification causes multitrophic homogenization of grassland communities. *Nature* 540(7632), 266-269.
- Gotelli, N.J., and Graves, G.R. (1996). *Null models in ecology*. Smithsonian Institution.
- Gotelli, N.J., and McCabe, D.J. (2002). Species co-occurrence: A meta-analysis of JM Diamond's assembly rules model. *Ecology* 83(8), 2091-2096.
- Green, J., and Bohannan, B.J. (2006). Spatial scaling of microbial biodiversity. *Trends in Ecology & Evolution* 21(9), 501-507.
- Green, J.L., Bohannan, B.J., and Whitaker, R.J. (2008). Microbial biogeography: from taxonomy to traits. *Science* 320(5879), 1039-1043.

- Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., et al. (2004). Spatial scaling of microbial eukaryote diversity. *Nature* 432(7018), 747-750.
- Grenon, F., Bradley, R.L., Jones, M.D., Shipley, B., and Peat, H. (2005). Soil factors controlling mineral N uptake by *Picea engelmannii* seedlings: the importance of gross NH_4^+ production rates. *New Phytologist* 165(3), 791-800.
- Grenyer, R., Orme, C.D.L., Jackson, S.F., Thomas, G.H., Davies, R.G., Davies, T.J., et al. (2006). Global distribution and conservation of rare and threatened vertebrates. *Nature* 444(7115), 93-96.
- Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., and Whiteley, A.S. (2011). The bacterial biogeography of British soils. *Environmental Microbiology* 13(6), 1642-1654.
- Grubb, P. (1977). Control of forest growth and distribution on wet tropical mountains: with special reference to mineral nutrition. *Annual Review of Ecology and Systematics* 8(1), 83-107.
- Guidot, A., Verner, M.C., Debaud, J.C., and Marmeisse, R. (2005). Intraspecific variation in use of different organic nitrogen sources by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Mycorrhiza* 15(3), 167-177.
- Guieysse, B., and Wuertz, S. (2012). Metabolically versatile large-genome prokaryotes. *Current Opinion in Biotechnology* 23(3), 467-473. doi: 10.1016/j.copbio.2011.12.022.
- Guo, G., Kong, W., Liu, J., Zhao, J., Du, H., Zhang, X., et al. (2015). Diversity and distribution of autotrophic microbial community along environmental gradients in grassland soils on the Tibetan Plateau. *Applied Microbiology and Biotechnology* 99(20), 8765-8776.
- Hales, T., and Roering, J.J. (2005). Climate-controlled variations in scree production, Southern Alps, New Zealand. *Geology* 33(9), 701-704.
- Hamdali, H., Hafidi, M., Virolle, M.J., and Ouhdouch, Y. (2008). Rock phosphate-

- solubilizing *Actinomycetes*: screening for plant growth-promoting activities. *World Journal of Microbiology and Biotechnology* 24(11), 2565-2575.
- Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., and Martiny, J.B. (2012). Beyond biogeographic patterns: processes shaping the microbial landscape. *Nature Reviews Microbiology* 10(7), 497-506. doi: 10.1038/nrmicro2795.
- Harrison, S., and Cornell, H. (2008). Toward a better understanding of the regional causes of local community richness. *Ecology Letters* 11(9), 969-979.
- Hättenschwiler, S., Tiunov, A.V., and Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 191-218.
- Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J.F., Kaufman, D.M., et al. (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology* 84(12), 3105-3117.
- He, H.S., Hao, Z., Mladenoff, D.J., Shao, G., Hu, Y., and Chang, Y. (2005). Simulating forest ecosystem response to climate warming incorporating spatial effects in north-eastern China. *Journal of Biogeography* 32(12), 2043-2056.
- He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., et al. (2007). GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *The ISME Journal* 1(1), 67.
- Heaney, L.R. (2001). Small mammal diversity along elevational gradients in the Philippines: an assessment of patterns and hypotheses. *Global Ecology and Biogeography* 10(1), 15-39.
- Hendershot, J.N., Read, Q.D., Henning, J.A., Sanders, N.J., and Classen, A.T. (2017). Consistently inconsistent drivers of patterns of microbial diversity and abundance at macroecological scales. *Ecology* 98(7), 1757-1763.
- Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., and Andersson, A.F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME Journal* 5(10), 1571-1579.

- Hermans, S.M., Buckley, H.L., Case, B.S., Curran-Cournane, F., Taylor, M., and Lear, G. (2017). Bacteria as emerging indicators of soil condition. *Applied and Environmental Microbiology* 83(1), e02826-02816. doi: 10.1128/AEM.02826-16.
- Hewson, I., Steele, J.A., Capone, D.G., and Fuhrman, J.A. (2006). Temporal and spatial scales of variation in bacterioplankton assemblages of oligotrophic surface waters. *Marine Ecology Progress Series* 311, 67-77.
- Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *The American Naturalist* 163(2), 192-211.
- Hillebrand, H., Soininen, J., and Snoeijs, P. (2010). Warming leads to higher species turnover in a coastal ecosystem. *Global Change Biology* 16(4), 1181-1193.
- Hillebrand, H., Watermann, F., Karez, R., and Berninger, U.G. (2001). Differences in species richness patterns between unicellular and multicellular organisms. *Oecologia* 126(1), 114-124.
- Hiscox, J., Clarkson, G., Savoury, M., Powell, G., Savva, I., Lloyd, M., et al. (2016). Effects of pre-colonisation and temperature on interspecific fungal interactions in wood. *Fungal Ecology* 21, 32-42. doi: 10.1016/j.funeco.2016.01.011.
- Holyoak, M., Leibold, M.A., and Holt, R.D. (2005). *Metacommunities: spatial dynamics and ecological communities*. University of Chicago Press.
- Horner-Devine, M.C., Lage, M., Hughes, J.B., and Bohannan, B.J. (2004). A taxa–area relationship for bacteria. *Nature* 432(7018), 750-753.
- Hortal, J., Rodríguez, J., Nieto-Díaz, M., and Lobo, J.M. (2008). Regional and environmental effects on the species richness of mammal assemblages. *Journal of Biogeography* 35(7), 1202-1214.
- Hubbell, S.P. (2001). *The unified neutral theory of biodiversity and biogeography (Monographs in population biology)*.
- Hug, K., Maher, W.A., Stott, M.B., Krikowa, F., Foster, S., and Moreau, J.W. (2014). Microbial contributions to coupled arsenic and sulfur cycling in the acid-sulfide

- hot spring Champagne Pool, New Zealand. *Frontiers in Microbiology* 5, 569.
- Hugenholtz, P., and Tyson, G.W. (2008). Microbiology: metagenomics. *Nature* 455(7212), 481.
- Hui, L., Xiao-Qin, W., Jia-Hong, R., and Jian-Ren, Y. (2011). Isolation and identification of phosphobacteria in poplar rhizosphere from different regions of China. *Pedosphere* 21(1), 90-97.
- Hutchison, M., Walters, L., Moore, A., Crookes, K., and Avery, S. (2004). Effect of length of time before incorporation on survival of pathogenic bacteria present in livestock wastes applied to agricultural soil. *Applied and Environmental Microbiology* 70(9), 5111-5118.
- Illumina (2013). "16S Metagenomic Sequencing Library Preparation". Illumina Inc.
- Ingham, E.R. (2011). Soil biology. *United States Department of Agriculture Natural Resources Conservation Service. Web* 19.
- Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C.F., et al. (2009). Ecological networks—beyond food webs. *Journal of Animal Ecology* 78(1), 253-269.
- Ives, A.R., and Cardinale, B.J. (2004). Food-web interactions govern the resistance of communities after non-random extinctions. *Nature* 429(6988), 174-177.
- Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., et al. (2008). Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biology and Biochemistry* 40(11), 2843-2853. doi: 10.1016/j.soilbio.2008.07.030.
- Jarvis, S.G., Woodward, S., and Taylor, A.F. (2015). Strong altitudinal partitioning in the distributions of ectomycorrhizal fungi along a short (300 m) elevation gradient. *New Phytologist* 206(3), 1145-1155.
- Jesus, E.D., Marsh, T.L., Tiedje, J.M., and Moreira, F.M.D. (2009). Changes in land use alter the structure of bacterial communities in Western Amazon soils. *The ISME*

