The spatial dynamics of below-ground fungal communities: a study of pattern and process

A thesis submitted in fulfillment of requirements for the award of the degree of Doctor of Philosophy

by

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in part or in full, for a degree at this or any other institution.

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Abbreviations

AM	arbuscular mycorrhizal
ARDRA	amplified ribosomal DNA restriction analysis
ARISA	amplified ribosomal intergenic spacer analysis
BSA	bovine serum albumin
CCA	canonical correspondence analysis
COCA	co-correspondence analysis
dATP	deoxyadenosine triphosphate
dbMEM	distance-based Moran's eigenvector map
dCTP	deoxycytidine triphosphate
DGGE	denaturing gradient gel electrophoresis
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
ECM	ectomycorrhizal

I	immigration parameter
IDR	inter-decile range
IQR	inter-quartile range
ITS	internal transcribed spacer
LSU	large subunit
MEM	Moran's eigenvector map
MIDs	multiplex identifiers
NPWS	NSW National Parks and Wildlife Service
OTU	operational taxonomic unit
PCA	principal components analysis
PCNM	principal co-ordinates of neighbor matrices
PCoA	principal co-ordinates analysis
PCR	polymerase chain reaction
PFTs	plant functional types
RDA	redundancy analysis
rRNA	ribosomal ribonucleic acid
TGGE	temperature gradient gel electrophoresis
TRF	terminal restriction fragment

T-RFLP	terminal restriction fragment length polymorphism
VWC	volumetric water content
Θ	neutral diversity parameter

Abstract

Soil fungi are an important part of terrestrial ecosystems, having invaluable roles in decomposition, carbon storage and nutrient cycling processes. Despite this, their ubiquitous nature and the methodological challenges associated with studying their diversity, have led to the traditional belief that fungal communities show little spatial structure. However, with the rapid development of molecular methods, research is now indicating that below-ground fungal communities are spatially heterogeneous in response to environmental variables and that the spatial structure of such communities has important above-ground consequences, such as influencing plant community structure and plant productivity.

Spatial patterns displayed by below-ground fungal communities are alone insufficient in order to gain an accurate insight into how the community dynamics contribute to ecosystem functioning. The importance of spatial and environmental factors is often strongly interrelated and their relative importance is generally context and scale dependent. The research presented in this thesis, therefore, combines the analysis of fungal community spatial patterns with models based on mechanisms that drive their assembly over a range of spatial scales and levels of community organisation.

Patterns of soil fungal community assembly were sampled from separation distances of 20 cm to 500 km, over three experimental designs, and analysed by T-RFLP and high-throughput sequencing. The roles of spatial distance, habitat type, edaphic characteristics, climatic conditions, vegetation type and the diversity of the plant

community, as well as phylogenetic relatedness, in shaping the observed fungal communities were considered by a range of multivariate and spatial statistics. Large scale fungal community patterns were found, spanning distances of between 100 and 500 km along the south-east coast of Australia, as well as at smaller scales of 20 cm to approximately 100 m, sampled in temperate and alpine/subalpine ecosystems. A distinct lack of spatial patterning existed for the sampled below-ground fungal communities at intermediate scales. Spatial distance was found to have an important role in shaping the detected community patterns, while environmental factors did not play a dominant role in shaping the fungal community, independently of other measured variables, at the scales at which spatial patterns were detected.

The functional processes that shape community assembly were additionally considered by looking at the role of niche and neutral dynamics as well as by explicitly characterising the role of dispersal in shaping the metacommunity. Neutrality modeling suggested that niche-based assembly contributed to soil fungal community assembly at the investigated scales. However, the role of stochastic or deterministic assembly mechanisms in driving community structure depended on the strength of dispersal and the degree of niche overlap experienced by the community, ultimately supporting the continuum hypothesis of niche and neutral assembly. Moreover, differences in the characteristics of the metacommunity were shown to influence the processes by which local communities were structured, emphasising that ecological processes do not act at the same scale/s at which community patterns are evident.

Overall, understanding both pattern and process of fungal community assembly is essential to contribute to predicting changes in fungal community structure and function, at spatially relevant scales. With this knowledge it will be possible to better

recognise the effects of environmental change on fungal communities, in order to manage and conserve the surrounding terrestrial environments accordingly.

Chapter 1 General Introduction

1.1. Literature Review

1.1.1. Fungi in the below-ground environment

Fungi account for a significant proportion of the microbial biomass in terrestrial environments. In 1995, over 72 000 fungal species had been formally identified and described worldwide (Hawksworth *et al.* 1995). Global estimates of fungal species richness based on pre-molecular data sat at 1.5 million species, however with the inclusion of cryptic species detected by molecular methods, it has been estimated that there could be as many as 5.1 million species worldwide (Hawksworth 2001; Peay *et al.* 2008). The soil environment is fundamental for the success of terrestrial fungi, as the majority of identified fungal species are present in soil for at least part of their life cycle, either as actively growing organisms or as dormant propagules (Bridge and Spooner 2001). The success of particular fungal species is, however, greatly influenced by the characteristics of the surrounding soil environment, with environmental factors such as vegetation type (e.g. Anderson *et al.* 2003) and soil properties (e.g. Lauber *et al.* 2008), as well as external factors such as altered fire regimes (e.g. Bastias *et al.* 2006), petrochemical pollution (e.g. Van Elsas *et al.* 2000) and heavy metal contamination (e.g. Cairney and Meharg 2003), having been shown to influence the composition of soil fungal communities.

Soil fungal species have been broadly classified into three categories: mycorrhizal, saprotrophic and pathogenic fungi. While these classifications are based on their primary role in ecosystem processes, all groups of fungi also contribute to a number of general ecosystem functions (summarised in Table 1.1). Moreover, individual species can be considered in multiple classifications, for example, a number of ectomycorrhizal (ECM) species are also known to have saprotrophic capabilities (Cullings and Courty 2009); thus emphasizing the importance of all soil fungal groups in ecosystem functioning.

Table 1.1: Summary of ecosystem services provided by soil fungi (adapted from Dighton 2003)

Ecosystem function/service	Primary fungal group
Soil formation	Mycorrhizae/Saprotrophs
Providing soil fertility for primary production	Saprotrophs
Regulation of primary production (plants) and plant community structure	Mycorrhizae /Pathogens
Regulation of secondary production (animals)	Mycorrhizae/Saprotrophs/Pathogens
Regulation of anthropogenic and environmental change	Mycorrhizae/Saprotrophs

Mycorrhizal fungi form mutualistic symbiotic associations with the roots of many terrestrial plants, relying on the plant as its carbon source. In return, mycorrhizal fungi assist their host plants in the acquisition of mineral nutrients from the soil and are thus largely responsible for promoting primary production within the ecosystem (Smith 2008). Mycorrhizal fungi increase plant yield in terms of both growth and fitness

(Dighton 2003), and can also improve water uptake and pathogen resistance of the plant (Cairney 2000). Furthermore, mycorrhizal fungi are known to influence plant community structure and dynamics through the development of mycelial networks between plants. These networks facilitate the potential transfer of carbon, mineral nutrients and water between plants, and different plant species are suggested to differ in their responses to these networks; thus influencing the structure of the emergent plant communities (van der Heijden and Horton 2009). Pathogenic fungi equally influence primary production and plant community structure. By causing disease in plants, arthropods, nematodes or other fungi, pathogenic fungi act to regulate plant biomass and populations, as well as introducing selective pressures on members of plant communities (Bridge and Spooner 2001; Dighton 2003).

Saprotrophic fungi provide fertility for primary production by the decomposition of dead organic matter. Plant and animal remains are degraded by the excretion of extracellular enzymes and absorbed by the fungus for its metabolism. Excess mineral nutrients are released in an inorganic form into the soil pool, by means of nutrient mineralisation, and this is regulated by the fungus through the processes of translocation and immobilisation (Dighton 2003). Mineral nutrients can be transported through the fungal hyphae away from the source of decomposition and later released, depending on the fertility of the soils at any one point in space or time. The rate of decomposition and release therefore influences the resulting soil type, with more rapid decomposition leading to more fertile soils, and subsequently determining the vegetation type (Dighton 2003). Plant communities that dominate nutrient poor soils rely on specific adaptations for these conditions, such as the development of ericoid mycorrhizal associations in Ericaceae species (Cairney and Meharg 2003).

Both mycorrhizal and saprotrophic fungi assist in the formation of soils. Either alone, or in association with plants and bacteria (as is the case for many mycorrhizal fungi in more established soils), some fungi can produce organic acids that break down parent rock into its mineral components (Dighton 2003). Fungi also act to stabilise the mineral particles by the penetration of hyphae amongst the soil particles. Polysaccharide secretions and the hydrophobic nature of the fungi additionally bind the soil particles and restrict water movement, thus minimising soil erosion (Dighton 2003). Fungi are a major component of terrestrial food webs by promoting and regulating secondary production. Fungal mycelia are a prominent carbon source for many grazing soil invertebrates and fungal fruiting bodies are consumed by many larger animals (Dighton 2003). Some fungal species also acquire nitrogen through predation of soil invertebrates including groups of nematodes, tardigrades, collembola, copepods and rotifers, and along with pathogenic fungi, act to regulate these populations (Peay *et al.* 2008). Moreover, fungi are known to ameliorate the effects of heavy metal pollutants (Cairney and Meharg 2003) and have the ability to sequestrate elements such as carbon (Dighton 2003), thus potentially being able to regulate the effects of environmental change.

1.1.2. Methodologies used to study fungal community diversity

Traditionally, the study of soil fungal diversity was limited to using culture-based isolation and enumeration techniques. However, the development of direct soil nucleic acid extraction coupled with polymerase chain reaction (PCR) amplification of specific DNA sequences has enabled the detection of both culturable and unculturable species, and thus has significantly advanced our understanding of soil fungal diversity (Anderson and Cairney 2004; Bidartondo and Gardes 2005). Genes and spacer regions within the ribosomal RNA (rRNA) gene cluster are commonly

used in such studies, especially the internal transcribed spacer (ITS) region, which lies between the 18S and the 28S rRNA genes and incorporates the 5.8S rRNA gene (Bridge and Spooner 2001; Bidartondo and Gardes 2005). The ITS region is multicopy and, due to the faster evolutionary rate of the non-coding ITS1 and ITS2 regions that flank the 5.8S rRNA gene, it provides sufficient sequence variation between closely related fungal species for identification to the genus, and at times, species level (Anderson and Cairney 2004).

A number of community profiling techniques can be used, in combination with ITS-PCR amplification, that enable the structure and diversity of fungal assemblages to be assessed. Gel-based methods such as denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE), as well as those which detect fluorescently labelled DNA fragments such as terminal restriction fragment length polymorphism (T-RFLP), amplified rDNA restriction analysis (ARDRA) and amplified ribosomal intergenic spacer analysis (ARISA) have been successfully applied to soil fungal ecology (reviewed by Anderson and Cairney 2004). Cloning and sequencing of the ITS region has additionally enabled taxonomic identification and phylogenetic analysis of species within soil fungal communities. These approaches have been fundamental in demonstrating shifts in fungal community structure in response to a range of environmental changes (e.g. Lauber *et al.* 2008), and have been particularly useful in the research of ECM fungal mycelia within soil (e.g. Genney *et al.* 2006), as well as for gaining a greater insight into soil fungal diversity (e.g. O'Brien *et al.* 2005).

Despite the fact that molecular surveys have uncovered a large pool of fungal diversity, with reduced sampling effort, large-scale soil sequencing projects have yet to produce results that correspond with asymptotic estimates of fungal richness (O'Brien *et al.*

2005; Fierer *et al.* 2007). More recently developed high-throughput next-generation sequencing technologies such as 454 and Illumina pyrosequencing, however, provide great potential for in-depth sampling of such species rich communities. Fungal diversity analyses of forest soils, using these methods have revealed far greater species diversity than previously expected using previous approaches (Buée *et al.* 2009; Lim *et al.* 2010). Next-generation sequencing technologies are now being implemented to understand the diversity of fungal communities in response to pH (e.g. Rousk *et al.* 2010; Carrino-Kyker *et al.* 2016), habitat type (Gottel *et al.* 2011; Lentendu *et al.* 2011) and various land use practices (e.g. Lumini *et al.* 2010; Fay *et al.* 2016), as well as investigating vertical stratification (Jumpponen *et al.* 2010a) and temporal changes (Jumpponen *et al.* 2010b; Dumbrell *et al.* 2011) in fungal community composition. However, the application of high-throughput sequencing to study fungal taxonomic diversity is still challenging because bioinformatics tools are in their infancy in terms of their ability to analyse the volume of sequence data generated by such methods (Peay *et al.* 2008; Parrent *et al.* 2010). Nonetheless, since DNA from mixed environmental samples can be used as the template in next-generation sequencing, and thus eliminating the need for cloning, this high-throughput technology is promising for processing the large datasets required to understand the spatiotemporal dynamics of fungal communities.

1.1.3. Spatial structure in ecological communities

A primary aim in community ecology is to understand patterns of species diversity and abundance across spatial and temporal scales. Structured patterns in species distributions, or spatial heterogeneity, is known to exist at a range of spatial scales — from large scale trends and gradients (i.e. many kilometers over landscapes or continents) to smaller scale discontinuous community structures, such as aggregations,

patchiness or random structures (i.e. over distances of millimetres, centimetres, or metres), that are nested within larger landscape scale patterns (Ettema and Wardle 2002; Fortin and Dale 2005). Furthermore, understanding the processes that give rise to these spatial patterns in species diversity is fundamental to explaining the mechanisms that drive and maintain biodiversity (Fortin and Dale 2009). Explanations for the assembly of species across space and time have been studied from the perspective of two dominant ecological theories: the niche theory and the neutral theory.

1.1.3.1. Niche and neutral theory

The niche theory of species coexistence developed from the formalisation of the 'competitive exclusion principle' in which similar species differ in their preferences for habitat and resource use so as to prevent competitive elimination (MacArthur and Levins 1964). Niches, or stabilising mechanisms, act more negatively on intraspecific interactions than interspecific interactions; therefore as a species' relative abundance increases within the community, its per capita growth rate decreases, and the competitive exclusion of other species is limited (Chesson 2000; Adler *et al.* 2007). Mechanisms that influence the relative strength of inter and intraspecific competition can either be fluctuation independent (i.e. the mechanism functions irrespective of the presence of environmental variation), such as resource partitioning and frequency dependant predation, or be directly dependant on fluctuations in population densities and environmental factors over space and time (Chesson 2000). Adler *et al.* (2007) emphasised that under the niche theory, coexistence is not solely dependent on stabilising mechanisms but also depends on fitness differences between species and the balance between these differences and the stabilising processes.

Niche models of species abundance have, however, been criticised as being unable to explain the great diversity present in species-rich communities such as tropical rainforests, because insufficient niches are present to support all species (Hubbell 2005; Zhou and Zhang 2008). Neutral theory, most famously developed as the 'unified neutral theory of biodiversity and biogeography' (Hubbell 2001b) accurately predicts many of the patterns of biodiversity observed in natural communities (Hubbell 2001b; Chave 2004). Rather than predict species patterns from deterministic processes, neutral theory models predict species coexistence primarily from stochastic speciation and dispersal events (Hubbell 2006; Adler *et al.* 2007). The underlying assumption of the neutral theory suggests that all species within a community are ecologically equivalent in their probability of giving birth, dying, migrating and undergoing speciation; thus the theory proposes that species similarities, rather than niche-based differences, are responsible for supporting high community diversity (Hubbell 2001b; Adler *et al.* 2007). Neutral theory is often accepted as a null model based on its assumption of ecological equivalence, and hence can be analysed in such a way (Etienne 2007, 2009). The use of null models in this way, therefore becomes more insightful than just a random statistical model, and provides a mechanistic perspective in the analysis of community assembly. It is the assumption of ecological equivalence, however, that has caused the most debate over the validity of neutral models, and, subsequently niche and neutral theory perspectives of species coexistence are presently suggested to be two extremes of a continuum (Gravel *et al.* 2006; Adler *et al.* 2007). Both niche and neutral processes are therefore thought to contribute to structuring species assemblages, and this depends on the relative differences in, and interactions between, stabilising mechanisms and species fitness within the community (Alonso *et al.* 2006; Gravel *et al.* 2006; Adler *et al.* 2007).

1.1.3.2. Spatial dependence and spatial autocorrelation

Modeling the assembly of species in response to niche-based and/or stochastic mechanisms provides insight into the factors that drive biodiversity, including the importance of space in structuring the community. Alternatively, a spatially-explicit approach can also be applied to understanding patterns of species diversity; achieved through describing and quantifying the spatial variation of ecological communities, as well as studying the role of ecological processes in shaping the spatial component of species distribution patterns. Spatial patterns are attributed to the combination of exogenous (those that are independent of the measured variable, e.g. a temperature gradient) and endogenous (those which are inherent to the measured variable, e.g. dispersal) factors acting on the species or community of interest and, as such, two broad models are used to describe the source of spatial structure in an ecological system (Fortin and Dale 2005). The terminology used to describe these models has, however, caused some debate. For example, spatial dependence of a species or community is said to result from their response to a combination of both exogenous and endogenous processes, however the same term has been used to more specifically refer to the response to only exogenous environmental factors, which display their own spatial structure (Legendre *et al.* 2002; Legendre and Legendre 2012). Fortin and Dale (2005) attempted to alleviate this confusion by introducing the term 'induced spatial dependence' to describe the latter. Spatial autocorrelation strictly describes the spatial patterns of a species or community in response to endogenous processes. It indicates the degree of correlation, or similarity, of the measured variable with itself, as a function of separation distance between samples (Koenig 1999; Fortin and Dale 2005; Legendre and Legendre 2012). It is the study of these two models, spatial

autocorrelation and spatial dependence, which form the basis of spatially explicit ecological investigations.

1.1.3.3. Scale

A vital consideration in studying the spatial structure of an ecological system is choosing an appropriate scale for the investigation. The scale of the study is influenced by three important aspects of the sampling design: the grain size, the sampling interval and the extent (Figure 1.1) (Legendre and Legendre 2012). The scale at which spatial structure is observed essentially limits the amount of detail perceived in the system of interest and therefore the scale of the sampling design must be optimised to match the hypotheses being tested (Levin 1992; Fortin and Dale 2005; Franklin and Mills 2007). Quantifying the scales at which spatial patterning occurs is essential in order to understand the complexity of the underlying mechanisms responsible for spatial patterns in population dynamics and community structure (Borcard *et al.* 2011; Legendre and Legendre 2012).

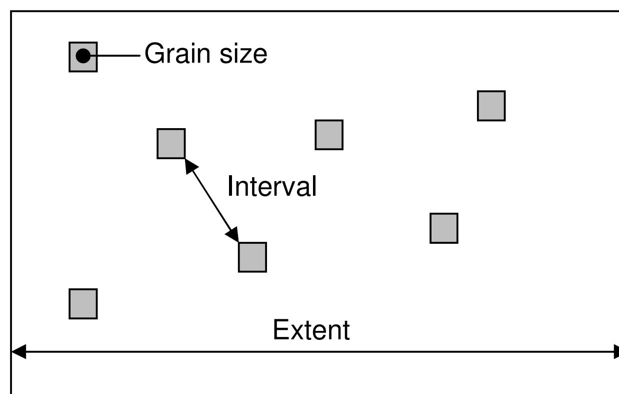


Figure 1.1: Components of sampling design that influence scale. Grain size is the size of the individual sampling unit. Interval is the average distance between sampling units. Extent is the total distance over which the study is conducted. (Franklin and Mills, 2007b; Legendre and Legendre 2012)

1.1.3.4. Analysing and modelling spatial structure

A fundamental challenge in studying spatial patterns of an ecological system results from the lack of independence among samples, which traditional statistical tests assume (Franklin and Mills 2007; Fortin and Dale 2009; Legendre and Legendre 2012). A range of statistical methods have subsequently been developed to analyse the spatial component of variation within a community, using one of three main approaches: calculation of structure functions, mapping and modelling.

Spatial dependence in relation to separation distance is most often analysed by the calculation of structure functions. While these analyses do not strictly distinguish between the patterns that are a result of spatial autocorrelation and those that are a result of induced spatial dependence, structure functions provide an effective method of describing and quantifying the spatial structure of the system of interest (Fortin and Dale 2005). Spatial correlation co-efficients for univariate datasets such as Moran's I or Geary's c indices are calculated based on the similarity of a pair of observations at a particular distance interval. Similarly, the Mantel test is used for multivariate datasets, in which a Euclidean distance matrix and a species similarity matrix are compared between pairs of sites, to give a series of Mantel statistics (r_M). These values can then be plotted for each distance class by means of a correlogram (Koenig 1999; Fortin and Dale 2005). Geostatistical approaches can also be used to quantify the spatial dependence in an ecological system, including that of the variogram (or semi-variogram). Like correlograms, variograms graphically present the spatial variation in a system as a function of separation distance; however this is achieved by computing the semi-variance of the dataset at each distance interval. Variograms can potentially be fitted to models of spatial structure and used to predict spatial patterns at unsampled locations (Fortin and Dale 2005).

The spatial structure of communities can be further described by mapping their patterns across geographic space. Interpolation methods such as kriging and trend surface analysis, enable spatial patterns to be predicted at unsampled locations within the extent of the study area (Fortin and Dale 2005). Variograms provide the basis for producing interpolation maps by kriging, whereby values are calculated using the semi-variances (from the model variogram) corresponding to the separation distances of pairs of points across the sampling area (Franklin and Mills 2007; Legendre and Legendre 2012). These values are then assigned to a regular grid of nodes and the series of point estimates are plotted to form a contour map of spatial heterogeneity over the extent of the study area (Legendre and Legendre 2012). Trend-surface analysis is an older method of mapping spatial community patterns. Smoothed maps are produced by calculating values at unsampled locations using linear regression when the pattern is a linear trend and polynomial regression when the spatial pattern is non-linear (Fortin and Dale 2005; Legendre and Legendre 2012).

While such mapping techniques can be used to predict the spatial patterns in species diversity at unsampled locations, they produce very coarse representations of spatial structure at these points. As spatial heterogeneity is a result of a number of environmental and biotic factors that operate at a range of scales, a primary aim of spatial analysis is to discriminate between the sources of variation acting on the community and model those that are relevant at various spatial scales (Borcard *et al.* 2011; Legendre and Legendre 2012). Causal modeling of spatial patterns in multivariate data has been achieved in the past by approaches such as partial Mantel analysis and partial canonical analysis, whereby the effects of explanatory environmental variables on the response variable can be analysed independently from the effects of spatial variables (Legendre and Legendre 2012). In the case of partial

canonical analysis, the variation present in the dataset can then be decomposed into purely spatial and environmental components, a spatial component that is confounded or influenced by the environment, and the remaining unexplained variation, by means of variation partitioning (Borcard *et al.* 1992). These methods, however, are largely used to model large-scale spatial structures and their ability to consider both small and large-scale patterns is limited (Borcard and Legendre 2002; Legendre and Legendre 2012).

Causal modeling at multiple spatial scales is possible with the recent ability to calculate spatial eigenfunctions, which can be used in the multiscale analysis of spatial structures in ecosystems, either in a univariate or multivariate context. Moran's eigenvector maps (MEM) are spatial eigenfunctions where the eigenvalues are equivalent to Moran's I coefficients of spatial correlation across multiple scales. Distance-based Moran's eigenvector maps (dbMEM), formally described as principal co-ordinates of neighbour matrices (PCNM) (Borcard and Legendre 2002; Borcard *et al.* 2004), are a specialised form of the more general MEM, whereby eigenvalues are specifically calculated using geographic distances (Legendre and Legendre 2012). A truncated Euclidean distance matrix of the geographic co-ordinates is constructed using a threshold of maximum distance between closest neighbours and eigenvalues are then obtained by principal co-ordinates analysis (PCoA) of the truncated matrix. The spatial patterns that are reconstructed therefore are representative of all scales that are significant, starting from the smallest scale relationships to the largest scale trends (Borcard and Legendre 2002; Borcard *et al.* 2004). These values can then be used as spatial descriptors of various spatial scales in other multivariate analyses (e.g. redundancy analysis (RDA) or canonical correspondence analysis (CCA)) and variation partitioning (e.g. Legendre *et al.* 2009).

Analytical advancements in the field of spatial ecology have thus made it possible to explicitly consider the importance of space in structuring ecological communities. Going beyond simply documenting patterns of species diversity, researchers now have the tools to distinguish between the sources of spatial variation in community structure and the importance of environmental heterogeneity and/or biotic interactions in shaping spatial patterns. Linking how spatial structure relates to ecosystem functioning will provide opportunity to develop the field of ecology as a predictive science, as well as to integrate current ecological theories and concepts with more traditional fields of the natural sciences, such as microbiology and mycology.

1.1.4. Spatial patterns of fungal communities

Despite the importance of understanding spatial patterns in biodiversity, and how these patterns are generated and maintained, the majority of spatial ecological studies to date have focused on above-ground organisms such as plants or animals. The ubiquitous nature of micro-organisms and the methodological challenges associated with studying their diversity (see Anderson and Cairney 2004), have resulted in the traditional belief that below-ground micro-organisms show little spatial structure (Ettema and Wardle 2002). The hypothesis of Baas Becking published in 1934, 'everything is everywhere, but the environment selects' has commonly been cited to describe microbial biogeography, inferring that micro-organisms have the ability to disperse globally and proliferate in a range of habitats (De Wit and Bouvier 2006). However with advances in molecular methods, an increasing number of studies have investigated the biogeography of micro-organisms and, while the outcomes are still somewhat debated (e.g. Finlay 2002; Taylor *et al.* 2006), many suggest that below-ground micro-organisms display discrete spatial structures (e.g. Green *et al.* 2004). Furthermore, the spatial structure of such communities has been shown to have

important above-ground consequences, such as influencing plant community structure and plant productivity (Wardle *et al.* 2004; Van Der Heijden *et al.* 2008; van der Heijden and Horton 2009).

Spatial variability in soil fungal community structure exists both vertically and horizontally. Vertical stratification along the soil profile has been noted, both in terms of decreasing abundance with increasing depth (Genney *et al.* 2006), and changes in community composition. For example, Lindahl *et al.* (2007) found a distinct transition in the distribution of saprotrophic versus mycorrhizal fungi in the L (surface litter) and F (fragmented litter) soil horizons. Differences in the community composition with increasing soil depth has also been observed for general soil communities to depths of 15 cm (Robinson *et al.* 2009) as well as for ECM fungal communities to depths of 50 cm (Rosling *et al.* 2003; Tedersoo *et al.* 2003). Tedersoo *et al.* (2003) also found significant horizontal turnover at similar spatial scales as was detected for vertical stratification, emphasising that ECM fungal communities display significant spatial autocorrelation at less than 2-3 m (Lilleskov *et al.* 2004). AM fungal communities are also reported to show significant horizontal spatial structure at scales less than 1-2 m (Carvalho *et al.* 2003; Whitcomb and Stutz 2007).

At larger spatial scales, patterns commonly observed for macro-organism communities (see Gaston 2000) are the basis for many studies of microbial biogeography. Patterns of increasing diversity approaching the equator, and that species' latitudinal ranges increase towards the poles, which are known patterns for plants and animals, have also been noted for fungal communities (Tedersoo *et al.* 2014). Some fungal species are suggested to display a cosmopolitan distribution, with low levels of endemism evident at global scales (Tedersoo *et al.* 2014; Davison *et al.* 2015). Regional diversity patterns have also been investigated, such as the influence of increasing sampling area on

diversity. For example, Green *et al.* (2004) investigated this in ascomycetes, noting a distance-decay relationship in community similarity from 1 m - 100 km. The calculated taxa-area relationship ($z = 0.074$) suggested that high fungal diversity exists at a local scale while decreasing at a regional scale; thus the spatial differences in community composition operate over greater geographic distances than for larger organisms. In contrast, much stronger taxa-area relationships that are similar to those reported for macro-organisms ($z = 0.2-0.23$) have been described for ECM fungi, using 'tree islands' ranging in size from < 10 to > 10 000 m^2 (Peay *et al.* 2007). Sample size and sampling effort have been noted to dramatically impact on the assessment of microbial diversity and influence the size of observed taxa-area relationships (Woodcock *et al.* 2006; Whitcomb and Stutz 2007), emphasising the importance of these considerations when making conclusions from, or comparing such studies.

Fungal community diversity has also received attention regarding the influence of environmental variability on their spatial patterns. Studies across environmental gradients such as elevation (Kernaghan and Harper 2001) and vegetation type (Bougoure *et al.* 2007) indicate significant shifts in fungal community structure with corresponding changes in environmental variables, however the explicit effect of spatial distance was not considered in these cases. Environmental conditions have also been noted to dominate soil fungal community assembly at regional scales, as defined by (Kivlin *et al.* 2014). Zinger *et al.* (2011) investigated the relative contribution of vegetation type, environmental conditions and geographic distance on alpine soil fungal communities across 11 habitat types across distances of 100 m - 1000 m. Fungal diversity correlated with soil organic matter content and pH, as well as the composition of plant communities, while geographic isolation did not significantly impact upon fungal community composition. In contrast, Green *et al.* (2004) suggested that

geographic distance was a stronger predictor for fungal community turnover than habitat variation (based on soil and vegetation type), from their distance-decay relationship observed for desert ascomycetes across distances of 1 m - 100 km. As noted by Zinger *et al.* (2011), such contradictory conclusions may be attributed to both differences in scale and taxonomic resolution of these studies. Therefore it is necessary to further our understanding of the relative roles of environmental and spatial factors in shaping fungal biodiversity and the scales at which various factors operate.

1.1.5. Linking spatial patterns and community assembly processes

The role of deterministic factors, such as environmental variation, relative to spatially influenced factors such as stochastic demographics and dispersal limitation, is closely linked to niche and neutral perspectives of community assembly. However, making inferences from spatial patterns and the control of spatial and environmental factors about the driving mechanisms of community assembly is not easily possible. These factors are often strongly interrelated and their relative importance is generally context and scale dependant (Powell *et al.* 2015). Changes to neutral dynamics can influence both spatial and environmental contributions to variation in community structure, and thus can influence the patterns explained by both niche and neutral dynamics (Smith and Lundholm 2010; Caruso *et al.* 2012b). Moreover, neutral patterns can originate from non-neutral based processes, where the assumption of ecological equivalence, which is intrinsic to neutral dynamics, is not met for various reasons (Alonso *et al.* 2006). Therefore spatial patterns need to be additionally studied from a mechanistic perspective, to gain an accurate insight into how the community dynamics contribute to ecosystem functioning.

Selection, drift, dispersal and mutation, as described by (Hanson *et al.* 2012), are four fundamental processes that drive the biogeographic patterns of microbial biodiversity. The role of selection can equate to the importance of niche dynamics in community assembly, while drift and mutation incorporate the occurrence of stochastic events under neutral assembly. Dispersal is the process by which individual communities are connected to one another; it effectively links the patterns and processes that occur within, and among, individual communities. These linkages across multiple scales can be explicitly considered from the perspective of the metacommunity (Leibold *et al.* 2004). A metacommunity is defined as a set of local communities that are linked by the dispersal of multiple potentially interacting species (Leibold *et al.* 2004). The metacommunity concept provides an underlying process-based framework that supports many of the spatial patterns of species diversity observed to date, such as species-area relationships, distribution-abundance relationships and species diversity along latitudinal and environmental gradients (Maurer 2009). The metacommunity framework can therefore complement the study of spatial dynamics of local communities, because the processes that drive spatial patterns interrelate across scales. While many of the above mentioned patterns have now been discovered in fungal communities (e.g. Green *et al.* 2004; Bougoure *et al.* 2007; Peay *et al.* 2007; Tedersoo and Nara 2010), applying the metacommunity framework to below-ground fungi has received relatively little attention.

The main approaches to evaluating the processes driving microbial spatial patterns have been reviewed as the use of *(i)*, variation partitioning the effects of space vs environment, *(ii)*, metacommunity theory, or *(iii)*, neutral theory of biodiversity (Hanson *et al.* 2012). Each of these provides a different emphasis on which general processes are important for structuring and maintaining biodiversity at different scales.

The research presented in this thesis uniquely incorporates all three approaches, in an attempt to more comprehensively understand the mechanisms driving fungal community assembly over a range of spatial scales and levels of organisation. By contributing to the knowledge required to predict changes in fungal community structure and function, at spatially relevant scales, it will be possible to recognise the effects of environmental change on fungal communities, in order to manage and conserve the surrounding terrestrial environments accordingly.

1.2. Research aims and objectives

Three overarching aims provide structure to the research undertaken for this thesis. These are as follows:

1. To determine the spatial structure of below ground fungal communities across a range of spatial scales.

2. To determine the role of environmental factors and spatial distance in shaping the community structure at different spatial scales.

3. To investigate the functional processes that are actively involved in structuring the observed fungal communities.

Each experimental chapter addresses the above objectives with a different focus which are outlined below:

- Experiment 1 focuses on the effect of habitat type for the assembly of below-ground fungal communities over a range of spatial scales, comparing the assembly of soil and root associated fungal communities and the functional processes driving their assembly.

- Experiment 2 focuses on investigating soil fungal community structure at small spatial scales and the relationship between the diversity and assembly of the plant community and the corresponding fungal community at these scales.

- Experiment 3 focuses on investigating the role of climatic conditions in shaping fungal community assembly by assessing soil fungal community patterns along an altitudinal gradient, across an alpine to subalpine ecotone.

- Experiment 4 considers the spatial dynamics of fungal community assembly at the scale of the metacommunity, rather than individual local communities. It explores the roles of geographic distance, environmental variables and phylogenetic relatedness in structuring the metacommunity, and how metacommunity patterns compare to the local community assembly patterns along the altitudinal gradient described in the previous chapter.

Chapter 2 The role of deterministic and stochastic factors in the assembly of simultaneous fungal communities

2.1. Introduction

Studying the spatial patterns that exist in ecological communities is fundamental to understanding how communities function and the mechanisms that drive and maintain their biodiversity. Spatial patterns in community structure are known to exist at a range of spatial scales (Ettema and Wardle 2002; Fortin and Dale 2005). Typically, large-scale heterogeneity exists as a gradual change in community structure with little patchiness, while small scale heterogeneity (small, discontinuous shifts in community structure) is nested within larger landscape scale patterns (Ettema and Wardle 2002; Legendre and Legendre 2012). By determining the scales at which spatial patterning occurs for individual communities, it is possible to gain insight into the complexity of the underlying mechanisms responsible for structuring these communities (Borcard *et al.* 2011; Legendre and Legendre 2012).

The mechanisms that drive community dynamics have recently been the subject of vigorous debate, with two seemingly opposing perspectives dominating the literature: niche-based and neutral assembly. The niche theory of species coexistence developed from the formalisation of the 'competitive exclusion principle' in which similar species differ in their preferences for habitat and resource use so as to prevent competitive

elimination (MacArthur and Levins 1964). Niches, or stabilising mechanisms, act more negatively on intraspecific interactions than interspecific interactions; therefore as a species' relative abundance increases within the community, its per capita growth rate decreases, and the competitive exclusion of other species is limited (Chesson 2000; Adler *et al.* 2007). As long as species have relatively narrow niche breadths, deterministic niche-based competitive exclusion would result in species replacement along environmental gradients. Niche models of species abundance have however been criticised as being unable to explain the great diversity present in species-rich communities such as tropical rainforests, because insufficient niches are present to support all species (Hubbell 2005; Zhou and Zhang 2008). This perspective has been challenged recently by neutral theories of community assembly, in which species' ecological differences are functionally unimportant ('fitness equivalence') and community dynamics are instead dependent on processes linked to dispersal limitation and demographic stochasticity.

While the applicability of such theories has been debated, neutral theories have been shown to accurately predict many of the patterns of biodiversity observed in natural communities (Hubbell 2001a; Chave 2004). However, niche and neutral perspectives are now suggested to be two extremes of a continuum (Gravel *et al.* 2006; Adler *et al.* 2007), and the focus of studying the mechanisms driving community assembly now lies around the question of what proportion of niche or neutral based mechanisms are actively involved in shaping natural communities. If community assembly were driven solely by niche dynamics, patterns of community dissimilarities would be dictated by environmental conditions and would generally result in spatial autocorrelation in community structure corresponding with the spatial structure of environmental variables. On the contrary, under neutral dynamics community dissimilarity would be

in response to increasing geographical distance, independent of patterns in environmental variables. Measuring purely spatial and purely environmental variation in a dataset is therefore meaningful to understand the relative importance of spatial variation and/or environmental control in shaping communities, but drawing conclusions about the processes involved in community dynamics from these patterns has been criticised (reviewed in Smith and Lundholm (2010)) .