- Journal* 3(9), 1004-1011. doi: 10.1038/ismej.2009.47.
- Johnson, M., Lee, K., and Scow, K. (2003). DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. *Geoderma* 114(3-4), 279-303.
- Jongbloed, R.H., and Borstpauwels, G. (1990). Effects of ammonium and pH on growth of some ectomycorrhizal fungi *in vitro*. *Acta Botanica Neerlandica* 39(4), 349-358.
- Jump, A.S., Matyas, C., and Penuelas, J. (2009). The altitude-for-latitude disparity in the range retractions of woody species. *Trends in Ecology & Evolution* 24(12), 694-701. doi: 10.1016/j.tree.2009.06.007.
- Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* 28(1), 27-30.
- Kara, E.L., Hanson, P.C., Hu, Y.H., Winslow, L., and McMahon, K.D. (2013). A decade of seasonal dynamics and co-occurrences within freshwater bacterioplankton communities from eutrophic Lake Mendota, WI, USA. *The ISME Journal* 7(3), 680-684.
- Kassambara, A. (2017). ggpubr:“ggplot2” based publication ready plots. *Version 0.1 2*.
- Keiser, A.D., and Bradford, M.A. (2017). Climate masks decomposer influence in a cross-site litter decomposition study. *Soil Biology and Biochemistry* 107, 180-187. doi: 10.1016/j.soilbio.2016.12.022.
- Keller, G. (1996). Utilization of inorganic and organic nitrogen sources by high-subalpine ectomycorrhizal fungi of *Pinus cembra* in pure culture. *Mycological Research* 100(8), 989-998.
- Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., et al. (2015). Climate change impacts on bumblebees converge across continents. *Science* 349(6244), 177-180.
- Kessler, M., Herzog, S.K., Fjeldså, J., and Bach, K. (2001). Species richness and endemism of plant and bird communities along two gradients of elevation,

- humidity and land use in the Bolivian Andes. *Diversity and Distributions* 7(1-2), 61-77.
- King, A.J., Freeman, K.R., McCormick, K.F., Lynch, R.C., Lozupone, C., Knight, R., et al. (2010). Biogeography and habitat modelling of high-alpine bacteria. *Nature Communications* 1, 53. doi: 10.1038/ncomms1055.
- Klappenbach, J.A., Dunbar, J.M., and Schmidt, T.M. (2000). rRNA operon copy number reflects ecological strategies of bacteria. *Applied and Environmental Microbiology* 66(4), 1328-1333. doi: 10.1128/aem.66.4.1328-1333.2000.
- Kluge, J., Kessler, M., and Dunn, R.R. (2006). What drives elevational patterns of diversity? A test of geometric constraints, climate and species pool effects for pteridophytes on an elevational gradient in Costa Rica. *Global Ecology and Biogeography* 15(4), 358-371. doi: 10.1111/j.1466-822X.2006.00223.x.
- Kohler, F., Hamelin, J., Gillet, F., Gobat, J.-M., and Buttler, A. (2005). Soil microbial community changes in wooded mountain pastures due to simulated effects of cattle grazing. *Plant and Soil* 278(1-2), 327-340.
- Konstantinidis, K.T., and Tiedje, J.M. (2004). Trends between gene content and genome size in prokaryotic species with larger genomes. *Proceedings of the National Academy of Sciences* 101(9), 3160-3165. doi: 10.1073/pnas.0308653100.
- Koonin, E.V. (2002). The Clusters of Orthologous Groups (COGs) Database: phylogenetic classification of proteins from complete genomes. *The NCBI Handbook*.
- Korner, C. (2000). Why are there global gradients in species richness? Mountains might hold the answer. *Trends in Ecology & Evolution* 15(12), 513-514. doi: 10.1016/s0169-5347(00)02004-8.
- Koyama, A., Wallenstein, M.D., Simpson, R.T., and Moore, J.C. (2014). Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Frontiers in Microbiology* 5, 516.
- Kranabetter, J.M., Dawson, C.R., and Dunn, D.E. (2007). Indices of dissolved organic

- nitrogen, ammonium and nitrate across productivity gradients of boreal forests. *Soil Biology and Biochemistry* 39(12), 3147-3158.
- Kranabetter, J.M., Hawkins, B.J., Jones, M.D., Robbins, S., Dyer, T., and Li, T. (2015). Species turnover (beta-diversity) in ectomycorrhizal fungi linked to NH_4^+ uptake capacity. *Molecular Ecology* 24(23), 5992-6005. doi: 10.1111/mec.13435.
- Krause, A.E., Frank, K.A., Mason, D.M., Ulanowicz, R.E., and Taylor, W.W. (2003). Compartments revealed in food-web structure. *Nature* 426(6964), 282-285.
- Krause, S., Le Roux, X., Niklaus, P.A., Van Bodegom, P.M., Lennon, J.T., Bertilsson, S., et al. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Frontiers in Microbiology* 5, 251.
- Kreft, H., and Jetz, W. (2007). Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences* 104(14), 5925-5930.
- Kubota, U., Loyola, R.D., Almeida, A.M., Carvalho, D.A., and Lewinsohn, T.M. (2007). Body size and host range co-determine the altitudinal distribution of Neotropical tephritid flies. *Global Ecology and Biogeography* 16(5), 632-639.
- Kuramae, E.E., Yergeau, E., Wong, L.C., Pijl, A.S., van Veen, J.A., and Kowalchuk, G.A. (2012). Soil characteristics more strongly influence soil bacterial communities than land-use type. *FEMS Microbiology Ecology* 79(1), 12-24. doi: 10.1111/j.1574-6941.2011.01192.x.
- Lachat Instruments, I. (1998a). *Quik Chem Method 13-107-06-2-D*. Milwaukee, Wisconsin, USA.
- Lachat Instruments, I. (1998b). *Quik Chem Method 13-115-01-1-B*. Milwaukee, Wisconsin, USA.
- Land, M., Hauser, L., Jun, S.-R., Nookaew, I., Leuze, M.R., Ahn, T.-H., et al. (2015). Insights from 20 years of bacterial genome sequencing. *Functional and Integrative Genomics* 15(2), 141-161.
- Lanzen, A., Epelde, L., Blanco, F., Martin, I., Artetxe, U., and Garbisu, C. (2016).

- Multi-targeted metagenetic analysis of the influence of climate and environmental parameters on soil microbial communities along an elevational gradient. *Scientific Reports* 6, 28257. doi: 10.1038/srep28257.
- Lau, K.E., Washington, V.J., Fan, V., Neale, M.W., Lear, G., Curran, J., et al. (2015). A novel bacterial community index to assess stream ecological health. *Freshwater Biology* 60(10), 1988-2002.
- Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75(15), 5111-5120. doi: 10.1128/AEM.00335-09.
- Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* 40(9), 2407-2415. doi: 10.1016/j.soilbio.2008.05.021.
- Lavorel, S., and Garnier, É. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* 16(5), 545-556.
- Lawton, J.H. (1999). Are there general laws in ecology? *Oikos*, 177-192.
- Lear, G., Dickie, I.A., Banks, J.C., Boyer, S., Buckley, H.L., Buckley, T.R., et al. (2017a). Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples. *New Zealand Journal of Ecology* 41(1), 10–50A.
- Lear, G., Lau, K., Perchec, A.M., Buckley, H.L., Case, B.S., Neale, M., et al. (2017b). Following Rapoport's Rule: the geographic range and genome size of bacterial taxa decline at warmer latitudes. *Environmental Microbiology* 19(8), 3152-3162. doi: 10.1111/1462-2920.13797.
- Lear, G., Washington, V., Neale, M., Case, B., Buckley, H., and Lewis, G. (2013). The biogeography of stream bacteria. *Global Ecology and Biogeography* 22(5), 544-554.

- Leco. 2003. Total/organic carbon and nitrogen in soils. *LECO Corporation, St. Joseph, MO, Organic Application Note 203-821-165*.
- Lee, P.F., Ding, T.S., Hsu, F.H., and Geng, S. (2004). Breeding bird species richness in Taiwan: distribution on gradients of elevation, primary productivity and urbanization. *Journal of Biogeography* 31(2), 307-314.
- Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L., et al. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences* 112(35), 10967-10972. doi: 10.1073/pnas.1508382112.
- Legendre, P., and Anderson, M.J. (1999). Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69(1), 1-24.
- Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., et al. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7(7), 601-613.
- Lemon, J. (2006). Plotrix: a package in the red light district of R. *R-news* 6(4), 8-12.
- Lenoir, J., Gégout, J.C., Marquet, P., De Ruffray, P., and Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. *Science* 320(5884), 1768-1771.
- Lewthwaite, J.M.M., Debinski, D.M., and Kerr, J.T. (2017). High community turnover and dispersal limitation relative to rapid climate change. *Global Ecology and Biogeography* 26(4), 459-471. doi: 10.1111/geb.12553.
- Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J.M., et al. (2015). Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *The ISME Journal* 9(11), 2490.
- Li, L., Wang, Z., Zerbe, S., Abdusalih, N., Tang, Z., Ma, M., et al. (2013). Species richness patterns and water-energy dynamics in the drylands of northwest China. *PLOS One* 8(6), e66450.