Recently developed techniques to comparatively model community similarity under neutral dynamics (e.g. Etienne 2007, 2009) are promising to understand assembly processes from a mechanistic perspective. However, the mechanisms operating on community dynamics of any particular organism group may not be consistent, due to differences in factors such as dispersal mode or habitat specificity. Below-ground fungal communities are one example of this, in which their habitat associations may be broadly classified as predominantly soil-borne (e.g. saprotrophic fungi) or those that are able to form endophytic associations within, or partially within, plant roots (e.g. mycorrhizal fungi). Spatial structure has been noted in below-ground fungal communities including patterns of vertical niche distribution down the soil profile (Dickie *et al.* 2002; Genney *et al.* 2006; Lindahl *et al.* 2007), horizontal spatial autocorrelation at scales smaller than a few metres (Carvalho *et al.* 2003; Lilleskov *et al.* 2004; Whitcomb and Stutz 2007; Mummey and Rillig 2008; Pickles *et al.* 2010, 2012) and distance decay relationships at larger scales (Green *et al.* 2004). This research has largely focused on changes in biodiversity with respect to their functional role within the ecosystem. The work presented in this chapter, in contrast, is based from the perspective of the processes involved in community assembly (e.g. dispersal, competition, host effects) and the spatial patterns that result from these processes operating, and hence the goal of this study is quite different.

This chapter investigated whether community assembly processes are impacted following species sorting across a strong environmental filter (i.e. between the soil and the plant root), existing in the same geographic space. Simultaneously sampling both the soil and root-associated communities in the same geographic locations provided a unique opportunity in which to understand the mechanisms driving fungal community assembly in these two distinct community types, while minimising the variation in spatial and environmental factors among the selected sampling points. At each sampling location, two unique local communities are represented, a root and a soil associated community in each case. Species are drawn from a common metacommunity in order to construct these local communities. A species may colonise and persist in each community but its success in these two communities may differ (Figure 2.1). Therefore, each local root and soil associated community has the potential to be individually influenced by different community assembly processes. Two aims were addressed in order to compare community assembly among the two community types: *(i)* to investigate the relative importance of spatial and environmental factors, across a range of scales, in shaping patterns in beta diversity in soil and root associated fungal communities, and *(ii)* to understand the importance of deterministic and stochastic processes in shaping beta diversity patterns for each community type.

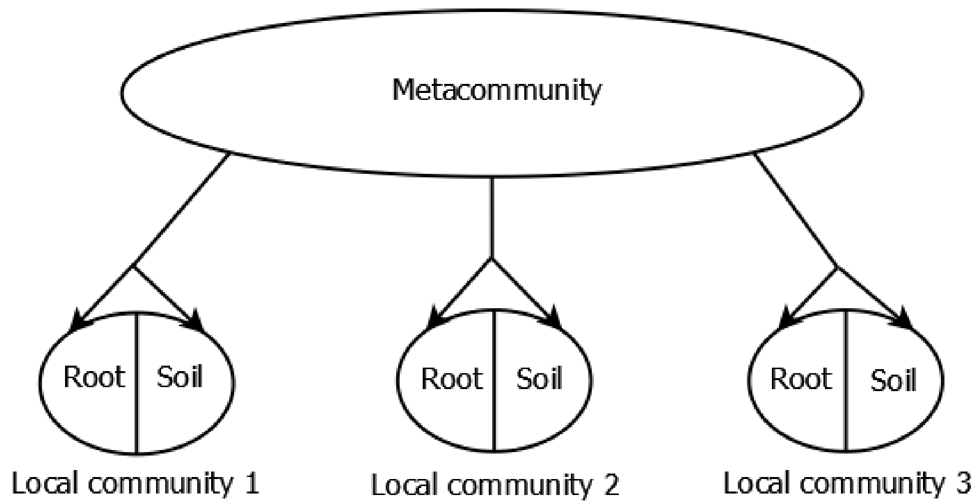


Figure 2.1: Representation of the relationship between the root and soil associated fungal communities. Each local community either in the soil or within the plant root is connected to a common metacommunity from which species are drawn. This may or may not be a single continuous metacommunity across the extent of the study, but rather it represents the greater species pool from which individual local communities are constructed, regardless of whether root or soil associated.

2.2. Methods

2.2.1. Study area and sampling design

Root and soil samples were collected from April to July 2010, according to a spatially explicit sampling design. A 500 km transect was established along the south-east coast of Australia, on which eight study sites were selected with exponentially increasing distance intervals between the sites. Samples were taken from one of four National Parks along the south-east coast of Australia, at distances of 0 m (Site 1), 40 m (Site 2), 200 m (Site 3), 1 km (Site 4), 5 km (Site 5), 25 km (Site 6), 100km (Site 7) and 500 km (Site 8), respectively along the transect (see Figure 2.2 and Table 2.1). Each site was characterised by similar soil and vegetation types - dry sclerophyll forest, graduating

into coastal heathlands, situated in sandstone basins (Keith 2004). Sampling locations were selected to minimise the environmental and biological variation present in the system, and hence maximise the ability to detect the spatial and neutral components of variation. Each site also had an abundance of juvenile *Woollsia pungens*, a native Australian heath species, chosen in this study for its wide geographic distribution along the coastal regions of south eastern Australia, as well as its ability to form mycorrhizal endophytic associations with a number of fungal species (e.g. Midgley *et al.* 2002; 2004).

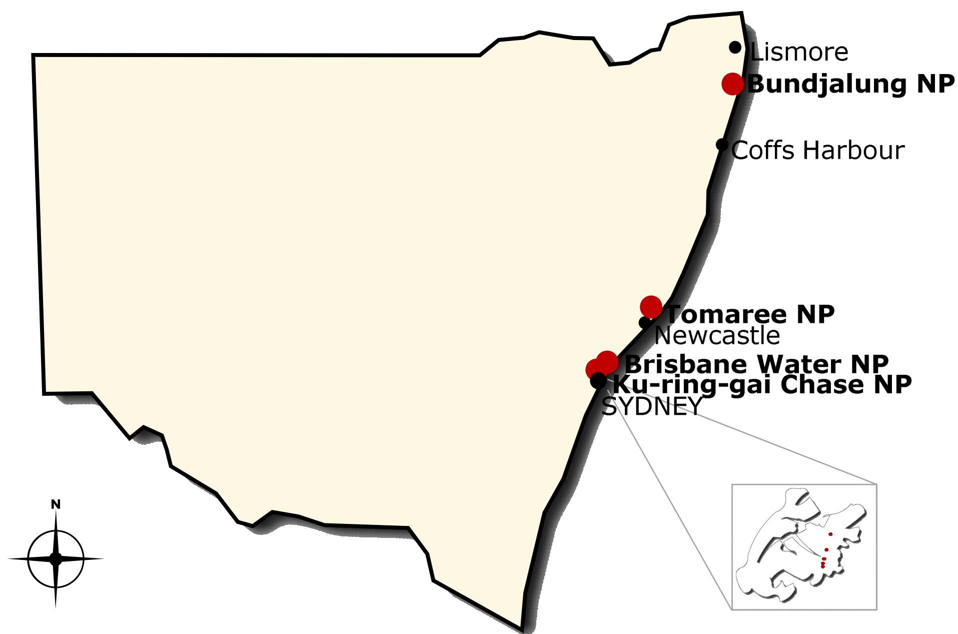


Figure 2.2: Sampling locations along the south-east coast of Australia displayed on a map of New South Wales. Red points indicate positions of study sites. Sites 1-5 are within Ku-ring-gai Chase NP (see inset), Site 6 within Brisbane Water NP, Site 7 within Tomaree NP, Site 8 within Bundjalung NP (not to scale).

Table 2.1: Geographical location, co-ordinates and position of each of the eight study sites, spanning the 500 km transect established along coastal NSW, Australia.

Study Site	National Park within which the study site is located	Geographical co-ordinates	Distance from origin of transect (km)
1	Ku-ring-gai Chase National Park	S 33°40'31.0" E 151°08'01.2"	0
2	Ku-ring-gai Chase National Park	S 33°40'36.8" E 151°08'05.5"	0.04
3	Ku-ring-gai Chase National Park	S 33°40'35.8" E 151°08'05.7"	0.2
4	Ku-ring-gai Chase National Park	S 33°40'10.7" E 151°08'39.0"	1
5	Ku-ring-gai Chase National Park	S 33°41'56.6" E 151°09'57.4"	5
6	Brisbane Water National Park	S 33°27'21.9" E 151°17'11.8"	25
7	Tomaree National Park	S 32°45'03.1" E 152°07'54.9"	100
8	Bundjalung National Park	S 29°12'38.1" E 153°22'32.8"	500

2.2.2. Sample collection and environmental analysis

Five *W. pungens* juvenile plants were randomly selected from within a 5 x 5 m quadrat at each of the eight sites. Distances between each plant in each quadrat were recorded. Entire plants were removed with intact root systems and the surrounding substrate was obtained to a depth of *ca.* 15 cm. The root systems were washed, hair roots excised, and then surface sterilised in a 100% commercial bleach solution (4.5% available chlorine) containing 100 $\mu l.l^{-1}$ of Tween 20 (Sigma-Aldrich Pty. Ltd., Sydney,

Australia) for 30 sec, followed by a 70% ethanol solution for 30 sec and three 1 min rinses in sterile MilliQ water as described by Bougoure and Cairney (2005).

Corresponding soil samples were sieved (< 2 mm) and a portion of each sample was stored at -80°C prior to DNA extraction, while the remainder was air dried and ground using a Mixer Mill MM400 (Retch – MEP Instruments Pty. Ltd., Gladesville, Australia) prior to measuring edaphic variables. Total C and N were analysed for each soil sample using a Leco TruSpec Micro, with oatmeal (%N 2.70 ± 0.04, %C 45.85 ± 0.40) and synthetic carbon (%C 4.9-5.1) as standards. C:N ratios were also calculated for each sample. Soil pH was determined using a standard protocol (Thomas 1996) in which distilled water was added to 5 g of air dried soil in a 1:1 w/v ratio, vortexed for 30 sec and allowed to settle for 10 min before taking readings from the supernatant using a CyberScan pH510 digital pH meter (Eutech Instruments Pty. Ltd., Singapore).

2.2.3. DNA extractions and T-RFLP analysis

DNA was extracted from *ca.* 100 mg of hair root material from each sample using the PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, USA). Hair roots were frozen in liquid nitrogen and ground to a fine powder in a 2 ml screw-cap tube using a micropestle before being added to the PowerBead tubes. DNA was subsequently extracted according to the manufacturer's instructions. Similarly, DNA was extracted from 0.5 g of soil using the PowerSoil® DNA Isolation Kit.

For T-RFLP analysis, the fungal ITS region was amplified using the fluorescently labelled primers ITS1F-6FAM and ITS4-HEX as previously described by Curlevski *et al.* (2010), with the exception that 30 ng of template DNA was used. Fluorescently labelled ITS-PCR products were purified using the UltraClean®-htp 96 Well PCR Clean-Up Kit (MOBIO Laboratories, Inc., Carlsbad, USA), and then digested for 3 h

using 5 units of the restriction enzymes *Hin*fI and *Taq*I (Promega Corporation, Sydney, Australia), incubated at 37°C and 65°C respectively. Restriction digests were analysed on an ABI 3730xl DNA analyser (Applied Biosystems, Warrington, UK) and each sample was run with a GeneScan 500 ROX internal size standard (Applied Biosystems, Warrington, UK) to facilitate the sizing of fragments. Terminal fragment lengths were determined using Genemapper v3.7 software (Applied Biosystems, Warrington, UK) in which peaks < 50 and > 500 bp were omitted from the analysis. Fragment lengths that were < 0.05 bp difference in size were identified as the same fragment. A peak height threshold of 50 fluorescence units was used. Terminal fragment lengths were exported as relative abundance data for each restriction enzyme (*Hin*fI and *Taq*I) and each dye (FAM and HEX) used.

2.2.4. Statistical analysis

As comparable datasets were produced from both *Hin*fI and *Taq*I enzymes (data not shown), OTU relative abundance matrices (fluorescence/peak height of the specific TRF relative to the total fluorescence of the complete T-RFLP profile) for root and soil datasets were calculated using the *Hin*fI HEX fragments (greater taxonomic diversity was observed in the HEX labelled datasets than the corresponding FAM labelled – data not shown). Relative abundance data were standardised using Hellinger distances, enabling the dataset to be analysed by Euclidean-based ordination methods, while not strongly weighting rare species (Legendre and Gallagher 2001).

In order to analyse the influence of both spatial and environmental factors on the structure of root and soil associated communities, pairwise distances for each sample were determined from the measured inter-plant distances within each plot and the GPS co-ordinates for distances between sites. A series of spatial variables was then

constructed using distance-based Moran's eigenvector mapping (dbMEM) analysis(Legendre and Legendre 2012). This method performs a spectral decomposition of the spatial relationships among the sampling sites in a study, creating a series of variables that correspond to all spatial scales contained within a given sampling design (Borcard and Legendre 2002). In this instance, the dbMEM eigenvectors do not show regular sine-shaped patterns, due to the irregular nature of the sampling design, but instead represent a general sequence of broad to medium scale variation over the extent of the study (Borcard and Legendre 2002; Borcard *et al.* 2004). The significance of the constructed dbMEM eigenvectors, together with the environmental variables measured at each sampling point (total C, total N, C:N ratio and pH), was tested in each dataset using 999 permutations. Only significant ($P < 0.05$) environmental and spatial variables were included in subsequent analyses. Variation partitioning was used to disentangle the role of the included environmental and spatial variables in shaping the root and soil associated communities over the entire extent of the study, while redundancy analysis (RDA) was performed to interpret the importance of each selected variable in shaping the observed structure in each community, across the various scales included in the sampling design.

As the proportion of variation attributable to spatial and environmental variables may not map directly to the relative contribution of neutral and niche-based processes in shaping community structure (Smith and Lundholm 2010), neutral models were formulated to estimate the role of deterministic and stochastic processes in shaping the root and soil associated communities. Neutral diversity (Θ) and immigration (I) parameters for each of the root and soil datasets were estimated using a recent development of the neutral sampling formula for multiple samples by Etienne (2009). Using the PARI/GP codes given in Etienne (2007), an artificial set of local

communities (total of 1000 simulations) of the same size as the observed communities were created for each dataset, using the above neutral parameters, enabling these communities simulated under neutral dynamics to be directly compared to the corresponding observed community. Bray-Curtis dissimilarities were calculated among each pair of observed communities, as well as among the communities in each simulation expected under purely neutral dynamics. The distributions generated by these distances were summarised into two test statistics. The first was the effect size of the mean observed dissimilarity relative to the average of the mean dissimilarity across all of the simulations, indicating a shift in the distribution of the community compared to what would be expected under the neutral hypothesis (Caruso *et al.* 2012a). The second was the effect size of the standard deviation of the distribution of observed dissimilarities relative to the average of the standard deviations across the distributions representing the simulated communities, indicating a change in the dispersion of the community distribution. Standard errors were calculated as bootstrapped 95% confidence intervals.

The distribution of dissimilarities can be consistent with the prediction under the neutral hypothesis or differ significantly from this prediction, indicating a lack of support for neutrality (Figure 2.3). For the latter, the sign of the estimated effect size can provide information on the role of the niche during community assembly (Caruso *et al.* 2012b). A shift in the mean of the distribution of dissimilarities (Figure 2.3b) that has larger distances among communities signals divergence, possibly due to species sorting or deterministic outcomes following stochastic colonisation events (e.g., priority effects). Whereas, a shift in the mean showing smaller distances among communities signals convergence, possibly due to the presence of a common environmental filter. Increased dispersion in the distribution of dissimilarities (Figure 2.3c) signals that both

divergence and convergence have occurred, in which subgroups of communities will tend to converge upon a common composition but then form clusters that diverge from one another within the entire community.

RDA ordinations were constructed using CANOCO v4.5 software (Ter Braak and Šmilauer 2002). All other analyses were performed in R v2.14.2 (R Development Core Team 2012), using the 'vegan' package (Oksanen et al. 2012).

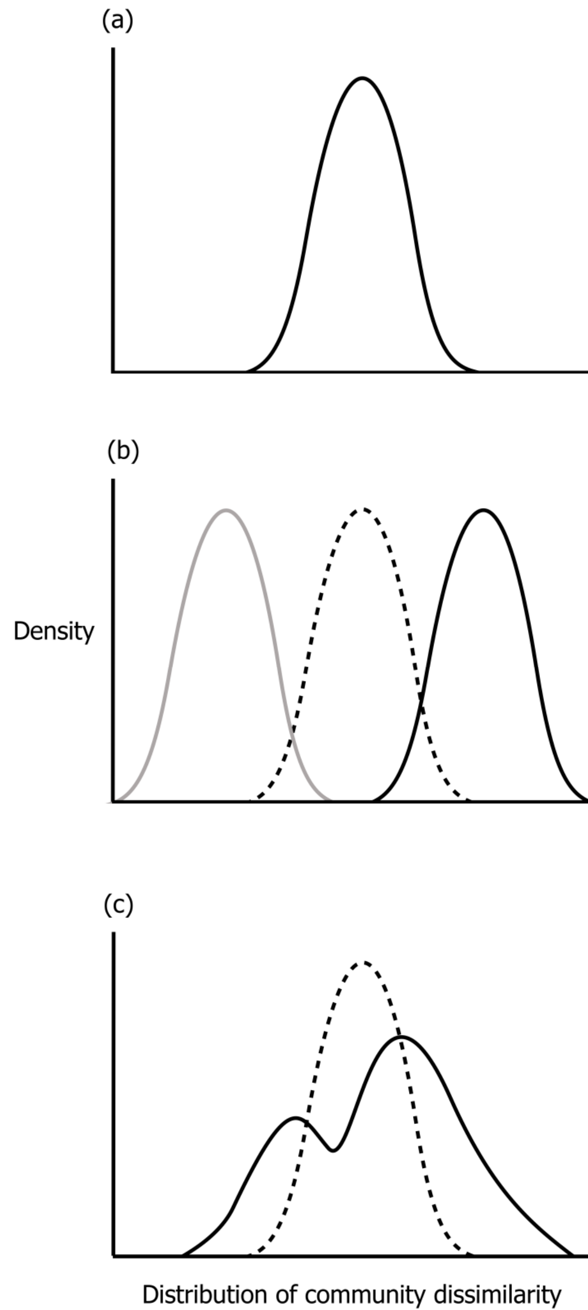


Figure 2.3: Conceptual diagram indicating distribution of community dissimilarity expected under neutral dynamics compared with those differing from the neutral prediction. Superimposed over the neutral distribution *(a)*, is *(b)* the shift in the distribution in accordance with a difference in the mean dissimilarity, resulting in either convergence (grey solid line) or divergence (black solid line) in the community, and *(c)* a change in the dispersion of the community distribution in accordance with a difference in the standard deviation of the distribution of dissimilarities.