- Li, P., Lang, M., Li, C., Thomas, B.W., and Hao, X.Y. (2016). Nutrient leaching from soil amended with manure and compost from cattle fed diets containing wheat dried distillers' grains with solubles. *Water, Air and Soil Pollution* 227(10), 393.
- Liaho, O. (1970). Paxillus involutus as a mycorrhizal symbiont of forest trees. *Acta Forestalia Fennica* (106).
- Liang, C., Gutknecht, J., and Balser, T. (2015). Microbial lipid and amino sugar responses to long-term simulated global environmental changes in a California annual grassland. *Frontiers in Microbiology* 6, 385.
- Lim, C.H., and Flint, K. (1989). The effects of nutrients on the survival of *Escherichia coli* in lake water. *Journal of Applied Microbiology* 66(6), 559-569.
- Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., et al. (2015). Determinants of community structure in the global plankton interactome. *Science* 348(6237), 1262073.
- Lindsay, E.A., and Cunningham, S.A. (2009). Livestock grazing exclusion and microhabitat variation affect invertebrates and litter decomposition rates in woodland remnants. *Forest Ecology and Management* 258(2), 178-187.
- Lindström, E.S., and Langenheder, S. (2012). Local and regional factors influencing bacterial community assembly. *Environmental Microbiology Reports* 4(1), 1-9.
- Linnaeus, C. (1781). On the increase of the habitable earth. *Amoenitates Academicæ* 2, 17-27.
- Litchman, E. (2010). Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecology Letters* 13(12), 1560-1572. doi: 10.1111/j.1461-0248.2010.01544.x.
- Litchman, E., Edwards, K.F., and Klausmeier, C.A. (2015). Microbial resource utilization traits and trade-offs: implications for community structure, functioning, and biogeochemical impacts at present and in the future. *Frontiers in Microbiology* 6, 254.
- Liu, L., Hart, M.M., Zhang, J., Cai, X., Gai, J., Christie, P., et al. (2015). Altitudinal

- distribution patterns of AM fungal assemblages in a Tibetan alpine grassland. *FEMS Microbiology Ecology* 91(7). doi: 10.1093/femsec/fiv078.
- Liu, Y., Shi, G., Mao, L., Cheng, G., Jiang, S., Ma, X., et al. (2012). Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on *Glomeromycota* in an alpine meadow ecosystem. *New Phytologist* 194(2), 523-535.
- Logue, J.B., Findlay, S.E., and Comte, J. (2015). Editorial: Microbial responses to environmental changes. *Frontiers in Microbiology* 6, 1364. doi: 10.3389/fmicb.2015.01364.
- Lomolino, M. (2001). Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography* 10(1), 3-13.
- Lomolino, M., Riddle, B., and Brown, J. (2006). Biogeography, 3rd edn Sinauer Associates. *Sunderland, MA, USA* 752.
- Lomolino, M., Riddle, B., Whittaker, R., and Brown, J. (2010). Biogeography. Sinauer. *Sunderland, MA, USA*.
- Looby, C.I., Maltz, M.R., and Treseder, K.K. (2016). Belowground responses to elevation in a changing cloud forest. *Ecology and Evolution* 6(7), 1996-2009.
- Lopez-Madrigal, S., Latorre, A., Porcar, M., Moya, A., and Gil, R. (2011). Complete genome sequence of "*Candidatus Tremblaya princeps*" strain PCVAL, an intriguing translational machine below the living-cell status. *Journal of Bacteriology* 193(19), 5587-5588. doi: 10.1128/JB.05749-11.
- Loreau, M., Naeem, S., and Inchausti, P. (2002). *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press on Demand.
- Lozupone, C.A., and Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences* 104(27), 11436-11440. doi: 10.1073/pnas.0611525104.
- Lu, J., Sanchez, S., Hofacre, C., Maurer, J.J., Harmon, B.G., and Lee, M.D. (2003). Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. *Applied and*

Environmental Microbiology 69(2), 901-908.

- Lugo, M.A., Ferrero, M., Menoyo, E., Estevez, M.C., Sineriz, F., and Anton, A. (2008). Arbuscular mycorrhizal fungi and rhizospheric bacteria diversity along an altitudinal gradient in South American Puna grassland. *Microbial Ecology* 55(4), 705-713. doi: 10.1007/s00248-007-9313-3.
- Lundeberg, G. (1970). Utilisation of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi.
- Lupatini, M., Suleiman, A.K.A., Jacques, R.J.S., Antoniolli, Z.I., de Siqueira Ferreira, A.o., Kuramae, E.E., et al. (2014). Network topology reveals high connectance levels and few key microbial genera within soils. *Frontiers in Environmental Science* 2. doi: 10.3389/fenvs.2014.00010.
- Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z., et al. (2016). Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *The ISME Journal* 10(8), 1891-1901. doi: 10.1038/ismej.2015.261.
- Ma, X., Wang, S., Wang, Y., Jiang, G., and Nyren, P. (2006). Short-term effects of sheep excrement on carbon dioxide, nitrous oxide and methane fluxes in typical grassland of Inner Mongolia. *New Zealand Journal of Agricultural Research* 49(3), 285-297.
- MacArthur, R.H. (1984). *Geographical ecology: patterns in the distribution of species*. Princeton University Press.
- MacArthur, R.H., and Wilson, E.O. (1967). *The theory of island biogeography*. Princeton University Press.
- Madsen, E.L. (2011). Microorganisms and their roles in fundamental biogeochemical cycles. *Current Opinion in Biotechnology* 22(3), 456-464.
- Maggi, O., Persiani, A.M., Casado, M.A., and Pineda, F.D. (2005). Effects of elevation, slope position and livestock exclusion on microfungi isolated from soils of Mediterranean grasslands. *Mycologia* 97(5), 984-995.

- Mamakani, K., Myrvold, W.J., and Ruskey, F. (2011). "Generating all simple convexly-drawable polar symmetric 6-venn diagrams", in: *International Workshop on Combinatorial Algorithms*, 275-286.
- Mandakovic, D., Rojas, C., Maldonado, J., Latorre, M., Travisany, D., Delage, E., et al. (2018). Structure and co-occurrence patterns in microbial communities under acute environmental stress reveal ecological factors fostering resilience. *Scientific Reports* 8(1), 5875. doi: 10.1038/s41598-018-23931-0.
- Mander, C., Wakelin, S., Young, S., Condrón, L., and O'Callaghan, M. (2012). Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. *Soil Biology and Biochemistry* 44(1), 93-101.
- Mao, S., Zhang, M., Liu, J., and Zhu, W. (2015). Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. *Scientific Reports* 5, 16116. doi: 10.1038/srep16116.
- Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., et al. (2005). Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437(7057), 376-380.
- Martiny, J.B., Bohannan, B.J., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., et al. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology* 4(2), 102-112. doi: 10.1038/nrmicro1341.
- Martiny, J.B., Jones, S.E., Lennon, J.T., and Martiny, A.C. (2015). Microbiomes in light of traits: a phylogenetic perspective. *Science* 350(6261), aac9323.
- Matz, C., and Jürgens, K. (2005). High motility reduces grazing mortality of planktonic bacteria. *Applied and Environmental Microbiology* 71(2), 921-929.
- Mau, R.L., Liu, C.M., Aziz, M., Schwartz, E., Dijkstra, P., Marks, J.C., et al. (2015). Linking soil bacterial biodiversity and soil carbon stability. *The ISME Journal* 9(6), 1477-1480. doi: 10.1038/ismej.2014.205.
- Maule, A. (2000). Survival of verocytotoxigenic *Escherichia coli* O157 in soil, water

- and on surfaces. *Journal of Applied Microbiology* 88(S1), 71S-78S.
- Mazerolle, M.J. (2013). AICcmodavg: model selection and multimodel inference based on (Q) AIC (c). *R package version* 1, 35.
- McCain, C.M. (2004). The mid-domain effect applied to elevational gradients: species richness of small mammals in Costa Rica. *Journal of Biogeography* 31(1), 19-31.
- McCain, C.M. (2005). Elevational gradients in diversity of small mammals. *Ecology* 86(2), 366-372.
- McCain, C.M. (2006). Could temperature and water availability drive elevational species richness patterns? A global case study for bats. *Global Ecology and Biogeography* 0(0), 1-13. doi: 10.1111/j.1466-822X.2006.00263.x.
- McCain, C.M., and Grytnes, J.A. 2010. Elevational gradients in species richness. *Encyclopedia of Life Sciences*.
- McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., et al. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal* 6(3), 610-618.
- McDowell, R. (2012). The rate of accumulation of cadmium and uranium in a long-term grazed pasture: implications for soil quality. *New Zealand Journal of Agricultural Research* 55(2), 133-146.
- McDowell, R., Taylor, M., and Stevenson, B. (2013). Natural background and anthropogenic contributions of cadmium to New Zealand soils. *Agriculture, Ecosystems & Environment* 165, 80-87.
- McGill, B.J., Enquist, B.J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution* 21(4), 178-185. doi: 10.1016/j.tree.2006.02.002.
- Meier, C.L., Rapp, J., Bowers, R.M., Silman, M., and Fierer, N. (2010). Fungal growth on a common wood substrate across a tropical elevation gradient: Temperature

- sensitivity, community composition, and potential for above-ground decomposition. *Soil Biology and Biochemistry* 42(7), 1083-1090. doi: 10.1016/j.soilbio.2010.03.005.
- Melián, C.J., and Bascompte, J. (2002). Food web structure and habitat loss. *Ecology Letters* 5(1), 37-46.
- Meng, H., Li, K., Nie, M., Wan, J.R., Quan, Z.X., Fang, C.M., et al. (2013). Responses of bacterial and fungal communities to an elevation gradient in a subtropical montane forest of China. *Applied Microbiology and Biotechnology* 97(5), 2219-2230. doi: 10.1007/s00253-012-4063-7.
- Merriam, C.H. (1890). Results of a biological survey of the San Francisco Mountain region and desert of the Little Colorado in Arizona. *North American Fauna*, 1-4.
- Mitsui, H., Gorlach, K., Lee, H.J., Hattori, R., and Hattori, T. (1997). Incubation time and media requirements of culturable bacteria from different phylogenetic groups. *Journal of Microbiological Methods* 30(2), 103-110. doi: 10.1016/s0167-7012(97)00052-3.
- Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., et al. (2007). Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters* 10(4), 315-331.
- Miyamoto, Y., Nakano, T., Hattori, M., and Nara, K. (2014). The mid-domain effect in ectomycorrhizal fungi: range overlap along an elevation gradient on Mount Fuji, Japan. *The ISME Journal* 8(8), 1739-1746. doi: 10.1038/ismej.2014.34.
- Mongodin, E.F., Shapir, N., Daugherty, S.C., DeBoy, R.T., Emerson, J.B., Shvartzbeyn, A., et al. (2006). Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. *PLOS Genetics* 2(12), e214. doi: 10.1371/journal.pgen.0020214.
- Morohoshi, T., Wang, W.Z., Someya, N., and Ikeda, T. (2011). Genome sequence of *Microbacterium testaceum* StLB037, an N-acylhomoserine lactone-degrading bacterium isolated from potato leaves. *Journal of Bacteriology* 193(8), 2072-

2073. doi: 10.1128/JB.00180-11.

Murty, D., Kirschbaum, M.U., Mcmurtrie, R.E., and Mcgilvray, H. (2002). Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. *Global Change Biology* 8(2), 105-123.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., and Renella, G. (2003). Microbial diversity and soil functions. *European Journal of Soil Science* 54(4), 655-670.

Navarrete, A.A., Cannavan, F.S., Taketani, R.G., and Tsai, S.M. (2010). A molecular survey of the diversity of microbial communities in different Amazonian agricultural model systems. *Diversity* 2(5), 787-809.

NCBI Resource Coordinators (2017). Database resources of the national center for biotechnology information. *Nucleic Acids Research* 45(D1), D12-D17. doi: 10.1093/nar/gkw1071.

Nekola, J.C., and White, P.S. (1999). The distance decay of similarity in biogeography and ecology. *Journal of Biogeography* 26(4), 867-878.

Newman, M.E. (2003). The structure and function of complex networks. *SIAM review* 45(2), 167-256.

Ney-Nifle, M., and Mangel, M. (2000). Habitat loss and changes in the species-area relationship. *Conservation Biology* 14(3), 893-898.

Nielsen, C.B., Groffman, P.M., Hamburg, S.P., Driscoll, C.T., Fahey, T.J., and Hardy, J.P. (2001). Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. *Soil Science Society of America Journal* 65(6), 1723-1730.

Novotny, V., Miller, S.E., Hulcr, J., Drew, R.A., Basset, Y., Janda, M., et al. (2007). Low beta diversity of herbivorous insects in tropical forests. *Nature* 448(7154), 692-695.

O'Brien, E.M. (2006). Biological relativity to water–energy dynamics. *Journal of Biogeography* 33(11), 1868-1888.

- O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.M., and Vilgalys, R. (2005). Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* 71(9), 5544-5550. doi: 10.1128/AEM.71.9.5544-5550.2005.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al. (2015). Package 'vegan'. *Community Ecology Package, version, 2.2-1*.
- Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E., and Fausch, K.D. (2004). Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution* 19(1), 18-24.
- Oliveira, C., Alves, V., Marriel, I., Gomes, E., Scotti, M., Carneiro, N., et al. (2009). Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry* 41(9), 1782-1787.
- Overbeek, R., Disz, T., and Stevens, R. (2004). The SEED: a peer-to-peer environment for genome annotation. *Communications of the ACM* 47(11), 46-51.
- Patra, A., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S., Loiseau, P., et al. (2005). Effects of grazing on microbial functional groups involved in soil N dynamics. *Ecological Monographs* 75(1), 65-80.
- Patterson, B., Pacheco, V., and Solari, S. (1996). Distribution of bats along an elevational gradient in the Andes of south-eastern Peru. *Journal of Zoology* 240(4), 637-658.
- Paula, F.S., Rodrigues, J.L., Zhou, J., Wu, L., Mueller, R.C., Mirza, B.S., et al. (2014). Land use change alters functional gene diversity, composition and abundance in Amazon forest soil microbial communities. *Molecular Ecology* 23(12), 2988-2999.
- Peay, K.G., Bruns, T.D., Kennedy, P.G., Bergemann, S.E., and Garbelotto, M. (2007). A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters* 10(6), 470-480. doi: 10.1111/j.1461-0248.2007.01035.x.

- Peay, K.G., von Sperber, C., Cardarelli, E., Toju, H., Francis, C.A., Chadwick, O.A., et al. (2017). Convergence and contrast in the community structure of Bacteria, Fungi and Archaea along a tropical elevation-climate gradient. *FEMS Microbiology Ecology* 93(5). doi: 10.1093/femsec/fix045.
- Pedros-Alio, C. (2006). Genomics and marine microbial ecology. *International Microbiology* 9(3), 191-197.
- Pellissier, L., Alvarez, N., Espíndola, A., Pottier, J., Dubuis, A., Pradervand, J.N., et al. (2013). Phylogenetic alpha and beta diversities of butterfly communities correlate with climate in the western Swiss Alps. *Ecography* 36(5), 541-550.
- Pellissier, L., Fiedler, K., Ndiribe, C., Dubuis, A., Pradervand, J.N., Guisan, A., et al. (2012). Shifts in species richness, herbivore specialization, and plant resistance along elevation gradients. *Ecology and Evolution* 2(8), 1818-1825.
- Pellissier, L., Niculita-Hirzel, H., Dubuis, A., Pagni, M., Guex, N., Ndiribe, C., et al. (2014). Soil fungal communities of grasslands are environmentally structured at a regional scale in the Alps. *Molecular Ecology* 23(17), 4274-4290.
- Peng, R.H., Xiong, A.S., Xue, Y., Fu, X.Y., Gao, F., Zhao, W., et al. (2008). Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiology Reviews* 32(6), 927-955.
- Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., et al. (2010). The ecological coherence of high bacterial taxonomic ranks. *Nature Reviews Microbiology* 8(7), 523-529. doi: 10.1038/nrmicro2367.
- Pianka, E.R. (1966). Latitudinal gradients in species diversity: a review of concepts. *The American Naturalist* 100(910), 33-46.
- Pickles, B.J., Genney, D.R., Anderson, I.C., and Alexander, I.J. (2012). Spatial analysis of ectomycorrhizal fungi reveals that root tip communities are structured by competitive interactions. *Molecular Ecology* 21(20), 5110-5123. doi: 10.1111/j.1365-294X.2012.05739.x.
- Podar, M., Gilmour, C.C., Brandt, C.C., Soren, A., Brown, S.D., Crable, B.R., et al.

- (2015). Global prevalence and distribution of genes and microorganisms involved in mercury methylation. *Science Advances* 1(9), e1500675.
- Pommier, T., Canbäck, B., Riemann, L., Boström, K., Simu, K., Lundberg, P., et al. (2007). Global patterns of diversity and community structure in marine bacterioplankton. *Molecular Ecology* 16(4), 867-880.
- Pope, P.B., Smith, W., Denman, S.E., Tringe, S.G., Barry, K., Hugenholtz, P., et al. (2011). Isolation of *Succinivibrionaceae* implicated in low methane emissions from Tammar wallabies. *Science* 333(6042), 646-648. doi: 10.1126/science.1205760.
- Post, W.M., and Mann, L. (1990). Changes in soil organic carbon and nitrogen as a result of cultivation. *Soils and the Greenhouse Effect*, 401-406.
- Prestat, E., David, M.M., Hultman, J., Tas, N., Lamendella, R., Dvornik, J., et al. (2014). FOAM (Functional Ontology Assignments for Metagenomes): a Hidden Markov Model (HMM) database with environmental focus. *Nucleic Acids Research* 42(19), e145. doi: 10.1093/nar/gku702.
- Preston, F.W. (1962). The canonical distribution of commonness and rarity: Part II. *Ecology* 43(3), 410-432.
- Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., et al. (2014). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters* 18(1), 85-95.
- Prosser, J.I., Bohannon, B.J., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., et al. (2007). The role of ecological theory in microbial ecology. *Nature Reviews Microbiology* 5(5), 384-392.
- Proulx, S.R., Promislow, D.E., and Phillips, P.C. (2005). Network thinking in ecology and evolution. *Trends in Ecology & Evolution* 20(6), 345-353.
- Qian, H., and Ricklefs, R.E. (2007). A latitudinal gradient in large-scale beta diversity for vascular plants in North America. *Ecology Letters* 10(8), 737-744.
- R Core Team (2015). R: A language and environment for statistical computing. R

Foundation for Statistical Computing, Vienna, Austria, [http://www/R-project.org/](http://www.R-project.org/).