2.3. Results

Despite a clear difference in composition between the root and soil associated fungal communities over all sampling sites (explaining 23.8% of variation - Figure 2.4, see also Appendix B for a summary of the T-RFLP data), similar spatial patterns in community structure over increasing geographic distances were observed in the two community types. All measured environmental variables (see Appendix C for a summary of these measured variables) were significantly related to both root and soil associated community structure (Table 2.2). Of the 15 dbMEM eigenvectors constructed for the distances incorporated within the sampling design, vectors 1-7 were found to be significant for the root associated community, and vectors 1-6 were significant for the soil associated community (Table 2.2).

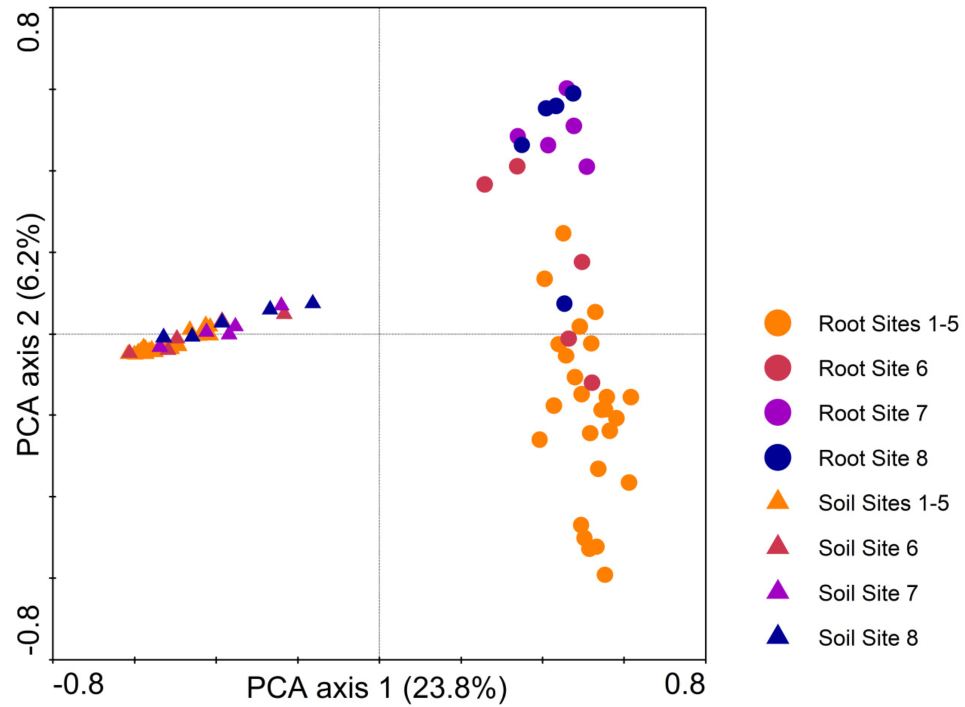


Figure 2.4: Principal components analysis (PCA) ordination of the ITS TRFs for the root and soil associated fungal communities sampled from eight spatially separated locations along the south-east coast of Australia.

Table 2.2: Significance values for the tested explanatory variables, based on their role in shaping the root and soil associated community structure along coastal NSW, Australia. Variables were forward selected based on 999 permutations.

Variable	Root		Soil	
	F statistic	P value	F statistic	P value
Total N	3.722	0.001	2.181	0.005
Total C	2.175	0.001	2.119	0.003
C:N ratio	2.044	0.001	1.860	0.007
pH	3.656	0.001	2.858	0.001
dbMEM1	2.638	0.001	2.399	0.001
dbMEM2	3.349	0.001	2.530	0.001
dbMEM3	1.879	0.006	2.417	0.001
dbMEM4	1.473	0.042	1.775	0.009
dbMEM5	1.498	0.041	1.913	0.007
dbMEM6	1.583	0.032	1.380	0.048
dbMEM7	1.609	0.033	-	ns
dbMEM8-15	-	ns	-	ns

Overall, variation partitioning indicated that the significant spatial and environmental variables accounted for 23% and 18% of the total variation present in the root and soil associated communities, respectively. However, the majority of the variation present in both systems remained unexplained by the included variables (Figure 2.5a, b). Despite this, the component of variation attributed to purely spatial variables was significant in both the root (P = 0.005) and soil (P = 0.005) associated communities, while the environmental variables, in the absence of the spatial component, explained little of

the overall variation observed (0.2% and 0% for the root and soil associated communities respectively) (Figure 2.5).

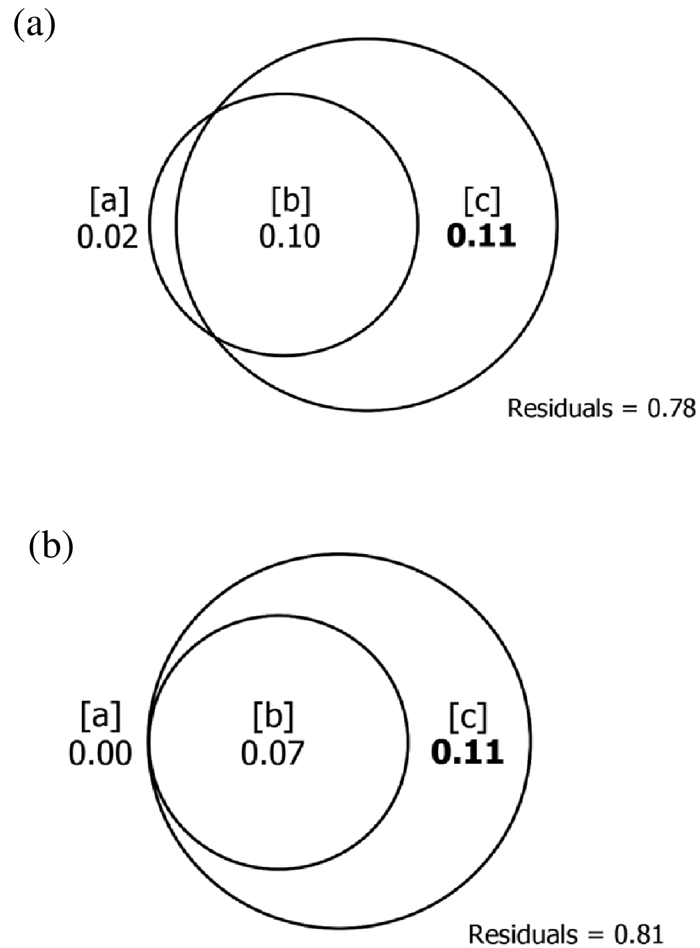


Figure 2.5: Variance partitioning of the proportion of variation in *(a)* root and *(b)* soil associated fungal community structure that can be explained by [a] purely environmental variables, [b] spatial and environmental variables that cannot be disentangled from one another, [c] purely spatial variables, and the residual unexplained variation. Significant values are indicated by bold type ($P < 0.05$). Note values do not sum to 1 due to rounding.

Spatial variables were responsible for shaping large scale structure in the root (dbMEM eigenvectors 1, 2 and 7) and soil (dbMEM eigenvectors 1 and 2) associated communities, evident by a correlation to the separation of samples from Sites 7 (100 km) and 8 (500 km) along axis 1 (Figure 2.6a, b). When considered together with these spatial variables, the measured environmental variables (pH, total C, total N, C:N ratio) were also most influential in shaping fungal community structure in both datasets at the largest spatial scales included in the sampling design, however the communities at Sites 1 and 5 also showed a positive correlation to total N (Figure 2.6). Spatial variables were further responsible for shaping fungal community structure at smaller spatial scales included within the sampling design (Sites 1-6; 40 m to 25 km), in the root (dbMEM eigenvectors 3-6; Figure 2.6a) and soil (dbMEM eigenvectors 3-6; Figure 2.6b) associated communities, along axis 2. Despite similar patterns of community structure across the scales included in the sampling design, the soil associated communities showed stronger clustering according to sampling site compared to that within the roots, indicating greater intra-site variability in the root associated communities, irrespective of sampling location (Figure 2.6).

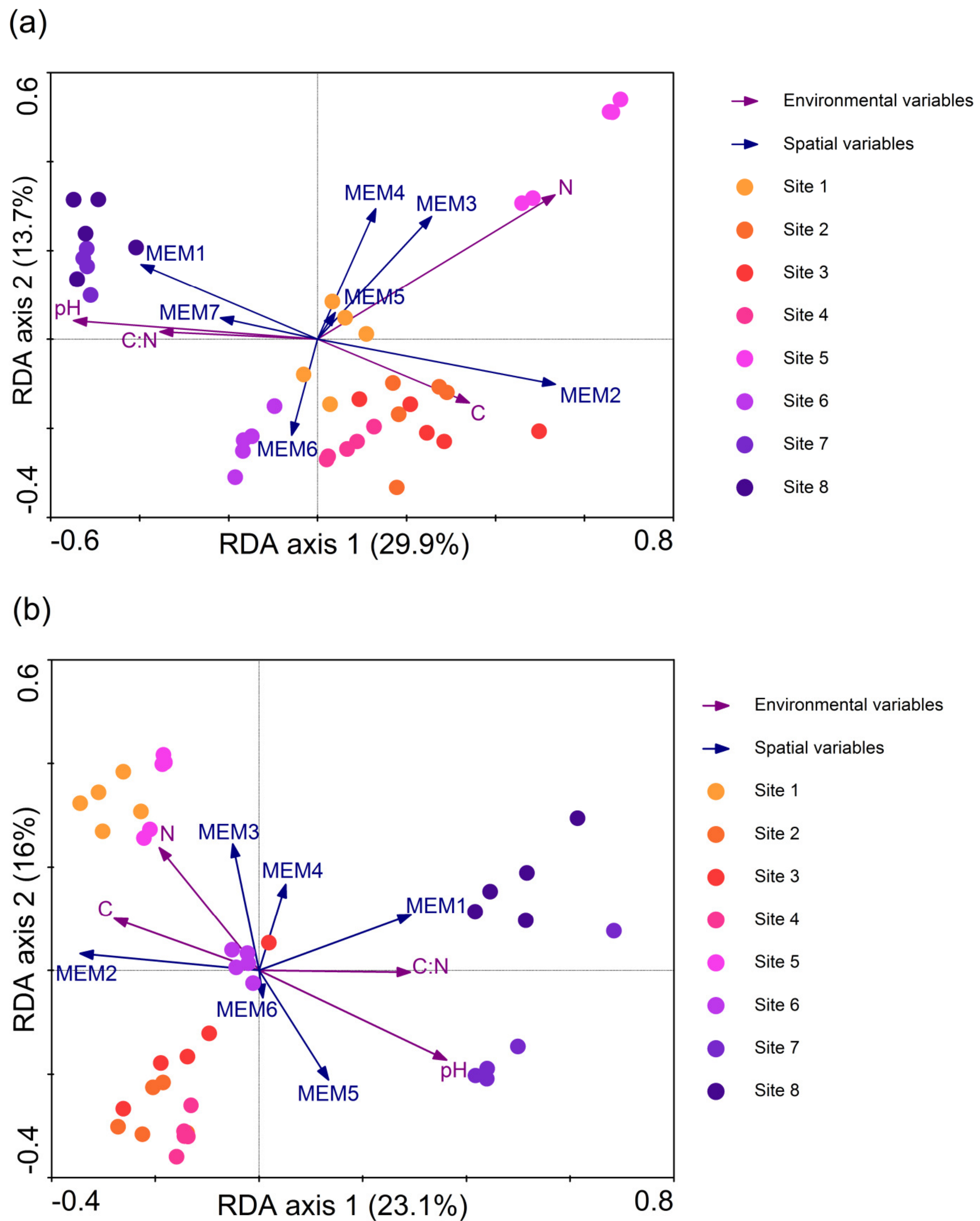


Figure 2.6: Redundancy analysis (RDA) ordination of the ITS TRFs from the *(a)* root and *(b)* soil associated fungal communities sampled from eight spatially separated locations along coastal NSW, Australia, and the significant ($P < 0.05$) environmental and spatial (MEM eigenvectors) variables responsible for shaping community structure at various scales.

Even though the measured environmental variables were poor predictors of fungal community structure, further investigation of the unexplained variation in the structure of root and soil associated communities, by means of simulating communities assembled under purely neutral dynamics using estimates of diversity (Θ) and immigration (I) parameters from the observed community, showed that neutral community dynamics were more influential in shaping the fungal community present within the roots of *W. pungens*. This was noted from the considerable overlap between the distributions of observed and simulated community dissimilarities (Figure 2.7 and Figure 2.8a). While there may have been a small reduction in the average dissimilarity among root associated fungal communities, suggesting convergence, the bootstrapped error bars overlapped with the null prediction of purely neutral dynamics. Conversely, there was a strong signal of niche-based assembly in the soil fungal community. Mean values of community dissimilarity were less than that expected from the simulated communities (Figure 2.7a and Figure 2.8b), indicating a degree of convergence of fungal communities to a common community structure. However, greater dispersion in the distribution of community dissimilarities than predicted under neutral dynamics was also observed for soil fungi (Figure 2.7b and Figure 2.8b), indicating a degree of divergence among soil fungal communities that is likely to be independent of purely spatial processes.

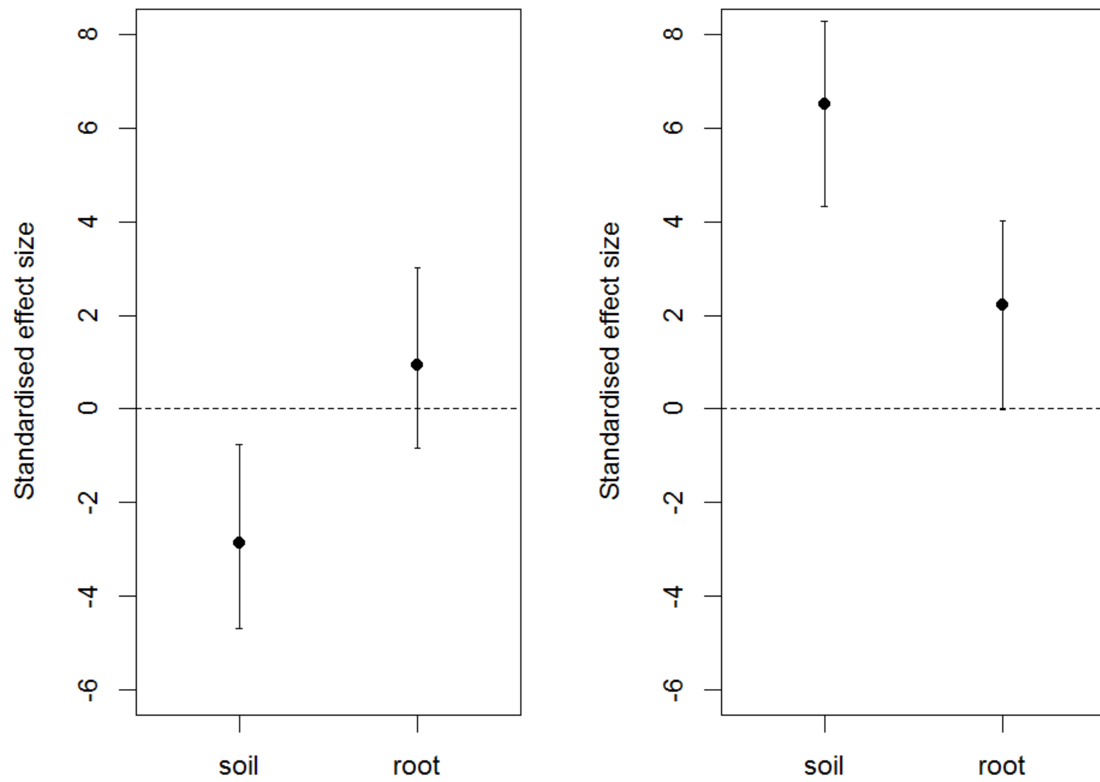
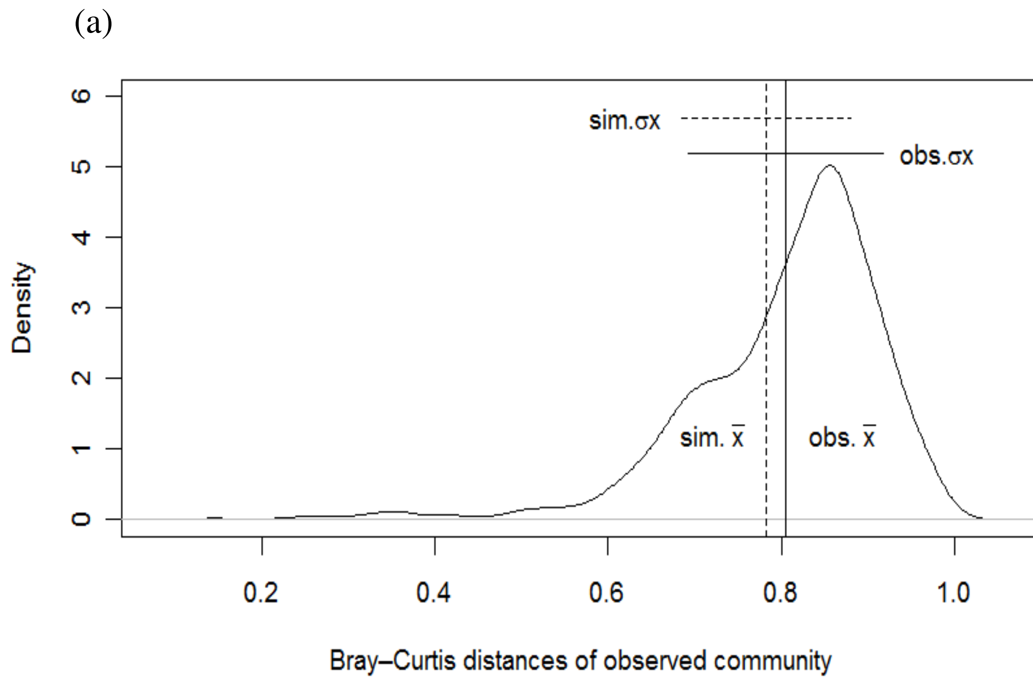


Figure 2.7: Standardised effect size ([observed dissimilarity – expected dissimilarity] / standard deviation of the expected dissimilarity) of the 'niche influence' on soil and root associated fungal communities, calculated using the dissimilarity (Bray-Curtis) distance *(a)* mean and *(b)* standard deviation values. The line at the origin of the y-axis indicates the prediction under neutral assembly (effect size = 0). A positive effect size indicates that the estimate is higher than predicted (divergence) while a negative effect size indicates the estimate is lower than predicted (convergence) under the neutral hypothesis. Error bars indicate bootstrapped 95% confidence intervals.