- Radl, V., Gättinger, A., Chroňáková, A., Němcová, A., Čuhel, J., Šimek, M., et al. (2007). Effects of cattle husbandry on abundance and activity of methanogenic archaea in upland soils. *The ISME Journal* 1(5), 443-452.
- Raes, J., Letunic, I., Yamada, T., Jensen, L.J., and Bork, P. (2011). Toward molecular trait-based ecology through integration of biogeochemical, geographical and metagenomic data. *Molecular Systems Biology* 7, 473. doi: 10.1038/msb.2011.6.
- Rahbek, C. (1995). The elevational gradient of species richness: a uniform pattern? *Ecography* 18(2), 200-205.
- Rahbek, C. (2004). The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters* 8(2), 224-239. doi: 10.1111/j.1461-0248.2004.00701.x.
- Rahbek, C. (2005). The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology letters* 8(2), 224-239.
- Rajaniemi, T.K. (2003). Explaining productivity-diversity relationships in plants. *Oikos* 101(3), 449-457.
- Ramette, A., and Tiedje, J.M. (2007). Biogeography: an emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. *Microbial Ecology* 53(2), 197-207.
- Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., and Fierer, N. (2010). Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* 91(12), 3463-3470.
- Ramirez, L., Diniz-Filho, J.A.F., and Hawkins, B.A. (2008). Partitioning phylogenetic and adaptive components of the geographical body-size pattern of New World birds. *Global Ecology and Biogeography* 17(1), 100-110.
- Randin, C.F., Engler, R., Normand, S., Zappa, M., Zimmermann, N.E., Pearman, P.B., et al. (2009). Climate change and plant distribution: local models predict high-

- elevation persistence. *Global Change Biology* 15(6), 1557-1569.
- Rangel-Castro, I.J., Danell, E., and Taylor, A.F. (2002). Use of different nitrogen sources by the edible ectomycorrhizal mushroom *Cantharellus cibarius*. *Mycorrhiza* 12(3), 131-137.
- Rapoport, A. (1982). *The meaning of the built environment: A nonverbal communication approach*. University of Arizona Press.
- Rapoport, E.H. (1975). *Aerografía: estrategias geográficas de las especies*. Fondo de Cultura Económica Mexico City, Mexico.
- Rastin, N., Schlechte, G., and Hüttermann, A. (1990). Soil macrofungi and some soil biological, biochemical and chemical investigations on the upper and lower slope of a spruce forest. *Soil Biology and Biochemistry* 22(8), 1039-1047.
- Ravasz, E., Somera, A.L., Mongru, D.A., Oltvai, Z.N., and Barabási, A.L. (2002). Hierarchical organization of modularity in metabolic networks. *Science* 297(5586), 1551-1555.
- Rayment, G.E., and Lyons, D.J. (2011). *Soil chemical methods: Australasia*. CSIRO publishing.
- Reche, I., Pulido-Villena, E., Morales-Baquero, R., and Casamayor, E.O. (2005). Does ecosystem size determine aquatic bacterial richness? *Ecology* 86(7), 1715-1722.
- Renella, G., Mench, M., Landi, L., and Nannipieri, P. (2005). Microbial activity and hydrolase synthesis in long-term Cd-contaminated soils. *Soil Biology and Biochemistry* 37(1), 133-139.
- Richards, T.A., Vepritskiy, A.A., Gouliamova, D.E., and Nierzwicki-Bauer, S.A. (2005). The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake reveals diverse, distinctive and globally dispersed lineages. *Environmental Microbiology* 7(9), 1413-1425.
- Richardson, A.E., and Simpson, R.J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology* 156(3), 989-996.

- Rickart, E.A. (2001). Elevational diversity gradients, biogeography and the structure of montane mammal communities in the intermountain region of North America. *Global Ecology and Biogeography* 10(1), 77-100.
- Riesenfeld, C.S., Schloss, P.D., and Handelsman, J. (2004). Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics* 38, 525-552. doi: 10.1146/annurev.genet.38.072902.091216.
- Rinnan, R., Rousk, J., Yergeau, E., Kowalchuk, G.A., and Bååth, E. (2009). Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. *Global Change Biology* 15(11), 2615-2625.
- Rodrigues, J.L.M., Pellizari, V.H., Mueller, R., Baek, K., Jesus, E.D., Paula, F.S., et al. (2013). Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. *Proceedings of the National Academy of Sciences* 110(3), 988-993. doi: 10.1073/pnas.1220608110.
- Rodríguez, M.Á., Belmontes, J.A., and Hawkins, B.A. (2005). Energy, water and large-scale patterns of reptile and amphibian species richness in Europe. *Acta Oecologica* 28(1), 65-70.
- Rohde, K. (1992). Latitudinal gradients in species diversity: the search for the primary cause. *Oikos*, 514-527.
- Rohner, P.T., Bächli, G., Pollini Paltrinieri, L., Duelli, P., Obrist, M.K., Jochmann, R., et al. (2015). Distribution, diversity gradients and Rapoport's elevational rule in the black scavenger flies of the Swiss Alps (Diptera: Sepsidae). *Insect Conservation and Diversity* 8(4), 367-376.
- Romero-Alcaraz, E., and Ávila, J.M. (2000). Effect of elevation and type of habitat on the abundance and diversity of Scarabaeoid dung beetle (Scarabaeoidea) assemblages in a Mediterranean area from Southern Iberian Peninsula. *Zoological Studies* 39(4), 351-359.
- Rosenzweig, M.L. (1968). The strategy of body size in mammalian carnivores. *American Midland Naturalist*, 299-315.

- Rosenzweig, M.L. (1995). *Species diversity in space and time*. Cambridge University Press.
- Rosenzweig, M.L. (2001). Loss of speciation rate will impoverish future diversity. *Proceedings of the National Academy of Sciences* 98(10), 5404-5410.
- Rothberg, J.M., Hinz, W., Rearick, T.M., Schultz, J., Mileski, W., Davey, M., et al. (2011). An integrated semiconductor device enabling non-optical genome sequencing. *Nature* 475(7356), 348-352.
- Rothstein, D.E. (2009). Soil amino-acid availability across a temperate-forest fertility gradient. *Biogeochemistry* 92(3), 201-215.
- Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., et al. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* 4(10), 1340-1351.
- Rousk, J., Brookes, P.C., and Baath, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology* 75(6), 1589-1596. doi: 10.1128/AEM.02775-08.
- Ruan, Q., Dutta, D., Schwalbach, M.S., Steele, J.A., Fuhrman, J.A., and Sun, F. (2006). Local similarity analysis reveals unique associations among marine bacterioplankton species and environmental factors. *Bioinformatics* 22(20), 2532-2538.
- Ruiz-González, C., Niño-García, J.P., Lapierre, J.F., and del Giorgio, P.A. (2015). The quality of organic matter shapes the functional biogeography of bacterioplankton across boreal freshwater ecosystems. *Global Ecology and Biogeography* 24(12), 1487-1498.
- Saito, A., Mitsui, H., Hattori, R., Minamisawa, K., and Hattori, T. (1998). Slow-growing and oligotrophic soil bacteria phylogenetically close to *Bradyrhizobium japonicum*. *FEMS Microbiology Ecology* 25(3), 277-286. doi: 10.1111/j.1574-6941.1998.tb00480.x.

- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., et al. (2000). Global biodiversity scenarios for the year 2100. *Science* 287(5459), 1770-1774.
- Sánchez-Cordero, V. (2001). Elevation gradients of diversity for rodents and bats in Oaxaca, Mexico. *Global Ecology and Biogeography* 10(1), 63-76.
- Sanchez-Rodriguez, J., and Baz, A. (1995). The effects of elevation on the butterfly communities of a Mediterranean mountain, Sierra de Javalambre, central Spain. *Journal of the Lepidopterists Society* 49(3), 192-207.
- Sanders, N.J. (2002). Elevational gradients in ant species richness: area, geometry, and Rapoport's rule. *Ecography* 25(1), 25-32.
- Sanders, N.J., Moss, J., and Wagner, D. (2003). Patterns of ant species richness along elevational gradients in an arid ecosystem. *Global Ecology and Biogeography* 12(2), 93-102.
- Sariyildiz, T., Anderson, J., and Kucuk, M. (2005). Effects of tree species and topography on soil chemistry, litter quality, and decomposition in Northeast Turkey. *Soil Biology and Biochemistry* 37(9), 1695-1706.
- Schaetzl, R.J., and Anderson, S. (2005). *Soils: Genesis and Geomorphology*. Cambridge: Cambridge Univ Press.
- Schimel, J.P., and Schaeffer, S.M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology* 3, 348. doi: 10.3389/fmicb.2012.00348.
- Schipper, L.A., Sparling, G.P., Fisk, L., Dodd, M., Power, I., and Littler, R.A. (2011). Rates of accumulation of cadmium and uranium in a New Zealand hill farm soil as a result of long-term use of phosphate fertilizer. *Agriculture, Ecosystems & Environment* 144(1), 95-101.
- Schloss, P.D., and Handelsman, J. (2004). Status of the microbial census. *Microbiology and Molecular Biology Reviews* 68(4), 686-691.
- Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., et al. (2012). Who is who in litter decomposition?

- Metaproteomics reveals major microbial players and their biogeochemical functions. *The ISME Journal* 6(9), 1749-1762.
- Schwab, C., Berry, D., Rauch, I., Rennisch, I., Ramesmayer, J., Hainzl, E., et al. (2014). Longitudinal study of murine microbiota activity and interactions with the host during acute inflammation and recovery. *The ISME Journal* 8(5), 1101-1114.
- Schwalbach, M.S., and Fuhrman, J.A. (2005). Wide-ranging abundances of aerobic anoxygenic phototrophic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR. *Limnology and Oceanography* 50(2), 620-628.
- Sekercioglu, C.H., Schneider, S.H., Fay, J.P., and Loarie, S.R. (2008). Climate change, elevational range shifts, and bird extinctions. *Conservation Biology* 22(1), 140-150.
- Selvakumar, G., Joshi, P., Mishra, P.K., Bisht, J.K., and Gupta, H.S. (2009). Mountain aspect influences the genetic clustering of psychrotolerant phosphate solubilizing Pseudomonads in the Uttarakhand Himalayas. *Current Microbiology* 59(4), 432-438. doi: 10.1007/s00284-009-9456-1.
- Serna-Chavez, H.M., Fierer, N., and Bodegom, P.M. (2013). Global drivers and patterns of microbial abundance in soil. *Global Ecology and Biogeography* 22(10), 1162-1172.
- Shade, A., and Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology* 14(1), 4-12. doi: 10.1111/j.1462-2920.2011.02585.x.
- Shade, A., Jones, S.E., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N., et al. (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *mBio* 5(4), e01371-01314.
- Shange, R.S., Ankumah, R.O., Ibekwe, A.M., Zabawa, R., and Dowd, S.E. (2012). Distinct soil bacterial communities revealed under a diversely managed agroecosystem. *PLOS One* 7(7), e40338. doi: 10.1371/journal.pone.0040338.

- Sharp, C.E., Brady, A.L., Sharp, G.H., Grasby, S.E., Stott, M.B., and Dunfield, P.F. (2014). Humboldt's spa: microbial diversity is controlled by temperature in geothermal environments. *The ISME Journal* 8(6), 1166-1174.
- Shen, C., Liang, W., Shi, Y., Lin, X., Zhang, H., Wu, X., et al. (2014). Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. *Ecology* 95(11), 3190-3202.
- Shen, C., Ni, Y., Liang, W., Wang, J., and Chu, H. (2015). Distinct soil bacterial communities along a small-scale elevational gradient in alpine tundra. *Frontiers in Microbiology* 6, 582. doi: 10.3389/fmicb.2015.00582.
- Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., et al. (2013). Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biology and Biochemistry* 57, 204-211. doi: 10.1016/j.soilbio.2012.07.013.
- Shokralla, S., Spall, J.L., Gibson, J.F., and Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology* 21(8), 1794-1805.
- Sikorski, J., Brambilla, E., Kroppenstedt, R.M., and Tindall, B.J. (2008). The temperature-adaptive fatty acid content in *Bacillus simplex* strains from 'Evolution Canyon', Israel. *Microbiology* 154(8), 2416-2426.
- Siles, J.A., and Margesin, R. (2016). Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: What are the driving factors? *Microbial Ecology* 72(1), 207-220. doi: 10.1007/s00248-016-0748-2.
- Siles, J.A., and Margesin, R. (2017). Seasonal soil microbial responses are limited to changes in functionality at two Alpine forest sites differing in altitude and vegetation. *Scientific Reports* 7(1), 2204. doi: 10.1038/s41598-017-02363-2.
- Singh, B.K., Bardgett, R.D., Smith, P., and Reay, D. (2010). Microorganisms and climate change: feedbacks and mitigation options. *Nature Reviews Microbiology* 8(11), 779-790.

- Singh, D., Takahashi, K., Kim, M., Chun, J., and Adams, J.M. (2012). A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. *Microbial Ecology* 63(2), 429-437. doi: 10.1007/s00248-011-9900-1.
- Šizling, A.L., Storch, D., and Keil, P. (2009). Rapoport's rule, species tolerances, and the latitudinal diversity gradient: geometric considerations. *Ecology* 90(12), 3575-3586.
- Smith, A.P. (1988). Does the correlation of elevation with plant taxonomic richness vary with latitude? *Biotropica* 20(3), 259-261.
- Smith, K.F., and Gaines, S.D. (2003). Rapoport's bathymetric rule and the latitudinal species diversity gradient for Northeast Pacific fishes and Northwest Atlantic gastropods: evidence against a causal link. *Journal of Biogeography* 30(8), 1153-1159.
- Soares, B.S., Nepstad, D.C., Curran, L.M., Cerqueira, G.C., Garcia, R.A., Ramos, C.A., et al. (2006). Modelling conservation in the Amazon basin. *Nature* 440(7083), 520-523. doi: 10.1038/nature04389.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., et al. (2006). Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proceedings of the National Academy of Sciences* 103(32), 12115-12120.
- Soininen, J. (2012). Macroecology of unicellular organisms-patterns and processes. *Environmental Microbiology Reports* 4(1), 10-22. doi: 10.1111/j.1758-2229.2011.00308.x.
- Soininen, J., Korhonen, J.J., Karhu, J., and Vetterli, A. (2011). Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. *Limnology and Oceanography* 56(2), 508-520.
- Soininen, J., McDonald, R., and Hillebrand, H. (2007). The distance decay of similarity in ecological communities. *Ecography* 30(1), 3-12.
- Sørensen, L.I., Mikola, J., Kytöviita, M.-M., and Olofsson, J. (2009). Trampling and

- spatial heterogeneity explain decomposer abundances in a sub-arctic grassland subjected to simulated reindeer grazing. *Ecosystems* 12(5), 830-842.
- Spooner, P., Lunt, I., and Robinson, W. (2002). Is fencing enough? The short-term effects of stock exclusion in remnant grassy woodlands in southern NSW. *Ecological Management & Restoration* 3(2), 117-126.
- Staddon, W.J., Trevors, J.T., and Duchesne, L.C. (1998). Soil microbial diversity and community structure across a climatic gradient in western Canada. *Biodiversity & Conservation* 7(8), 1081-1092.
- Staley, J.T. (1997). Biodiversity: are microbial species threatened? *Current Opinion in Biotechnology* 8(3), 340-345.
- Staley, J.T., and Gosink, J.J. (1999). Poles apart: biodiversity and biogeography of sea ice bacteria. *Annual Reviews of Microbiology* 53(1), 189-215.
- Stark, S., Männistö, M.K., and Eskelinen, A. (2014). Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. *Plant and Soil* 383(1-2), 373-385. doi: 10.1007/s11104-014-2181-y.
- Steele, J.A., Countway, P.D., Xia, L., Vigil, P.D., Beman, J.M., Kim, D.Y., et al. (2011). Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *The ISME Journal* 5(9), 1414-1425.
- Steenwerth, K.L., Jackson, L.E., Calderón, F.J., Stromberg, M.R., and Scow, K.M. (2002). Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biochemistry* 34(11), 1599-1611.
- Stephenson, N.L. (1990). Climatic control of vegetation distribution: the role of the water balance. *The American Naturalist* 135(5), 649-670.
- Stevens, G.C. (1989). The latitudinal gradient in geographical range: how so many species coexist in the tropics. *The American Naturalist* 133(2), 240-256.
- Stevens, G.C. (1992). The elevational gradient in altitudinal range: an extension of

- Rapoport's latitudinal rule to altitude. *The American Naturalist* 140(6), 893-911.
- Stevenson, B. (2004). Changes in phosphorus availability and nutrient status of indigenous forest fragments in pastoral New Zealand hill country. *Plant and Soil* 262(1-2), 317-325.
- Stott, M.B., Crowe, M.A., Mountain, B.W., Smirnova, A.V., Hou, S., Alam, M., et al. (2008). Isolation of novel bacteria, including a candidate division, from geothermal soils in New Zealand. *Environmental Microbiology* 10(8), 2030-2041.
- Strickland, M.S., Keiser, A.D., and Bradford, M.A. (2015). Climate history shapes contemporary leaf litter decomposition. *Biogeochemistry* 122(2-3), 165-174.
- Stuart, R.K., Brahamsha, B., Busby, K., and Palenik, B. (2013). Genomic island genes in a coastal marine *Synechococcus* strain confer enhanced tolerance to copper and oxidative stress. *The ISME Journal* 7(6), 1139-1149. doi: 10.1038/ismej.2012.175.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S., Fischman, D.L., et al. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306(5702), 1783-1786.
- Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., et al. (2015). Structure and function of the global ocean microbiome. *Science* 348(6237), 1261359.
- Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., et al. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences* 111(15), 5610-5615. doi: 10.1073/pnas.1316145111.
- Sunohara, M.D., Topp, E., Wilkes, G., Gottschall, N., Neumann, N., Ruecker, N., et al. (2012). Impact of riparian zone protection from cattle on nutrient, bacteria, F-coliphage, *Cryptosporidium*, and *Giardia* loading of an intermittent stream. *Journal of Environmental Quality* 41(4), 1301-1314.