(a)



(b)

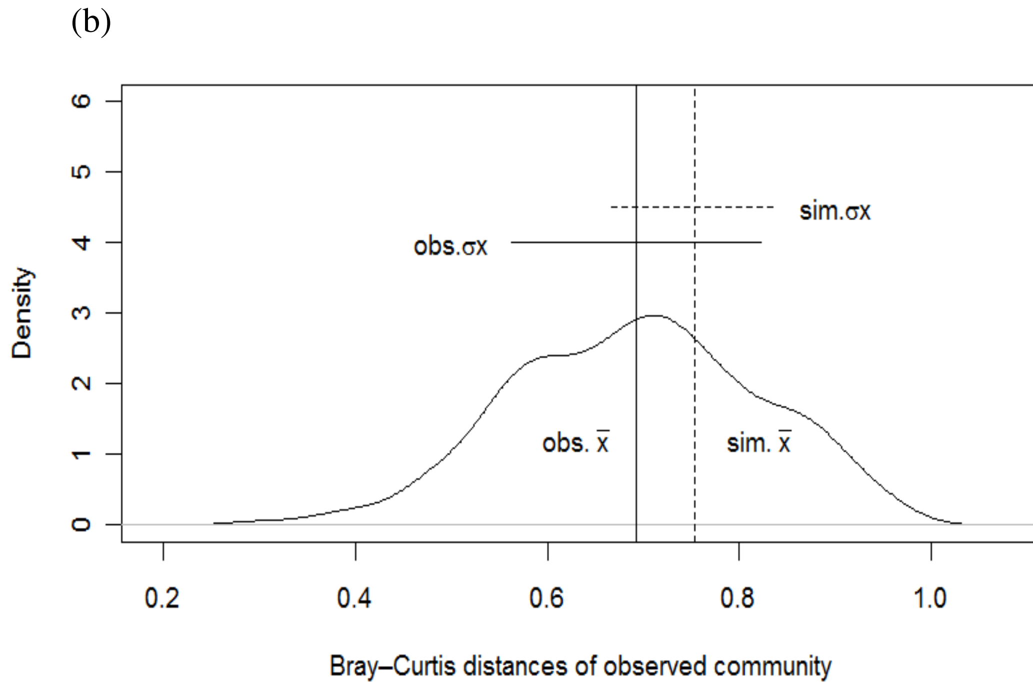


Figure 2.8: Distribution density curves of the observed *(a)* root and *(b)* soil community dissimilarity (Bray-Curtis) distances. Curves are plotted with the observed (solid line) dissimilarity mean and standard deviation values and are compared to the simulated (dashed line) mean and standard deviation values predicted under neutral community dynamics. Deviation from the simulated mean indicates a shift in the distribution of the community compared to what would be expected under the neutral hypothesis, while deviation from the simulated standard deviation indicates a change in the dispersion of the community distribution, compared to the expected distribution under neutral dynamics.

2.4. Discussion

Patterns in the structure of fungal beta diversity were found to be generally similar in both root and soil associated communities, across the scales included in the sampling design (40 m – 500 km). Spatial structure in both community types was most evident at large spatial scales (100 – 500 km), with some spatial structure also evident at smaller scales (more so in the soil than the root associated community). This work contributes to the growing evidence that below-ground fungal communities are spatially structured over a range of spatial scales; distance-decay relationships at similar spatial scales have previously been reported for soil borne ascomycete fungal communities in arid Australia at scales of 1 m – 100 km (Green *et al.* 2004), and taxa-area relationships have also been calculated for a number of fungal groups at various spatial scales (e.g. Green *et al.* 2004; Peay *et al.* 2007). The moderate levels of regional diversity observed in both community types, before an increase in species turnover at the largest scales sampled, is fitting with the triphasic model of the species-area relationship in which species diversity levels off at intermediate scales and increases again at continental scales (O'Dwyer and Green 2010). This lack of regional diversity was more evident in the root-associated communities where greater intra-site variability was apparent, irrespective of sampling location. This also makes sense considering the results of the neutral modelling where the important role of neutral processes was not rejected for the root associated fungal community.

From the variation partitioning analysis, purely environmental factors accounted for little of the overall variation in community structure in both the root and soil associated fungal communities. This indicates that, without considering their combined role with space in these systems, the measured environmental variables were not influential in the processes primarily involved in shaping these communities

over the scales included in the sampling design. The bulk of research to date has focussed on the role of environmental gradients in determining fungal diversity patterns (e.g. Anderson *et al.* 2003; Bougoure *et al.* 2007; Burke *et al.* 2009), and the influence of soil chemistry and nutrient status on soil microbial communities (Frey *et al.* 2004; Nilsson *et al.* 2007; Lauber *et al.* 2008). This work however confirms the importance of including a spatial perspective in studies aiming to understand the factors that are shaping community structure. Cottenie (2005) enforced this, reporting that disregarding spatial processes in communities would result in missing important patterns in 37% of the communities included in a meta-analysis. Despite explicitly considering space, a high proportion of the variation within both datasets of the current study remained unexplained. Similar results have been noted in studies of arbuscular mycorrhizal fungi (73% unexplained - Dumbrell *et al.* 2010), and while it is tempting to attribute this variation to either unmeasured environmental factors or random stochastic processes, making inferences about the role of niche or neutral processes in shaping community structure is not simplistic. The importance of spatial or environmental control on community assembly does not directly correspond to the importance of niche or neutral processes in community structure because the role of spatial and environmental factors in shaping community structure is often heavily intertwined. Changes in neutral mechanisms (e.g. migration and dispersal rates), for example, can influence both spatial and environmental contributions to variation in community structure, and thus can influence the patterns explained by both niche and neutral dynamics (Smith and Lundholm 2010; Caruso *et al.* 2012b). However, by complimenting variation partitioning analysis with neutral models, it was explicitly revealed that both niche and neutral processes have an important role in shaping below-ground fungal community structure.

Interestingly, these mechanisms responsible for driving community assembly differed in the root and soil associated communities, despite similar spatial patterns being observed for the two community types. Niche-based processes were most influential in shaping soil fungal community assembly, while the patterns in root associated community structure were driven by neutral mechanisms. Few other studies to date have focussed on how, in the same geographic space, community assembly is impacted by species sorting across a strong environmental filter. Farnon Ellwood *et al.* (2009) studied arthropod decomposer community dynamics in response to habitat gradients, seasonal variation and ecological succession, in which environmental heterogeneity could be controlled by sampling from tree epiphytes of the same height in the canopy, age and season. Null modelling results of that study indicated that, when environmental heterogeneity was controlled, community dynamics were operating stochastically, while deterministic processes dominated in heterogeneous environmental conditions (sampled at multiple heights within the canopy). In the current study, root associated communities were controlled by sampling a single plant species of similar developmental stage (juvenile *W. pungens*) across the extent of the sampling design. Soil associated communities, on the other hand, were potentially influenced by any number of plant species that have roots located within the sampling area. The current results suggest that, like Farnon Ellwood *et al.* (2009), community assembly can be driven by both niche and neutral dynamics, and which of these processes dominates community assembly may be influenced by the degree of environmental heterogeneity experienced by the individual local community.

Niche dynamics were more evident in the soil associated community, and both convergence and divergence in the soil associated community constituted this result. Convergence is the classical paradigm under niche dynamics, where local adaptation

occurs in a relatively homogeneous and natural environment. Divergence on the other hand is interpreted as species sorting in a heterogeneous environment, due to gradients in environmental variables or a disturbance regime characterised by high spatio-temporal variability (Caruso *et al.* 2012a). Determining whether communities converge, diverge or remain consistent to their neutral counterpart is an increasingly popular method in community ecology, ideally suiting the study of ubiquitous organisms (e.g. Dornelas *et al.* 2006; Caruso *et al.* 2011, 2012a,c). In such studies, the mean values of community dissimilarity are commonly used to compare the observed and simulated communities. In the work presented in this chapter, shifts in the distribution of dissimilarities relative to the neutral expectation may have been missed if the work was focussed solely on these mean values, due to the hierarchical nature of our sampling design (samples within sites). Indeed, evidence of convergence was observed using mean values of community dissimilarity, but evidence of divergence using estimates of the breadth of the distributions (standard deviations) was also seen. Therefore, while the mean community similarity shows a shift in the community distribution that reflects a specific mechanism, the standard deviation values of community dissimilarity are also important, indicating a shift in the variance within the community which could be the result of another mechanism or the interaction of several. These results enforce that care needs to be taken when interpreting how environmental heterogeneity is affecting community assembly at multiple scales, and shows that several community parameters are important for understanding the mechanisms behind community assembly.

Both niche and neutral processes are known to be influential in shaping below-ground fungal community structure (Dumbrell *et al.* 2010; Caruso *et al.* 2012a). The research presented in this chapter supports this and also suggests that the importance of niche

or neutral processes may differ following species sorting across a strong environmental filter. In the soil associated community, deterministic processes dominating local community assembly may be a result of greater environmental heterogeneity (as discussed above), whereas in the root associated community, stochastic processes are likely to be shaped by differences in immigration and dispersal histories that influence which species are drawn from the metacommunity. Such differences may result in priority effects on local community establishment, and in turn, influence the evolutionary dynamics of habitat specialization and generalization for the local community. The root associated fungi here seem to have a high niche overlap and therefore may be generalists in terms of their habitat preferences, despite their specialised ability to form endophytic associations; thus stochastic exclusion would dominantly be involved in their community assembly. Whereas for the soil associated fungi, the niche breadth would be much narrower as they are influenced by competitive exclusion in response to varying edaphic conditions. The relative importance of competitive or stochastic exclusion creates a continuum from niche structured communities to those structured by neutral dynamics (Gravel *et al.* 2006). The current results support this continuum hypothesis of community assembly, in which the relative importance of niche and neutral processes is dictated by the degree of environmental heterogeneity and strength of dispersal and immigration.

Note: The research presented in this chapter forms the basis for the below published article, of which I had the role of leading the development of the sampling design, field work, data generation and analysis, and writing. The community profiles of the root-associated fungal community were generated during the completion of a Bachelor of Science (Honours) degree, however the remainder of the data generation and all statistical analysis are original for this degree.

Beck, S., Powell, J. R., Drigo, B., Cairney, J. W. G. and Anderson, I. C. (2015). The role of stochasticity differs in the assembly of soil- and root-associated fungal communities. *Soil Biology and Biochemistry* 80: 18 – 25

Chapter 3 Small scale fungal community assembly and its relationship with plant diversity

3.1. Introduction

Understanding the spatial scales that are relevant to the distribution of fungal communities in the environment and their relationships with plant communities is vital for revealing how fungal communities contribute to ecosystem processes. Below-ground diversity and community structure is thought to have a major influence on plant communities, both in terms of productivity and diversity (Wardle *et al.* 2004; Van Der Heijden *et al.* 2008). Whether pathogenic or mutualistic, the effect of particular fungal species is not consistent across all members of a plant community, and therefore changes in fungal community assembly at relevant spatial scales can have carry-on effects to the above-ground community (Wardle *et al.* 2004). For example, mycorrhizal fungi can increase plant diversity by encouraging seedling establishment and enhancing the competitive ability of subordinate species compared to those dominant in the community (van der Heijden *et al.* 1998; 2008). The scales at which fungal community structure has an influence on the plant community is however unclear, especially with current knowledge gaps in how below-ground fungal communities are structured over a range of scales. The importance of choosing an appropriate scale for spatial ecological studies is paramount for understanding the mechanisms that are driving community assembly and the role of the community in

ecosystem interactions, and thus an understanding of community spatial patterns across scales is necessary.

In Chapter 2, fungal community assembly was investigated over a range of spatial scales, in both the soil and root associated communities. It was evident that variation existed in community assembly within each of the sampling sites, while a large proportion of variation in the fungal communities remained unexplained. This suggests that stochastic assembly processes may be involved in structuring these communities, as was tested in Chapter 2. However this pattern may also suggest that the fungal communities are structured at scales smaller than was the focus of Chapter 2 (< 5 m), as well as at the intermediate scales at some point between 40 m and 200 m. Based on the results presented in Chapter 2, spatial variables may play a more influential role than environmental variables in shaping the fungal community at these scales, however an intensive mechanistic study focused at a relevant scale is necessary to more fully understand the assembly of these communities at these smaller spatial scales.

To date, research into the spatial patterns of fungal communities at small spatial scales has largely focused on either arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungal communities (e.g. Tedersoo *et al.* 2003; Genney *et al.* 2006). ECM communities have been noted to show high species turnover at scales less than 50 cm (Tedersoo *et al.* 2003) and significant spatial autocorrelation at distances less than 2-3 m (Lilleskov *et al.* 2004). Differences in the spatial distribution of ECM mycelium compared to root tips (Genney *et al.* 2006) as well as a general patchy distribution at scales up to 20 m (Pickles *et al.* 2010) have also been noted. AM fungal communities have additionally been reported to show significant horizontal spatial structure at scales less than 1-2 m (Carvalho *et al.* 2003; Whitcomb and Stutz 2007; Mummey and Rillig

2008). Many such studies of small-scale patterns are based on that of individual species or genets, without consideration within the context of the whole fungal community. Spatial patterns evident at the species or genet level may not correspond to those across an entire fungal community (Pickles and Anderson 2016). In addition, measures of the distribution of species using different identification methods (i.e. using root tips vs sporocarps) can yield different results (Hortal *et al.* 2012), so it is not possible to make generalisations across these studies. Moreover, a range of functional types exist within a general fungal community, including mycorrhizal, saprotrophic and pathogenic groups, which all interact in a unique way with each other and other organisms, and this in turn may influence overall general community patterns. Little research has focused on consolidating the findings of small-scale spatial studies for specific fungal groups, in order to understand whole community patterns and the mechanisms that drive these patterns, within the context of other co-existing fungal groups present in the environment.

Potentially important mechanisms driving fungal community structure include abiotic niche differentiation, host specificity, competitive interactions, and dispersal ability, although the scale/s at which each of these is most important is uncertain. The plant community plays an important role by linking above-ground and below-ground biodiversity through these and other mechanisms (Wardle *et al.* 2004; Wardle 2006; Peay *et al.* 2008). However plant and fungal communities may also respond independently from one another, but in parallel, to similar environmental drivers, and thus their community patterns may appear to be interrelated more so than they actually are. Therefore, the additional influence of vegetation structure and diversity on fungal community structure is a valid consideration when attempting to understand the mechanisms driving fungal community patterns and the relationships that exist

between plant and fungal partners. The current chapter therefore also builds on the findings of the previous chapter by also considering the role of vegetation in structuring below-ground fungal diversity.

The work presented in this chapter aimed to understand the spatial structure of the soil fungal community as a whole, at scales of 20 cm – 200 m, and to determine what factors (spatial or environmental – edaphic or vegetation characteristics) are influential in shaping the observed community patterns. Because plant and soil fungal communities are importantly linked in their ecological functions, the research underlying this chapter also determined whether the patterns of beta diversity, or the variability in species composition among sampling units at a given scale (Anderson *et al.* 2006), showed any correlations with differences in the corresponding plant communities. Two further questions were therefore addressed in this chapter: *(i)*, are there changes in fungal and plant beta diversity within sites compared to among sites along each transect? *(ii)*, are these patterns in beta diversity comparable between the plant and fungal communities and across the two transects sampled?

3.2. Methods

3.2.1. Site description and sampling design

Sampling was conducted in March 2012, within Ku-ring-gai Chase National Park, approximately 20 km north-west of the city of Sydney, Australia (Table 3.1). The study location was chosen to correspond to the first three study sites of the experiment in Chapter 2, in order to enable comparison between the community patterns and scale of the sampling design. The site is characterised by a fire trail which separates two

contrasting vegetation types; a *Eucalyptus spp.* and *Banksia spp.* overstorey with an *Ericaceae spp.* dominated understorey on one side, while the overstorey is absent on the other side due to a history of disturbance in the area by means of frequent clearing (personal communication; NPWS 2011). A licence agreement with the NSW National Parks and Wildlife Service permitted the collection of plant and soil material from the selected locations (licence number SL100673).

Table 3.1: Location of the 12 chosen sampling sites. Transect 1 (T1) and Transect 2 (T2) were positioned parallel to one another in contrasting (open vs established canopy) vegetation types, within Ku-ring-gai Chase National Park, Australia.

Sampling Site	GPS co-ordinates	Sampling Site	GPS co-ordinates
T1 Site 1	S 33° 40.399’ E 151° 08.071’	T2 Site 1	S 33° 40.403’ E 151° 08.107’
T1 Site 2	S 33° 40.418’ E 151° 08.084’	T2 Site 2	S 33° 40.421’ E 151° 08.122’
T1 Site 3	S 33° 40.437’ E 151° 08.096’	T2 Site 3	S 33° 40.439’ E 151° 08.131’
T1 Site 4	S 33° 40.455’ E 151° 08.111’	T2 Site 4	S 33° 40.460’ E 151° 08.142’
T1 Site 5	S 33° 40.474’ E 151° 08.123’	T2 Site 5	S 33° 40.478’ E 151° 08.156’
T1 Site 6	S 33° 40.492’ E 151° 08.136’	T2 Site 6	S 33° 40.499’ E 151° 08.167’

Two parallel 200 m transects were established approximately 50 m apart, on either side of the fire trail. Transect 1 (T1) was located on the disturbed side of the trail, along which the overstorey was absent, while Transect 2 (T2) was positioned within the vegetation with an intact overstorey. Six evenly distributed sampling sites (every 40 m) were positioned along each transect, at which seven sampling points were established. The points at each site were spaced symmetrically within a distance of 5 m along the transect line, with separation distances of 2 m, 5 x 20 cm and 2 m, respectively (Figure 3.1).