- Symonds, M.R., and Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology* 65(1), 13-21.
- Takacs-Vesbach, C., Mitchell, K., Jackson-Weaver, O., and Reysenbach, A.L. (2008). Volcanic calderas delineate biogeographic provinces among Yellowstone thermophiles. *Environmental Microbiology* 10(7), 1681-1689.
- Tanca, A., Fraumene, C., Manghina, V., Palomba, A., Abbondio, M., Deligios, M., et al. (2017). Diversity and functions of the sheep faecal microbiota: a multi-omic characterization. *Microbial Biotechnology* 10(3), 541-554. doi: 10.1111/1751-7915.12462.
- Taylor, D., and Bruns, T. (1999). Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. *Molecular Ecology* 8(11), 1837-1850.
- Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., et al. (2014). Global diversity and geography of soil fungi. *Science* 346(6213), 1256688.
- Tedersoo, L., Bahram, M., Toots, M., Diedhiou, A.G., Henkel, T.W., Kjølner, R., et al. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* 21(17), 4160-4170.
- Telford, R.J., Vandvik, V., and Birks, H.J.B. (2006). Dispersal limitations matter for microbial morphospecies. *Science* 312(5776), 1015-1015.
- Ter Steege, H., Pitman, N., Sabatier, D., Castellanos, H., Van Der Hout, P., Daly, D.C., et al. (2003). A spatial model of tree α -diversity and tree density for the Amazon. *Biodiversity & Conservation* 12(11), 2255-2277.
- Terrat, S., Dequiedt, S., Horrigue, W., Lelievre, M., Cruaud, C., Saby, N., et al. (2015). Improving soil bacterial taxa–area relationships assessment using DNA meta-barcoding. *Heredity* 114(5), 468-475.
- Thion, C., Cébron, A., Beguiristain, T., and Leyval, C. (2012). Long-term in situ

- dynamics of the fungal communities in a multi-contaminated soil are mainly driven by plants. *FEMS Microbiology Ecology* 82(1), 169-181.
- Thomas, C.D., Bodsworth, E., Wilson, R.J., Simmons, A.D., Davies, Z.G., Musche, M., et al. (2001). Ecological and evolutionary processes at expanding range margins. *Nature* 411(6837), 577-581.
- Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., et al. (2004). Extinction risk from climate change. *Nature* 427(6970), 145-148.
- Thomas, T., Gilbert, J., and Meyer, F. (2012). Metagenomics-a guide from sampling to data analysis. *Microbial Informatics and Experimentation* 2(1), 3.
- Titshall, L., O'Connor, T., and Morris, C. (2000). Effect of long-term exclusion of fire and herbivory on the soils and vegetation of sour grassland. *African Journal of Range and Forage Science* 17(1-3), 70-80.
- Toljander, J.F., Eberhardt, U., Toljander, Y.K., Paul, L.R., and Taylor, A.F. (2006). Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist* 170(4), 873-883. doi: 10.1111/j.1469-8137.2006.01718.x.
- Tringe, S.G., von Mering, C., Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., et al. (2005). Comparative metagenomics of microbial communities. *Science* 308(5721), 554-557. doi: 10.1126/science.1107851.
- Trivedi, P., Anderson, I.C., and Singh, B.K. (2013). Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends in Microbiology* 21(12), 641-651. doi: 10.1016/j.tim.2013.09.005.
- Tu, Q., Yu, H., He, Z., Deng, Y., Wu, L., Van Nostrand, J.D., et al. (2014). GeoChip 4: a functional gene-array-based high-throughput environmental technology for microbial community analysis. *Molecular Ecology Resources* 14(5), 914-928.
- Tuomisto, H., Ruokolainen, K., and Yli-Halla, M. (2003). Dispersal, environment, and floristic variation of western Amazonian forests. *Science* 299(5604), 241-244.

- Tyrell, S.F., and Quinton, J.N. (2003). Overland flow transport of pathogens from agricultural land receiving faecal wastes. *Journal of Applied Ecology* 94, 87-93.
- van der Gast, C.J. (2015). Microbial biogeography: the end of the ubiquitous dispersal hypothesis? *Environmental Microbiology* 17(3), 544-546. doi: 10.1111/1462-2920.12635.
- van Nimwegen, K.J., van Soest, R.A., Veltman, J.A., Nelen, M.R., van der Wilt, G.J., Vissers, L.E., et al. (2016). Is the \$1000 genome as near as we think? A cost analysis of next-generation sequencing. *Clinical Chemistry* 62(11), 1458-1464.
- Vandenkoornhuyse, P., Baldauf, S.L., Leyval, C., Straczek, J., and Young, J.P.W. (2002). Extensive fungal diversity in plant roots. *Science* 295(5562), 2051-2051.
- Vellend, M. (2010). Conceptual synthesis in community ecology. *The Quarterly Review of Biology* 85(2), 183-206.
- Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304(5667), 66-74.
- Vetaas, O.R., and Ferrer-Castán, D. (2008). Patterns of woody plant species richness in the Iberian Peninsula: environmental range and spatial scale. *Journal of Biogeography* 35(10), 1863-1878.
- Vetaas, O.R., and Grytnes, J.A. (2002). Distribution of vascular plant species richness and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography* 11(4), 291-301.
- Vick-Majors, T.J., Priscu, J.C., and Amaral-Zettler, L.A. (2014). Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *The ISME Journal* 8(4), 778-789. doi: 10.1038/ismej.2013.190.
- Violle, C., Reich, P.B., Pacala, S.W., Enquist, B.J., and Kattge, J. (2014). The emergence and promise of functional biogeography. *Proceedings of the National Academy of Sciences* 111(38), 13690-13696.

- Von Humboldt, A. (1849). *Aspects of nature, in different lands and different climates; with scientific elucidations*. Lea and Blanchard.
- Von Linnaeus, C. (1743). On the growth of the habitable earth. *Select Dissertations from the Amoenitates Academicæ*.
- Vries, F.T., Manning, P., Tallowin, J.R., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., et al. (2012). Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters* 15(11), 1230-1239.
- Wakelin, S., Macdonald, L., Rogers, S., Gregg, A.L., Bolger, T., and Baldock, J. (2008). Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. *Soil Biology and Biochemistry* 40(3), 803-813.
- Wakelin, S.A., Gregg, A.L., Simpson, R.J., Li, G.D., Riley, I.T., and McKay, A.C. (2009). Pasture management clearly affects soil microbial community structure and N-cycling bacteria. *Pedobiologia* 52(4), 237-251.
- Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D., and Tilman, D. (2006). Resource availability controls fungal diversity across a plant diversity gradient. *Ecology Letters* 9(10), 1127-1135. doi: 10.1111/j.1461-0248.2006.00965.x.
- Walker, A.W., Duncan, S.H., Louis, P., and Flint, H.J. (2014). Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends in Microbiology* 22(5), 267-274. doi: 10.1016/j.tim.2014.03.001.
- Wallenstein, M., Allison, S.D., Ernakovich, J., Steinweg, J.M., and Sinsabaugh, R. (2010). "Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates," in *Soil Enzymology*, 245-258.
- Wang, J., Soininen, J., Zhang, Y., Wang, B., Yang, X., and Shen, J. (2011). Contrasting patterns in elevational diversity between microorganisms and macroorganisms. *Journal of Biogeography* 38(3), 595-603.
- Wang, J., Soininen, J., Zhang, Y., Wang, B., Yang, X., and Shen, J. (2012a). Patterns of elevational beta diversity in micro- and macroorganisms. *Global Ecology and*

- Biogeography* 21(7), 743-750. doi: 10.1111/j.1466-8238.2011.00718.x.
- Wang, J.J., Soininen, J., He, J.Z., and Shen, J. (2012b). Phylogenetic clustering increases with elevation for microbes. *Environmental Microbiology Reports* 4(2), 217-226. doi: 10.1111/j.1758-2229.2011.00324.x.
- Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73(16), 5261-5267.
- Ward, D.M., Weller, R., and Bateson, M.M. (1990). 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* 345(6270), 63-65.
- Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., et al. (2009). Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology* 75(7), 2046-2056. doi: 10.1128/AEM.02294-08.
- Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., et al. (2009). gplots: Various R programming tools for plotting data. *R package version* 2(4), 1.
- Watts, D.J., and Strogatz, S.H. (1998). Collective dynamics of ‘small-world’ networks. *Nature* 393(6684), 440-442.
- Wehrens, R., and Mevik, B.-H. (2007). The pls package: principal component and partial least squares regression in R.
- Wei, T., and Simko, V. (2013). corrplot: Visualization of a correlation matrix. *R package version* 230(231), 11.
- Weiss, S.B., and Weiss, A.D. (1998). Landscape-level phenology of a threatened butterfly: a GIS-based modeling approach. *Ecosystems* 1(3), 299-309.
- Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., et al. (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases* 13(2),

155-165.

- Whittaker, R.J., Grogan, D.W., and Taylor, J.W. (2003). Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* 301(5635), 976-978.
- Whittaker, K.A., and Rynearson, T.A. (2017). Evidence for environmental and ecological selection in a microbe with no geographic limits to gene flow. *Proceedings of the National Academy of Sciences* 114(10), 2651-2656. doi: 10.1073/pnas.1612346114.
- Whittaker, R.H. (1972). Evolution and measurement of species diversity. *Taxon*, 213-251.
- Whittaker, R.H., and Niering, W.A. (1965). Vegetation of the Santa Catalina Mountains, Arizona: a gradient analysis of the south slope. *Ecology* 46(4), 429-452.
- Wickham, H. (2009). ggplot2: Elegant graphics for data analysis. *New York*.
- Wilkins, D., Lauro, F.M., Williams, T.J., Demaree, M.Z., Brown, M.V., Hoffman, J.M., et al. (2013). Biogeographic partitioning of Southern Ocean microorganisms revealed by metagenomics. *Environmental Microbiology* 15(5), 1318-1333. doi: 10.1111/1462-2920.12035.
- Wilkinson, D.M., Koumoutsaris, S., Mitchell, E.A., and Bey, I. (2012). Modelling the effect of size on the aerial dispersal of microorganisms. *Journal of Biogeography* 39(1), 89-97.
- Willdenow, K.L. (1811). *The principles of botany, and of vegetable physiology*. Printed at the University Press for William Blackwood.
- Williams, R.J., Howe, A., and Hofmockel, K.S. (2014). Demonstrating microbial co-occurrence pattern analyses within and between ecosystems. *Frontiers in Microbiology* 5, 358. doi: 10.3389/fmicb.2014.00358.
- Willig, M.R., Kaufman, D.M., and Stevens, R.D. (2003). Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics* 34(1), 273-309.

- Willms, W.D. (1971). *Influence of forest edge, elevation, aspect, site index, and roads on deer use of logged and mature forest, Northern Vancouver Island*. University of British Columbia.
- Wolf, B., Zheng, X., Brüggemann, N., Chen, W., Dannenmann, M., Han, X., et al. (2010). Grazing-induced reduction of natural nitrous oxide release from continental steppe. *Nature* 464(7290), 881-884.
- Wooley, J.C., Godzik, A., and Friedberg, I. (2010). A Primer on Metagenomics. *PLOS Computational Biology* 6(2), e1000667. doi: 10.1371/journal.pcbi.1000667.
- Worm, B., Lotze, H.K., Hillebrand, H., and Sommer, U. (2002). Consumer versus resource control of species diversity and ecosystem functioning. *Nature* 417(6891), 848-851.
- Wright, D.H. (1983). Species-energy theory: an extension of species-area theory. *Oikos*, 496-506.
- Wu, J., Anderson, B.J., Buckley, H.L., Lewis, G., and Lear, G. (2017). Aspect has a greater impact on alpine soil bacterial community structure than elevation. *FEMS Microbiology Ecology* 93(3). doi: 10.1093/femsec/fiw253.
- Wu, T., Chellemi, D.O., Graham, J.H., Martin, K.J., and Rosskopf, E.N. (2008). Comparison of soil bacterial communities under diverse agricultural land management and crop production practices. *Microbial Ecology* 55(2), 293-310.
- Xu, M., Chen, J., and Qi, Y. (2002). Growing-season temperature and soil moisture along a 10 km transect across a forested landscape. *Climate Research* 22(1), 57-72.
- Xu, M., Wu, W.-M., Wu, L., He, Z., Van Nostrand, J.D., Deng, Y., et al. (2010). Responses of microbial community functional structures to pilot-scale uranium in situ bioremediation. *The ISME Journal* 4(8), 1060-1070.
- Xu, X., Thornton, P.E., and Post, W.M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography* 22(6), 737-749.

- Yabe, S., Aiba, Y., Sakai, Y., Hazaka, M., and Yokota, A. (2010). *Thermosporothrix hazakensis* gen. nov., sp. nov., isolated from compost, description of *Thermosporotrichaceae* fam. nov. within the class *Ktedonobacteria* Cavaletti et al. 2007 and emended description of the class *Ktedonobacteria*. *International Journal of Systematic and Evolutionary Microbiology* 60(8), 1794-1801.
- Yamulki, S., Jarvis, S., and Owen, P. (1998). Nitrous oxide emissions from excreta applied in a simulated grazing pattern. *Soil Biology and Biochemistry* 30(4), 491-500.
- Yan, Y., Kuramae, E.E., de Hollander, M., Klinkhamer, P.G., and van Veen, J.A. (2017). Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *The ISME Journal* 11(1), 56-66. doi: 10.1038/ismej.2016.108.
- Yang, Y., Gao, Y., Wang, S., Xu, D., Yu, H., Wu, L., et al. (2014). The microbial gene diversity along an elevation gradient of the Tibetan grassland. *The ISME Journal* 8(2), 430-440. doi: 10.1038/ismej.2013.146.
- Yang, Y., Wu, L., Lin, Q., Yuan, M., Xu, D., Yu, H., et al. (2013). Responses of the functional structure of soil microbial community to livestock grazing in the Tibetan alpine grassland. *Global Change Biology* 19(2), 637-648. doi: 10.1111/gcb.12065.
- Yang, Y.Q., Chen, N., and Chen, T. (2017). Inference of environmental factor-microbe and microbe-microbe associations from metagenomic data using a hierarchical Bayesian statistical model. *Cell Systems* 4(1), 129-137. doi: 10.1016/j.cels.2016.12.012.
- Yergeau, E., Newsham, K.K., Pearce, D.A., and Kowalchuk, G.A. (2007). Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environmental Microbiology* 9(11), 2670-2682.
- Yuan, H.Z., Ge, T.D., Chen, C.Y., O'Donnell, A.G., and Wu, J.S. (2012). Significant role for microbial autotrophy in the sequestration of soil carbon. *Applied and Environmental Microbiology* 78(7), 2328-2336. doi: 10.1128/aem.06881-11.

- Zeglin, L.H., Bottomley, P.J., Jumpponen, A., Rice, C.W., Arango, M., Lindsley, A., et al. (2013). Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. *Ecology* 94(10), 2334-2345. doi: 10.1890/12-2018.1.
- Zhang, B., Liang, C., He, H., and Zhang, X. (2013). Variations in soil microbial communities and residues along an altitude gradient on the northern slope of Changbai Mountain, China. *PLOS One* 8(6), e66184.
- Zhou, J., Deng, Y., Luo, F., He, Z., Tu, Q., and Zhi, X. (2010). Functional molecular ecological networks. *mBio* 1(4). doi: 10.1128/mBio.00169-10.
- Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., et al. (2016). Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communications* 7, 12083.
- Zhou, J.Z., Kang, S., Schadt, C.W., and Garten, C.T. (2008). Spatial scaling of functional gene diversity across various microbial taxa. *Proceedings of the National Academy of Sciences* 105(22), 7768-7773. doi: 10.1073/pnas.0709016105.
- Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews* 32(5), 723-735. doi: 10.1111/j.1574-6976.2008.00123.x.
- Zimmerman, N.B., and Vitousek, P.M. (2012). Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. *Proceedings of the National Academy of Sciences* 109(32), 13022-13027.
- Zinger, L., Boetius, A., and Ramette, A. (2014). Bacterial taxa-area and distance-decay relationships in marine environments. *Molecular Ecology* 23(4), 954-964. doi: 10.1111/mec.12640.
- Zinger, L., Lejon, D.P., Baptist, F., Bouasria, A., Aubert, S., Geremia, R.A., et al. (2011). Contrasting diversity patterns of crenarchaeal, bacterial and fungal soil communities in an alpine landscape. *PLOS One* 6(5), e19950.

- Zink, R., and Remsen Jr, J. (1986). Evolutionary processes and patterns of geographic variation in birds.
- Zumsteg, A., Bååth, E., Stierli, B., Zeyer, J., and Frey, B. (2013). Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. *Soil Biology and Biochemistry* 61, 121-132. doi: 10.1016/j.soilbio.2013.02.017.
- Zwart, G., Hiorns, W.D., Methé, B.A., van Agterveld, M.P., Huismans, R., Nold, S.C., et al. (1998). Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. *Systematic and Applied Microbiology* 21(4), 546-556.