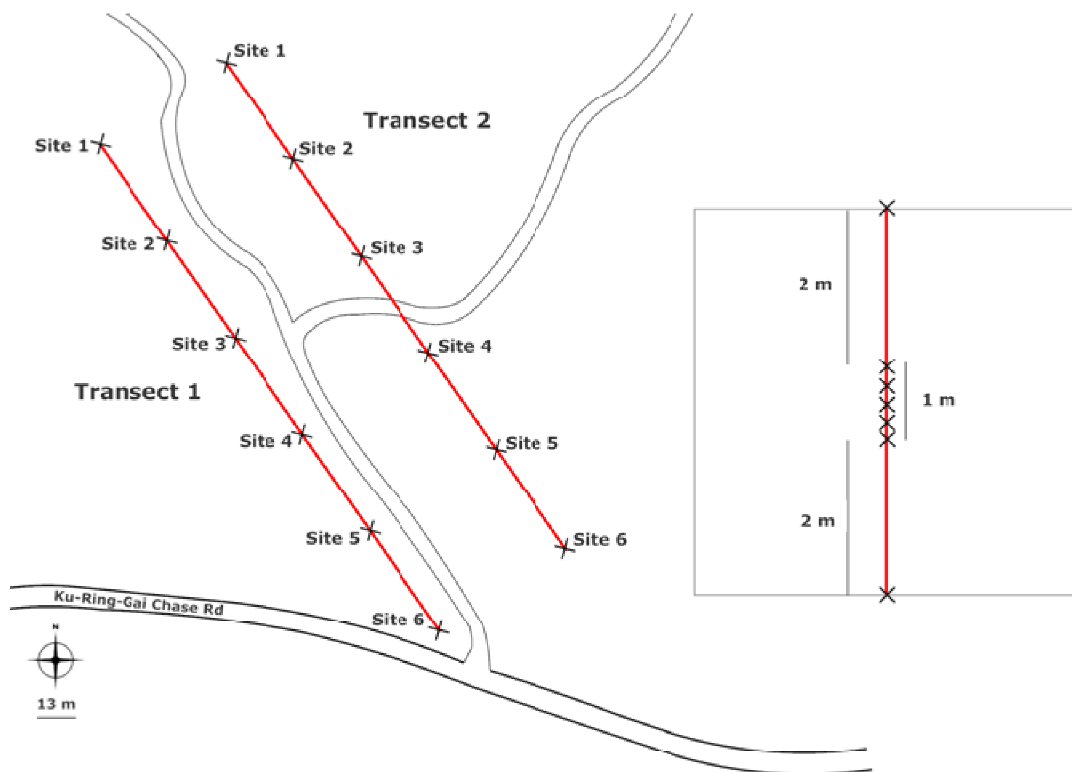


Figure 3.1: Map of study site in Ku-ring-gai Chase National Park, showing the location of the two 200 m transects along which the field work was carried out.

3.2.2. Sample collection and preparation

At each sampling location a 10 cm soil core (4 cm diameter) was taken and stored on ice, then 4°C upon return to the laboratory. Each core was homogenised, sieved (< 2 mm) and sub sampled, with a portion of each sample frozen at -80°C prior to molecular analysis, and the remainder air-dried at room temperature for environmental analysis. All samples were processed within 72 hours of collection.

3.2.3. Environmental analyses and vegetation survey

Soil moisture (measured as percentage volumetric water content) and soil temperature were measured and recorded over a single day, before each sample core was taken, using a HydroSense Soil Water Measurement System (12cm probe) (Campbell Scientific Australia Pty. Ltd., Thuringowa Central, Australia) and a LI-COR LI-1400 data logger (5 cm probe) (John Morris Scientific, Chatswood, Australia), respectively. A GPS elevation reading was also recorded at each sampling point, using a Garmin GPSMAP 62S (Garmin, Seven Hills, Australia). Once soil cores were collected, root material was separated by sieving, air dried and weighed to determine total root biomass for each sample (roots were stored at -80°C).

Air dried soil was ground using a Mixer Mill MM400 (Retch – MEP Instruments Pty. Ltd., Gladesville, Australia) for determination of total C and N content, and calculation of C:N ratio, using a TruSpec Micro CHN analyser (Leco Corporation, St. Joseph, Michigan, USA) with oatmeal (%N 2.70+/- 0.04, %C 45.85+/-0.40) and synthetic carbon (%C 4.9-5.1) as standards. Soil pH was also measured using a standard protocol (Thomas 1996) in which distilled water was added to 5 g of air dried soil in a 1:1 w/v ratio, vortexed for 30 sec and allowed to settle for 10 min before

taking readings from the supernatant using a CyberScan pH510 digital pH meter (Eutech Instruments Pty. Ltd., Singapore).

Vegetation characteristics at each site were determined by recording the presence or absence of ground cover, shrub (< 5 m in height) and tree (≥ 5 m in height) cover directly over each sampling location. In addition, in order to obtain beta diversity information for the plant community corresponding to the sampled fungal community, plant species diversity and abundance was recorded for a 5 x 5 m quadrat at each site, encompassing the seven sampling points.

3.2.4. DNA extractions and T-RFLP analysis

Total DNA was extracted from 0.5 g of soil from each core using the PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, USA), according to the manufacturer's instructions. Briefly, 60 µl of Solution C1 (cell lysis buffer) was added to each sample in the PowerBead tubes, and the samples were homogenised using the TissueLyser (QIAGEN Pty. Ltd., Doncaster, Australia) at 30 Hz for 10 min. The supernatant was separated by centrifugation at 10 000 x *g* for 1 min, then added to a series of inhibitor removal (Solutions C2 and C3) and salt (Solution C4) solutions. The samples were incubated at 4°C for 5 min and centrifuged at 10 000 x *g* for 1 min after each addition. The supernatant was loaded into a spin filter in three aliquots and centrifuged at 10 000 x *g* for 1 min after each addition. The spin filter membrane was then washed with an ethanol solution (Solution C5) and the DNA was eluted into 50 µl of sterile MilliQ water and stored at -20°C.

For T-RFLP analysis, the ITS region of the soil fungal rDNA was amplified using the fluorescently labelled primers ITS1F-6FAM and ITS4-HEX (Anderson *et al.* 2007) (Sigma-Aldrich Pty. Ltd., Sydney, Australia) in a 50 µl reaction volume containing:

100 ng of DNA; 20 pmol of each primer; 200 μM of each of dATP, dCTP, dGTP and dTTP; 10 μl of 5x reaction buffer (Promega Corporation, Sydney, Australia); 2.5 mM $MgCl_2$; 10 μg of bovine serum albumin (BSA) and 2.5 U GoTaq® Flexi DNA polymerase (Promega Corporation, Sydney, Australia). Reactions were performed using a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad Laboratories, Inc., California, USA) with cycling conditions of 95°C for 5 min, followed by 29 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 1 min, and a final extension of 72°C for 10 min. Negative controls containing no template DNA were also included in each PCR reaction. Amplification products were electrophoresed in 2% (w/v) agarose gels, stained with ethidium bromide and visualised under UV light.

Fluorescently labelled ITS-PCR products were purified using the UltraClean®-htp 96 Well PCR Clean-Up Kit (MOBIO Laboratories, Inc., Carlsbad, USA), according to the manufacturer's instructions. Briefly, five volumes of SpinBind was added to each PCR product, transferred to the wells of the Spin Plate and centrifuged at 2 500 x *g* for 3 min. Three hundred μl of SpinClean solution was then added to each well and centrifuged twice at 2 500 x *g* for 3 min, discarding the flowthrough after each centrifugation. The purified samples were eluted into 100 μl of 10mM Tris buffer and stored at -20°C. Samples were electrophoresed in 2% (w/v) agarose gels, stained with ethidium bromide and visualised under UV light.

All purified products were digested for 3 h using the restriction enzymes *Hin*fI (incubated at 37°C) and *Taq*I (incubated at 65°C) (Promega Corporation, Sydney, Australia). Each reaction contained 17 μl of purified ITS product; 2 μl of 10x reaction buffer (Buffer B for *Hin*fI – 6 mM Tris-HCl, 6 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT) (Buffer E for *Taq*I – 6 mM Tris-HCl, 6 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT); 2 μg of BSA and 5U of restriction enzyme, in a final reaction volume of 20 μl.

Restriction digests were analysed on an ABI 3500xl DNA analyser (Applied Biosystems, Warrington, UK). Each sample was run with GeneScan 600 LIZ internal size standard (Applied Biosystems, Warrington, UK) to facilitate the sizing of fragments. Terminal fragment lengths were determined using Genemapper v4.1 software (Applied Biosystems, Warrington, UK) in which peaks < 50 and > 500 bp were omitted from the analysis. Fragment lengths that were < 0.05 bp difference in size were identified as the same fragment. A peak height threshold of 50 fluorescence units was used. Terminal fragment lengths were exported as relative abundance data for each restriction enzyme (*Hin*fI and *Taq*I) and each dye (FAM and HEX) used.

3.2.5. Statistical analysis

A combined relative abundance matrix was calculated as the mean of the combined *Taq*I and *Hin*fI datasets, as there were no distinguishable differences in the number of fragments produced by each enzyme/dye combination (in contrast to the T-RFLP results in Chapter 2). This matrix was used as the community dataset of OTUs in subsequent analyses, so as to include the most diversity as possible while not over estimating differences in community patterns (see Appendix A – the large amounts of variation explained on axis 1 of the FAM datasets compared to the HEX datasets). Relative abundance data was also standardised using Hellinger distances, enabling the species data to be used in Euclidean-based ordination methods, while not strongly weighting rare species (Legendre and Gallagher 2001).

The similarity in community composition at each sampling site along each transect was analysed by principal components analysis (PCA) of the Hellinger standardised species dataset. The role of spatial separation distance on fungal community structure was then analysed by constructing a Mantel correlogram, in which the spatial variability

in the dataset is decomposed into specified distance classes within the extent of the sampling design (Legendre and Legendre 2012). This was complemented by the construction of a series of spatial variables that could be used alongside other explanatory variables in subsequent analyses, by distance-based Moran's eigenvector mapping (dbMEM) analysis (Legendre and Legendre 2012). This method performs a spectral decomposition of the spatial relationships among the sampling sites in a study, creating a series of variables that correspond to all spatial scales contained within a given sampling design (Borcard and Legendre 2002). The significance of the constructed dbMEM eigenvectors, together with the abiotic variables (total C, total N, C:N ratio, pH, soil temperature and soil moisture) and the vegetation variables (root biomass, as well as ground, shrub and tree cover), measured at each sampling point, was tested in each dataset by forward selection using 999 permutations. Only significant ($P < 0.05$) variables were included in subsequent analyses. Variation partitioning was used to disentangle the role of the included abiotic, vegetation and spatial variables in shaping the community assembly over the entire extent of the study, while redundancy analysis (RDA) was performed to interpret the importance of each selected variable in shaping the observed structure in the fungal community sampled at the various scales included in the sampling design, both within and between transects.

As high stochasticity in the community assembly was evident in the results of the current chapter, as was also the case in Chapter 2, neutral models were formulated to estimate the role of deterministic and stochastic processes in shaping the communities along the sampled transects. Neutral diversity (Θ) and immigration (I) parameters were estimated using a recent development of the neutral sampling formula for multiple samples by Etienne (2009). Using the PARI/GP codes given in Etienne (2007), an artificial set of local communities (total of 100 simulations) of the same size as the

observed communities were created, enabling these communities simulated under neutral dynamics to be directly compared to the corresponding observed community. Bray-Curtis dissimilarity distances were calculated among each pair of observed communities, as well as among the communities in each simulation expected under purely neutral dynamics. The distributions generated by these distances were summarised by the following test statistics: the median observed dissimilarity and the inter-quartile range (IQR) of the distribution of observed dissimilarities relative to the average of the median and IQR values across all of the simulations. Standard errors were calculated as bootstrapped 95% confidence intervals. These statistics were compared to those calculated under neutrality in order to indicate whether the community assembly is consistent with the prediction under the neutral hypothesis or differs significantly from this prediction, indicating a lack of support for neutrality, and thus suggesting the role of the niche in community assembly (see Chapter 2 for more detail).

In order to compare beta diversity of the fungal and plant communities within and between transects, as well as to see whether any patterns were consistent with the patterns of edaphic characteristics between and within transects, the homogeneity of multivariate dispersions (Anderson 2006; Anderson *et al.* 2006) was tested for each of the three datasets. Bray-Curtis similarity distance matrices were generated and the distance between each sample and its group centroid (samples were grouped by site) was estimated from the principal co-ordinate axes. Mean and standard error values were also calculated for each site. The distances of group members to the group centroid were then analysed by ANOVA ($P < 0.05$) to determine significant differences in the dispersions (variances) of each group. Correlations between the beta diversity of the fungal communities at each site, with the corresponding plant

communities and edaphic characteristics at each site, were tested by ordinary least squares linear regression of the mean dispersion distances at each site across both transects, using the 'lmodel2' package (Legendre 2014) in R v3.13.1 (R Development Core Team 2015).

The PCA and RDA were performed using CANOCO v4.5 software (Ter Braak and Smilauer 2002), while all other statistical analysis was performed using R v2.14.2 (R Development Core Team 2012) using the 'vegan' package (Oksanen *et al.* 2012), unless otherwise previously stated.

3.3. Results

Significant positive spatial correlation was observed in the fungal communities separated by 20 cm up to less than 100 m, while communities separated by distances greater than 100 m showed negative spatial autocorrelation (Figure 3.2). A summary of the T-RFLP data recorded for the fungal communities at each site is presented in Appendix D.

Forward selection revealed that all of the measured edaphic variables (a summary of the recorded edaphic and vegetation variables in presented in Appendices E and F), as well as the presence of tree and ground cover, were significantly related to fungal community structure across sites (

Table 3.2). Of the 37 dbMEM eigenvectors constructed for the distances incorporated within the entire sampling design, vectors 1-11 and 30 were significant, which mainly correspond to spatial structure at the larger scales included in the sampling design, as well as a portion of the smaller scale variation (

Table 3.2). Overall these significant spatial variables, followed by the edaphic characteristics, accounted for the majority of the explainable variation in community structure among the samples taken (32% and 19% respectively) (Figure 3.3). The effect of purely spatial variables on the community structure accounted for a significant 14% of the variation (***P*** = 0.005), while purely edaphic variables only accounted for 2%, although this was still significant (***P*** = 0.03). The transect from which samples were taken (a proxy for other differences between transects that was not explicitly measured) accounted for a small but significant proportion of the variation (1%: ***P*** = 0.017), while vegetation characteristics did not account for a significant proportion of the overall variation in the sampled fungal communities (***P*** > 0.05) (Figure 3.3).

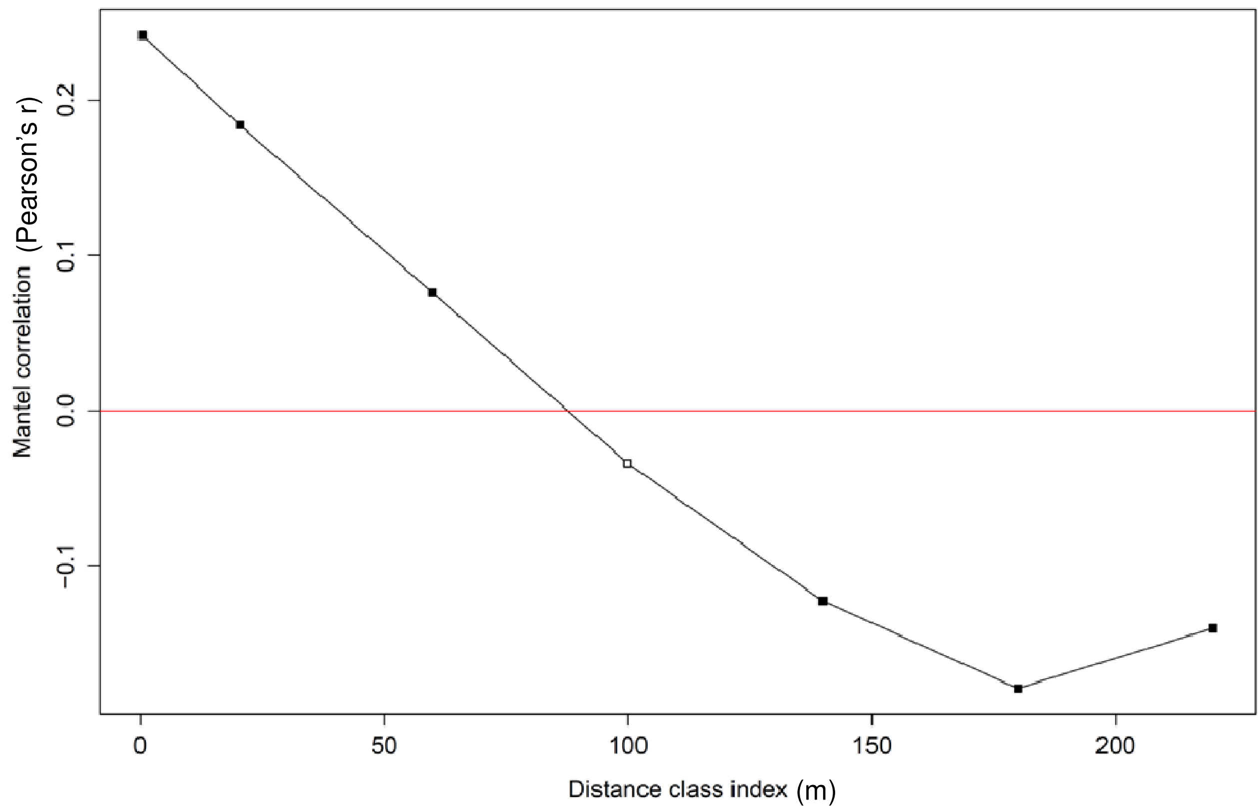


Figure 3.2: Mantel correlogram of the similarity in community composition among samples of increasing separation distances along the 200 m transects sampled in Ku-ring-gai Chase National Park, Australia. Positive Pearson *r* values indicate positive spatial autocorrelation at the specified distance class (i.e. samples are more similar to one another than expected by chance), while negative values indicate negative spatial autocorrelation at the specified distance class. Significant values are indicated as filled symbols ($P < 0.05$).

Table 3.2: Significance values for the tested explanatory variables, based on their role in shaping fungal community structure across all sampling sites in Ku-ring-gai Chase National Park, Australia. Variables were forward selected based on 999 permutations.

Variable	**F statistic**	**P value**
soil temperature	3.690	0.001
elevation	3.656	0.001
soil moisture	3.428	0.001
tree cover	2.986	0.001
C:N ratio	2.745	0.001
C	2.405	0.001
pH	2.338	0.001
ground cover	1.937	0.001
N	1.674	0.007
root biomass	1.392	0.041
shrub cover	-	ns
dbMEM1	4.748	0.001
dbMEM3	2.943	0.001
dbMEM2	2.900	0.001
dbMEM8	2.698	0.001
dbMEM4	2.505	0.001
dbMEM6	2.330	0.001
dbMEM5	2.006	0.001
dbMEM7	1.918	0.005
dbMEM9	1.708	0.005
dbMEM10	1.704	0.002
dbMEM11	1.660	0.006
dbMEM30	1.415	0.033
dbMEM12 - 29	-	ns
dbMEM31 - 37	-	ns

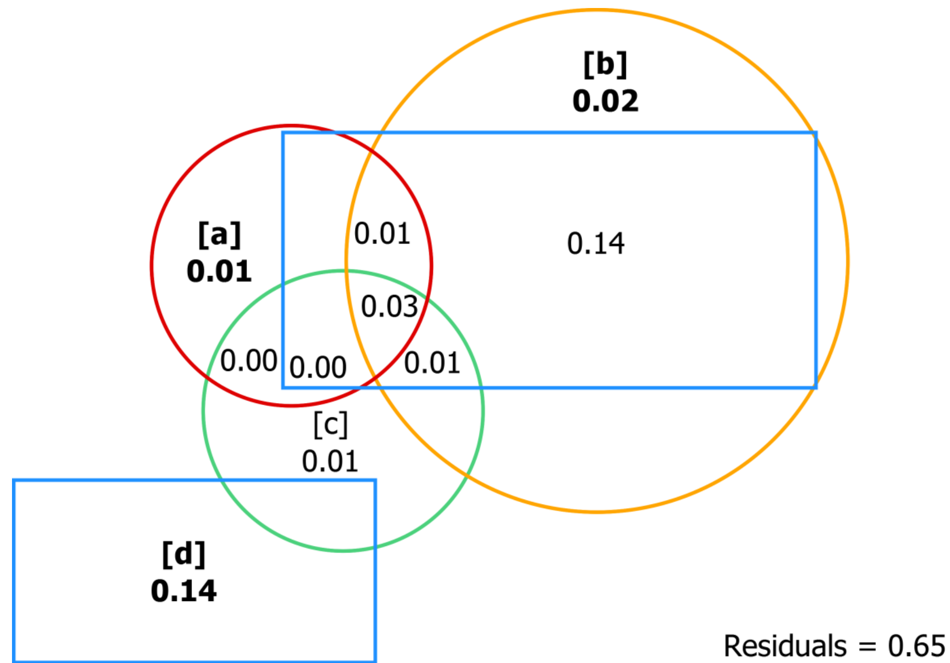


Figure 3.3: Variation partitioning of the proportion of variation in the fungal communities along two adjacent transects within Ku-ring-gai Chase National Park, Australia, that can be attributed the effect of transect (red), edaphic variables (orange), vegetation characteristics (green) and spatial variables (blue). The amounts of variation explained purely by each factor are specified by letters; transect [a], edaphic variables [b], vegetation characteristics [c] and spatial variables [d]. The proportions of variation that cannot be disentangled from their respective factors and the residual unexplained variation are also noted. Significant values are indicated in bold type ($P < 0.05$). Note values do not sum to 1 due to rounding.

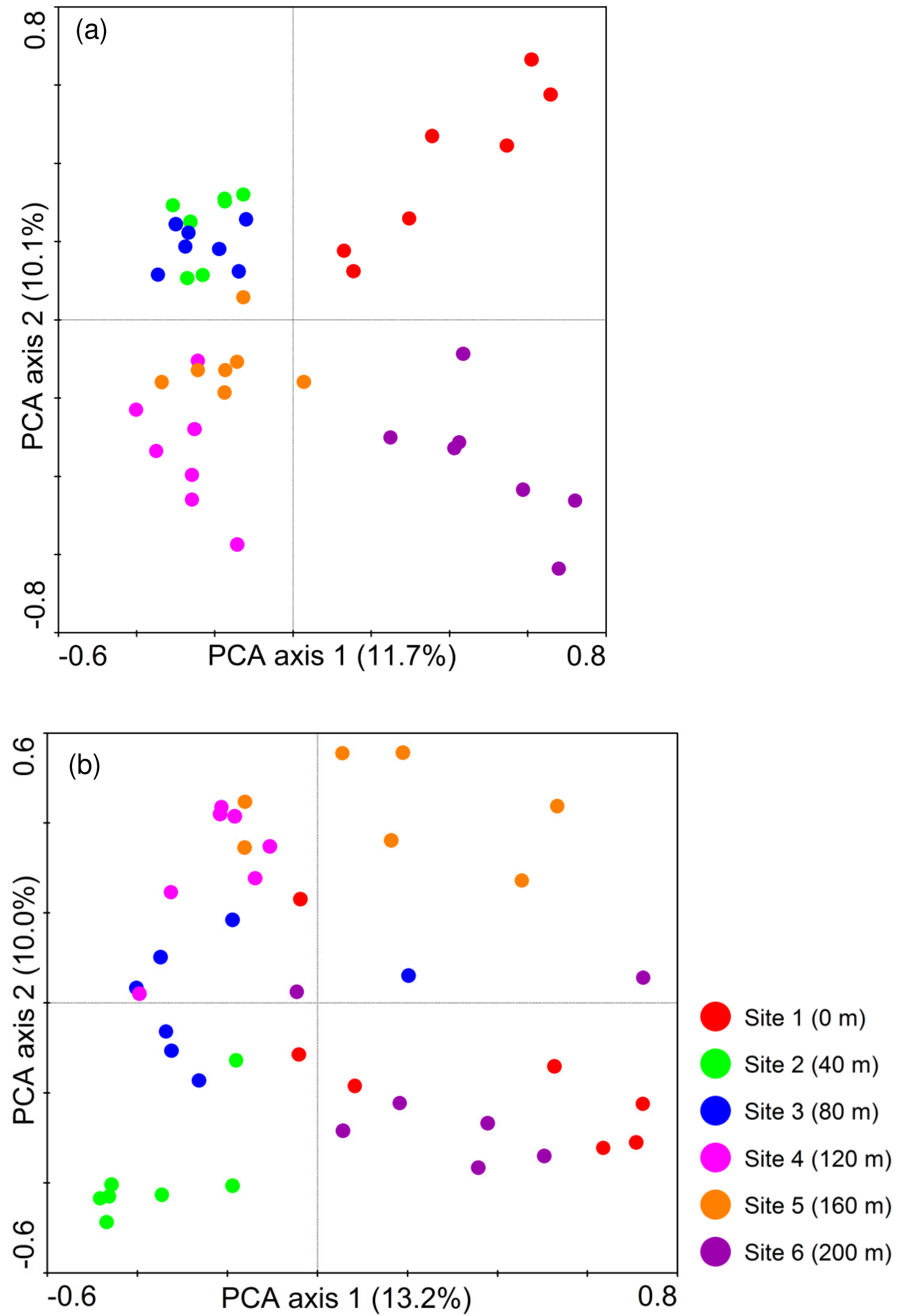


Figure 3.4: Principal components analysis (PCA) ordination of the ITS TRFs from the soil fungal communities sampled along two adjacent 200 m transects in Ku-ring-gai Chase National Park, Australia; (a) Transect 1 (open, cleared vegetation) and (b) Transect 2 (less disturbed vegetation with a canopy).

From the PCA ordinations, samples from Transect 1 showed a degree of clustering according to site, whereas the samples from the sites along Transect 2 were more variable in their composition (Figure 3.4). Across Transect 1, samples from Site 1 separated from Sites 2 and 3 along axis 1 of the PCA, which accounted for 11.7% of the explained variation, as did Sites 4 and 5 from Site 6. Separation of samples from Sites 1-3 from Site 4-6 was also evident along axis 2, which accounted for 10.1% of the explained variation (Figure 3.4a).

With the exception of Site 2, a separation according to transect was evident along axis 1 (16.1% of explained variation) which can be attributed to large scale spatial variables and vegetation variables, as well as soil moisture and soil temperature. Soil pH, C and C:N ratio, elevation, as well as smaller scale spatial variables attributed to the separation of Sites 4 and 5 from Sites 1-3 and 6 in Transect 1 and from Sites 1, 2 and 6 in Transect 2, along axis 2 (12.1% explained variation) (Figure 3.5).

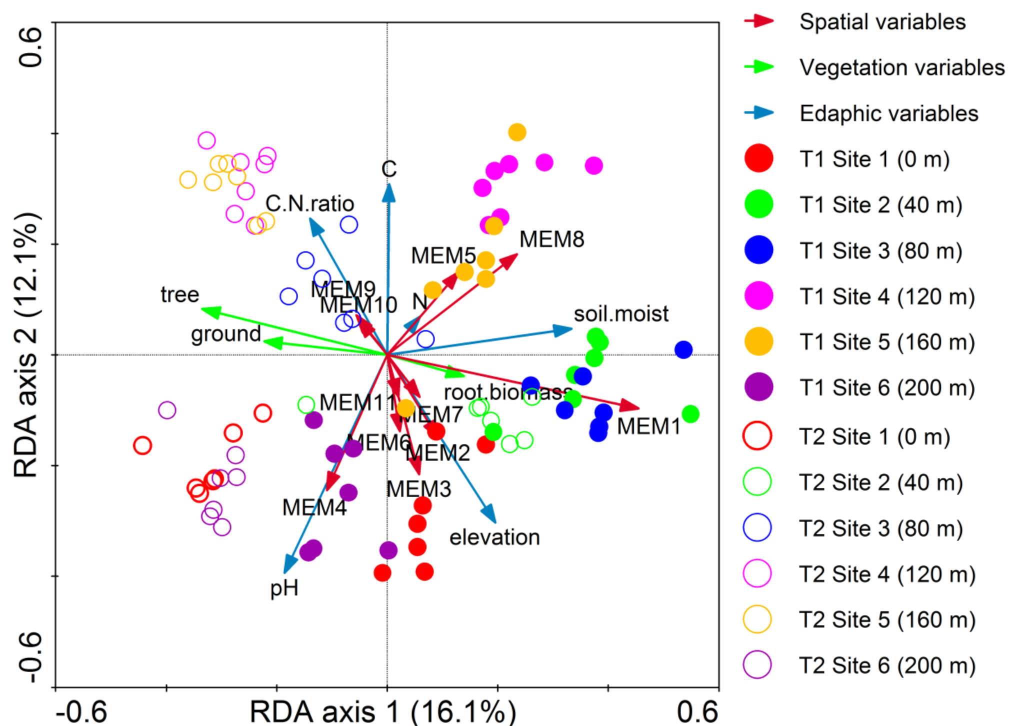


Figure 3.5: Redundancy analysis (RDA) ordination of the ITS TRFs from the soil fungal communities sampled along two adjacent 200 m transects with contrasting vegetation characteristics (Transect 1 and Transect 2), in Ku-ring-gai Chase National Park, Australia

The distribution of the observed community dissimilarities overlapped with the distribution of the communities simulated under neutral dynamics, thus being consistent with neutral assembly, however the dispersion of the observed communities was much greater than the neutral simulation (Figure 3.6a), as indicated by a greater IQR value relative to the neutral estimate (Figure 3.6b). Skew in the distribution of community dissimilarities was observed in both directions relative to the neutral scenario (Figure 3.6a), indicating that some samples show a high degree of clustering, while there are large differences in the similarity of certain pairs.

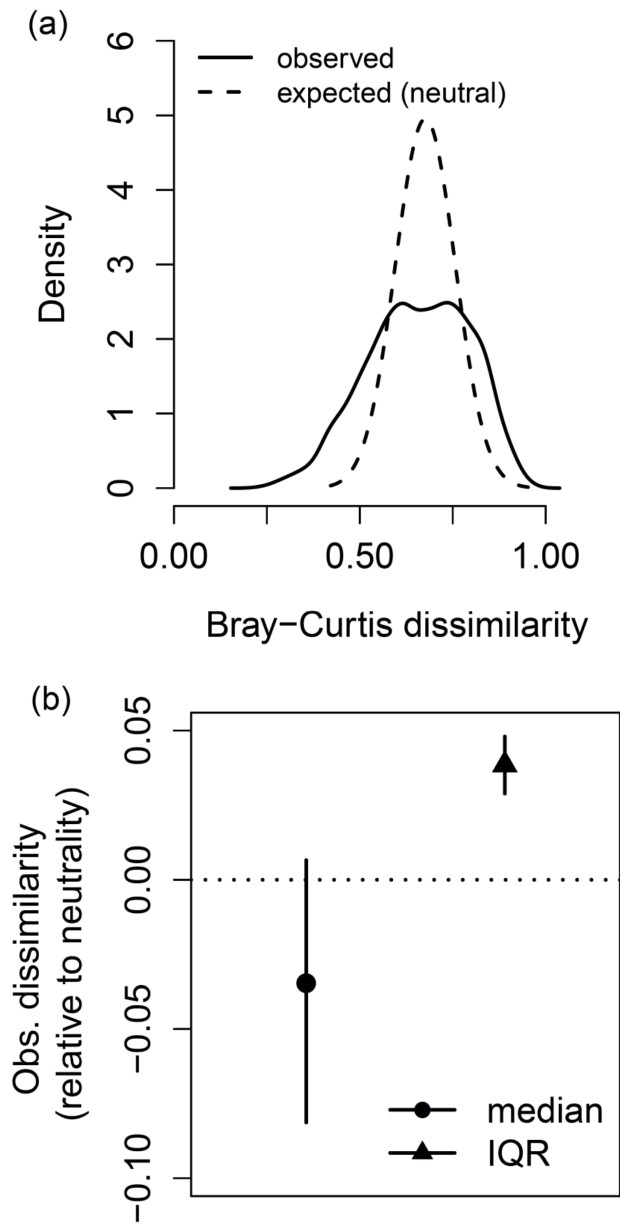


Figure 3.6: Comparison of the observed soil fungal communities in Ku-ring-gai Chase National Park, Australia, to a corresponding set of 100 simulated communities constructed under purely neutral dynamics. (a) Distribution density curves of the Bray-Curtis pairwise dissimilarity distances for the observed fungal community (solid line) and the corresponding simulated neutral communities (dotted line). The neutral distribution was calculated from the average of the mean and standard deviation values for each of the simulated communities. (b) Median dissimilarity values and inter-quartile range values relative to those predicted under neutrality (indicated by dotted line), plotted with the 95% confidence interval.

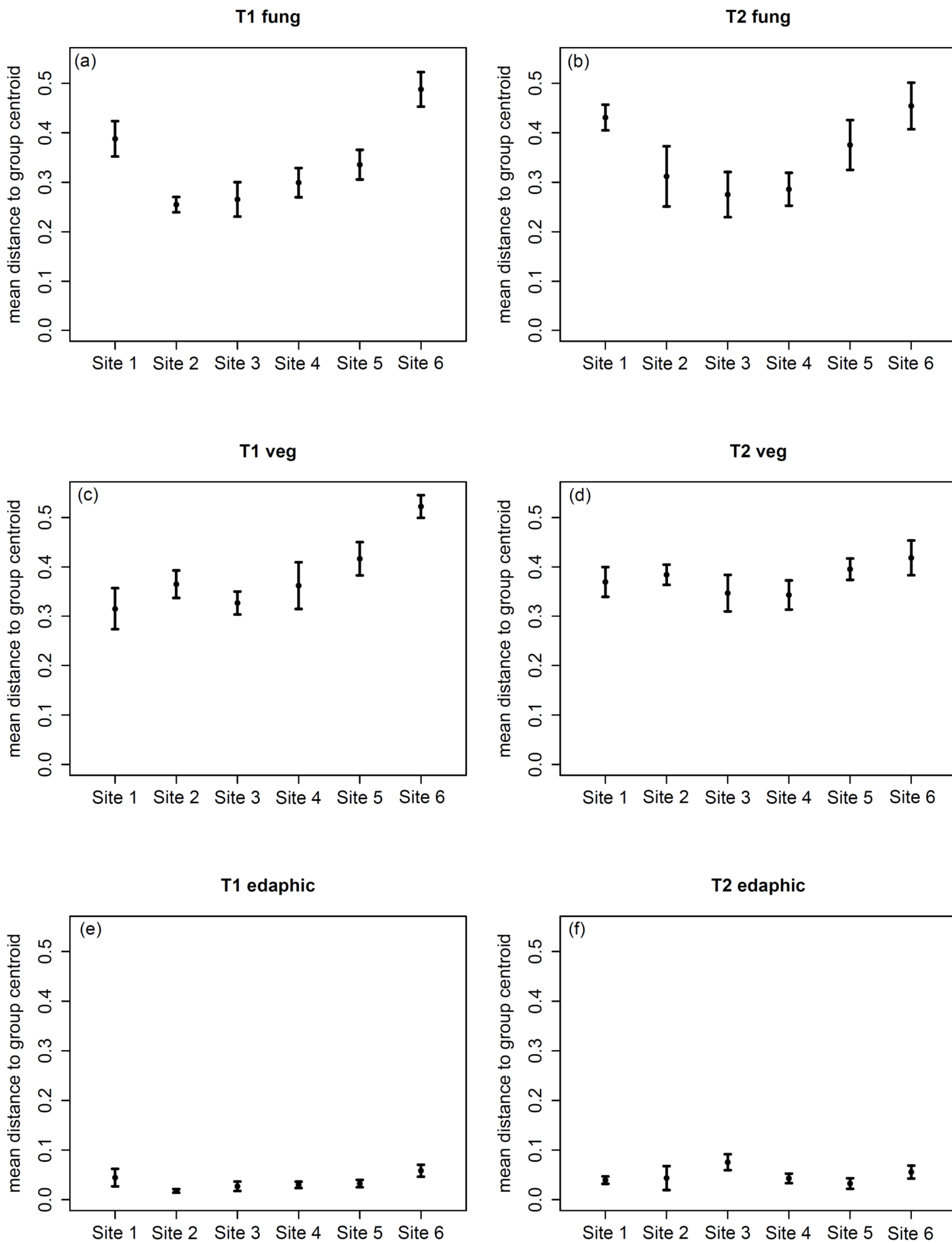


Figure 3.7: Multivariate dispersion analysis of (a) T1 fungal, (b) T2 fungal, (c) T1 plant and (d) T2 plant communities indicating the differences in beta diversity among the samples at each site along the transect and the variability of the corresponding (e) T1 edaphic and (f) T2 edaphic characteristics at each site. Mean distance to the group centroid in multivariate space are plotted for each site (± standard error).

Analysis of multivariate dispersions revealed that the variability of the fungal communities at each sampling location differed significantly among sites, along both transects (Transect 1: $P < 0.001$; Transect 2: $P = 0.030$). Levels of fungal beta diversity were highest at Sites 1 and 6, decreasing at the sites in between along each transect (Figure 3.7a,b). Dispersion of the plant communities within each site differed significantly among sites along Transect 1 ($P < 0.001$; Figure 3.7c), however this was not the case along Transect 2 ($P > 0.05$; Figure 3.7d). There was relatively little variability in the edaphic characteristics within and among sites, compared to the dispersion of the fungal and plant communities. No significant differences in the dispersions of the measured edaphic characteristics among sites were observed for either transect ($P > 0.05$; Figure 3.7e,f). A significant correlation between the mean beta diversity of the fungal communities and the plant communities was observed across both transects combined ($P = 0.011$; $R^2 = 0.438$), while a weaker, non-significant correlation was observed between the fungal communities and the edaphic characteristics ($P > 0.05$; $R^2 = 0.172$) (Figure 3.8).

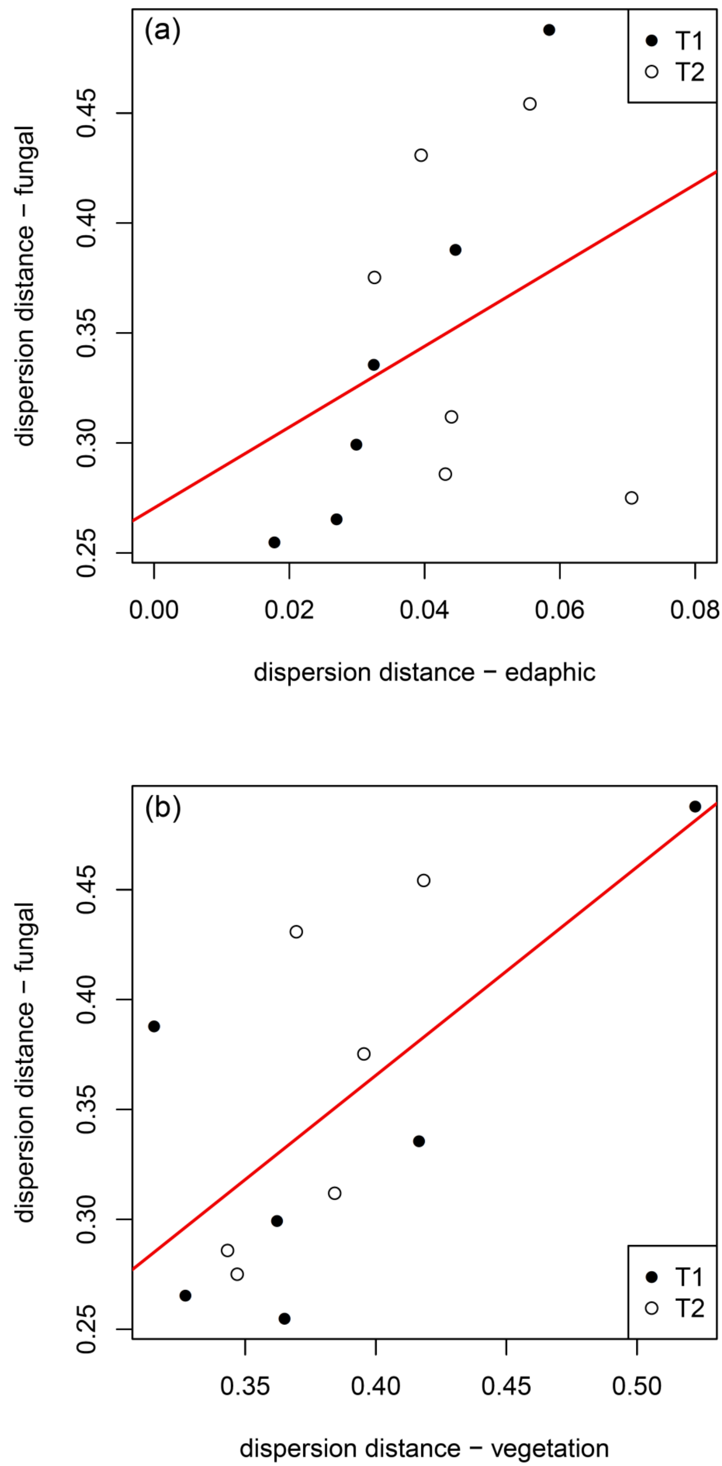


Figure 3.8: Mean multivariate dispersion calculated for (a) the fungal dataset vs the edaphic dataset, and (b) the fungal dataset vs the vegetation dataset, for both transects within Ku-ring-gai Chase National Park, Australia. Points are fitted with a linear regression model to indicate correlations in beta diversity among the datasets.

3.4. Discussion

3.4.1. Spatial patterns and mechanisms driving fungal community structure

The results presented in this chapter add to the growing evidence that soil fungal communities are spatially structured across a range of scales. Small scale spatial patterns in soil fungal communities were evident, in the form of positive spatial autocorrelation, at scales of 20 cm to less than 100 m. This is in keeping with the scales at which spatial structure has been previously noted in studies of fungal genets and specific functional groups. Spatial patterns in AM and ECM fungal communities, for example, have been noted at distances of 20 cm to a few metres (e.g. Tedersoo *et al.* 2003; Lilleskov *et al.* 2004; Pickles *et al.* 2010). The results of this chapter compliment the findings of Chapter 2 and fill the knowledge gaps that were presented in the previous chapter, indicating that spatial structure does exist in these communities at scales of < 5 m and between 40 and 200 m. These results also can be used to inform the design of future studies, as spatial patterns in soil fungal communities are likely to be detected between scales ranging from a few centimetres to up to distances of approximately 100 m.

Interestingly, negative spatial autocorrelation was also observed in the general soil fungal community at scales of 100 – 200 m. In contrast to the spatial patterns at the smaller scales mentioned above, the pattern of negative spatial autocorrelation at slightly larger spatial scales (at distances of 100 – 200 m compared to <100 m), may be a result of negative species interactions of closely related species that are not occurring at the smaller separation distances that were also investigated. Divergent resource requirements among species is also a possible explanation, however less likely, considering the subordinate role of edaphic variables in shaping fungal community

structure, as indicated by the following findings. Firstly, the variation partitioning results showed that the measured edaphic variables were less influential in explaining the variability of the fungal community compared to the roles of the other variables examined. In addition to this, only very weak correlations between the dispersion of edaphic variables and the diversity of the fungal community were evident from the multivariate dispersion analysis, thus supporting the fact that the measured edaphic variables were not primary drivers shaping the fungal community patterns at these scales.

Overall at the scales investigated, the patterns in fungal community structure that were observed are most strongly linked to spatial variables. Spatial distance is considered a proxy variable for community dynamics such as dispersal limitation (Legendre *et al.* 2002), thus indicating that such dynamics may have an important influence on how the communities are structured at the scales investigated. Evidence for strong competitive interactions has been noted for fungal communities previously (Wardle 2006), while the explicit role of dispersal has received less attention. Peay *et al.* (2007) measured fruit body abundance as a proxy for dispersal ability of selected species of ectomycorrhizal fungi, noting a positive correlation between the relative abundance of a species' fruiting bodies and the number of tree islands at which the species was recorded. Dispersal-competition trade-offs likely existed among species that could colonise the smallest versus the larger tree islands, and that these species had a greater abundance of fruiting body structures, thus investing more heavily in their dispersal ability than their competitors. A similar trade-off relationship may exist in this system, whereby members of the community with greater dispersal abilities are more successful at the scales investigated here, while those with a competitive advantage may be more successful at smaller or larger spatial scales, however a more extensive

understanding of the role of dispersal across such systems is necessary to make further conclusions.

Neutral dynamics were noted to have an important influence in community assembly at these scales, which is in keeping with the fact that spatial variables are influential in this system. The observation of greater dispersion in the community assembly than under neutral dynamics, together with significant multivariate dispersion results among samples, indicates that some communities are clustered in the similarity of their assembly, while other pairs have larger dissimilarity distances than would be expected under purely neutral dynamics. Thus it is evident that neutral dynamics are not the sole drivers of fungal community assembly. Other possible mechanisms involved in community assembly here may include resource partitioning (which is likely to have a relatively minor role; mentioned above and further discussed below), or the regulation of community structure by consumers in higher trophic levels. Little is known at this stage of the role of soil food web interactions on soil fungal communities in general, and understanding the relative importance of resource partitioning vs trophic level interactions as drivers of soil biodiversity is still in its infancy (Wardle 2006), however their role in driving soil fungal communities at the scales investigated here cannot be discounted, further indicating the complexity of the mechanisms driving soil fungal community structure.

3.4.2. Relationship between plant and fungal biodiversity

The role of plant diversity in structuring soil diversity has received only very recent attention (see Wardle 2006 for review). Evidence exists for below-ground microbial communities indirectly and directly influencing the productivity, diversity and composition of plant communities (van der Heijden *et al.* 1998; 2008), however

whether plant diversity and microbial diversity are coupled is less clear. Studies have generally concluded that plant diversity is unrelated to diversity of many groups of below-ground organisms, suggesting that that biodiversity above and below-ground may be unrelated despite their interdependence in ecosystem processes (Wardle 2006). Prober *et al.* (2015), when considering the relationship between soil microbial groups and plant diversity, both in terms of alpha and beta diversity patterns, reported that plant and fungal alpha diversity were unrelated, while plant beta diversity was significantly correlated with fungal beta diversity patterns. The current work also suggests that correlations do exist between the beta diversity of fungal communities and their corresponding above-ground plant communities. This is despite the diversity of fungal communities being found to differ among sites irrespective of the vegetation type from which they were sampled, whereas the diversity of the plant community was consistent across sites within a more established plant community compared to one with a history of disturbance. Therefore, whether the observed correlation is directly related to plant diversity is not obvious from these findings, as increased plant diversity can have indirect effects on microbial diversity through increased plant production associated with greater diversity rather than diversity itself (Zak *et al.* 2003). This correlation does suggest however that plant and fungal diversity are linked in some way; the characteristics of this relationship that can be concluded from the current results are discussed herein.

It has been proposed that the most likely mechanism for plant diversity to influence soil diversity is by an increase in the heterogeneity of soil resources with increasing plant diversity, which in turn promotes resource partitioning among the constituent soil organisms (Hooper *et al.* 2000; Wardle *et al.* 2004). While resource partitioning may be the primary mechanism relating plant diversity to the diversity of other soil

organisms, the current results suggest that the correlation between fungal diversity and the variation in edaphic characteristics among sampling sites was weaker than the relationship between fungal and plant diversity. Sample sites that had heterogeneous plant communities were also seen to contain heterogeneous fungal communities, while the same was not the case for the edaphic characteristics. Therefore, fungal community composition is more strongly influenced by the dispersion, or range, of vegetation properties, compared to the range of measured edaphic variables. Therefore when considering the relationship between the variation in fungal and plant diversity among sites, echanisms that do not involve resource partitioning, such as those in keeping with neutral dynamics, are therefore likely to be primarily responsible. Similarly, Prober *et al.* (2015) noted that plant beta diversity was a stronger predictor of soil microbial diversity (including fungal diversity) than any of the explanatory environmental variables that were included in their study, and also acknowledged that the strength of the responses of plant and microbial communities to environmental drivers may become more apparent at broader spatial scales due to distinct responses of the two community types to environmental factors. From the current work, it cannot be concluded with confidence that edaphic variation plays no significant role in shaping fungal beta diversity patterns, because the result of a non significant relationship between fungal beta diversity and edaphic variation may have been impacted by a potential outlier datapoint in a dataset with a small sample size, however it is likely that niche partitioning plays a subordinate role in any interactions between plant and fungal beta diversity at the scales investigated.

Variability in the diversity of the fungal community was evident among sites, regardless of the sampled transect, while the diversity of the plant community did not vary significantly in the established plant community, but did in the disturbed plant

community. The effect of disturbance itself on below-ground fungal communities has been reported with mixed conclusions, mainly focusing on AM community patterns (e.g. Schnoor *et al.* 2011; Lekberg *et al.* 2012). The current results cannot suggest a lot about the role of disturbance *per se* on this system, but it does indicate that the specificity of the relationship between plant and fungal diversity may be quite low. In other words, changes in the diversity of one community type can occur without strongly influencing the diversity of the other community type. It has been reported that below-ground organisms that are directly associated with plant roots, such as mycorrhizal fungi, have a higher degree of specificity than previously expected, suggesting that a higher diversity of plant species should be able to support a greater diversity of root-associated species (Wardle *et al.* 2004). This prediction may not be applicable however to soil fungi at a more general level, as was considered here, because the strength and specificity of the relationship between plants and their fungal partners may not be consistent for mutualistic vs pathogenic or saprotrophic groups. Moreover, if plants and the fungal community did have a highly specific relationship, this would support niche partitioning as a mechanism operating in this system, however, as mentioned above, it is likely that this may not be the strongest mechanism shaping the fungal community.

Correlations among plant and fungal diversity were found when the vegetation characteristics were considered at finer functional scales (i.e. grouping similar members of the plant community consistently according to their classification at a species level vs species within a community being grouped into broader functional types based on common ecological traits – i.e. tree, shrub or ground cover). This was despite vegetation being an insignificant descriptor of fungal community patterns when the variation of plant communities was considered in terms of general community

traits (i.e. the presence of ground cover, shrubs or trees). Therefore the relationship between the diversity of plant and fungal communities may also be only obvious at certain functional scales. The interdependency of above-ground and below-ground communities has been noted to be influenced by scale, with plants playing a central role in these interactions. The interactions between plants and the below-ground community may shift at a different rate, dependant on the diversity of the below-ground community, compared to the rate of the changing above-ground interactions with other plants as plant diversity changes (De Deyn and Van der Putten 2005). Therefore the linkages between the plant and fungal communities may only be apparent at some functional scales, as was observed in this case, and their strength may depend on the relative diversity of the above-ground and below-ground components.

Chapter 4 Spatial dynamics of soil fungal communities across an Australian alpine/subalpine altitudinal gradient

4.1. Introduction

Spatial patterns in fungal community structure are known to exist at a range of spatial scales, however the factors which are influential in driving these patterns are not well understood and their roles are not necessarily consistent across various geographic scales (Ettema and Wardle 2002; Green *et al.* 2004). Spatial separation distance, a proxy variable for factors such as dispersal limitation, together with environmental variables, such as plant community composition, edaphic variables and climatic variation, have a joint role in shaping fungal community dynamics, however the relative roles of such variables at different geographic scales is not well understood (Ramette and Tiedje 2007; Peay *et al.* 2010a).

In Chapter 2, it was demonstrated that fungal communities show spatially explicit patterns in community structure at large geographic scales (100 km – 500 km extent). These patterns were attributed to spatial variables as well as edaphic characteristics that were also spatially structured, however a large proportion of variation present within the dataset remained unexplained by the measured variables. While this is consistent with other results (e.g. Dumbrell *et al.* 2010), and indicates the stochastic nature of these systems, other unmeasured environmental variables may also be influential in

shaping these communities, in particular climatic variables acting over large geographic extents.

The influence of climatic variables on the diversity of species across latitudinal gradients is often similar to their effect on altitudinal gradients of diversity (Stevens 1992; Lomolino 2001). Therefore, the study of patterns of diversity over altitudinal gradients can be used to inform what role such environmental factors have on larger scale latitudinal patterns, while minimising the influence of spatial variation that exists across larger geographic distances. A unique opportunity to study the mechanisms that shape and maintain the diversity of fungal communities across a range of ecosystem types exists in high-elevation environments, which are characterised by sharp environmental gradients and high turnover of plant species composition over relatively short distances (Zinger *et al.* 2011).

Fungal community diversity has been studied across altitudinal gradients in a number of alpine regions across the globe (Kernaghan and Harper 2001; Zinger *et al.* 2011; Bahram *et al.* 2012; Timling *et al.* 2012; Davey *et al.* 2013; Yao *et al.* 2013). In general, fungal diversity appears to decrease with increasing altitude, but little consensus exists over which factors (temperature, soil pH, host plant identity, etc.) are most influential in shaping the community structure across various study regions. Given this lack of consensus, it is difficult to make generalisations about the influence of climatic factors on fungal community assembly. The Australian alpine region has received little attention in this respect, despite the fact that the region is highly endangered and holds both national and international significance (Office of Environment and Heritage 2014). Only 250 km^2 of the Australian mainland is above the natural treeline, equating to 0.001% of the total landmass, with Kosciuszko National Park being the largest continuous region within the Australian Alps (Costin *et*

al. 2000). Knowledge of fungal diversity in Kosciuszko National Park is currently limited to that collected using sporocarp samples and non-molecular identifications (Johnston and Ryan 2000; Trappe and Claridge 2006), and so the application of next generation sequencing technologies, as described in this chapter, have the potential to reveal a much greater diversity in the region than previously acknowledged (Buée *et al.* 2009).

The work described in this chapter is also the first to understand the spatial dynamics of soil fungal communities in the Australian alpine region, considering the effects of spatial distance, climatic variation, edaphic characteristics and plant community structure, at a range of scales (10 cm – 1200 m). The sampling design chosen enabled community patterns to be examined across a range of scales within each site as well as the changes among sites, with a uniform number of point pairs representing each distance class (Lister *et al.* 2000). Fungal community dynamics along the altitudinal gradient within Kosciuszko National Park, were therefore investigated, with two primary aims; *(i)* to investigate how fungal community structure differs with altitude at various spatial scales, above, at and below the biological treeline; and *(ii)* to determine the influence of variation in spatial and environmental (climatic, edaphic characteristics, and vegetation characteristics) factors in shaping the observed fungal community patterns.

4.2. Methods

4.2.1. Study design and sampling locations

Soil samples were collected from three distinct locations within Kosciuszko National Park, located in the Snowy Mountains region of the Great Dividing Range, in south-east Australia. Kosciuszko National Park is the largest national park in the Australian Alps and is both nationally significant and internationally recognised as a UNESCO Biosphere Reserve (Office of Environment and Heritage 2014). The region plays host to three highly endemic floristic zones that are strongly correlated with altitudinal/climatic gradients – montane, subalpine and alpine vegetation. Field sampling for the research presented in this chapter focused on the transition between alpine and subalpine communities across the biological treeline. The treeline in the Australian Alps occurs at an average elevation of 1830 m, above which the mean mid-summer temperature does not exceed 10°C and the physiological limits of tree growth is reached (Costin *et al.* 2000).

Sampling locations were accessed along the Dead Horse Gap trail within Kosciuszko National Park, which starts approximately 5 km south-west of Thredbo, NSW, Australia. The Dead Horse Gap trail climbs north-east over the Rams Head Ranges towards Mt Kosciuszko, covering an elevation range of 1560 – 2000 m. A change in elevation of approximately 75 m separated each site. Site 1 (referred to herein as Site 1-AB) was located above the treeline (S 36°30.044' E 148°16.470'; alt. 1966 m), Site 2 (Site 2-TL) was located on the treeline (S 36°30.319' E 148°16.389'; alt. 1890 m), and Site 3-BL (Site 3-BL) was located below the treeline (S 36°30.596' E 148°16.112'; alt. 1814 m) (Figure 4.1).

Plant communities varied among the three sites in keeping with their altitudinal position. Alpine and subalpine vegetation of the area has previously been described by Costin *et al.* (2000) and Keith (2004); the following observations were in keeping with their records. Tall alpine herbfield graduating to alpine heath was characteristic of Site 1-AB, dominated by a *Clemsia-Poa* alliance graduating to an *Oxylobium-Podocarpus* alliance (Figure 4.2a). Site 2-TL was distinguished by small numbers of *Eucalyptus niphophila* and an understorey of alpine heath dominated by *Epacris glacialis*, graduating to a raised bog with an *Epacris-Sphagnum* alliance (Figure 4.2b). Site 3-BL was subalpine woodland constituting of *Eucalyptus niphophila*, with a *Poa* dominated understorey (Figure 4.2c) (Costin *et al.* 2000; Keith 2004).

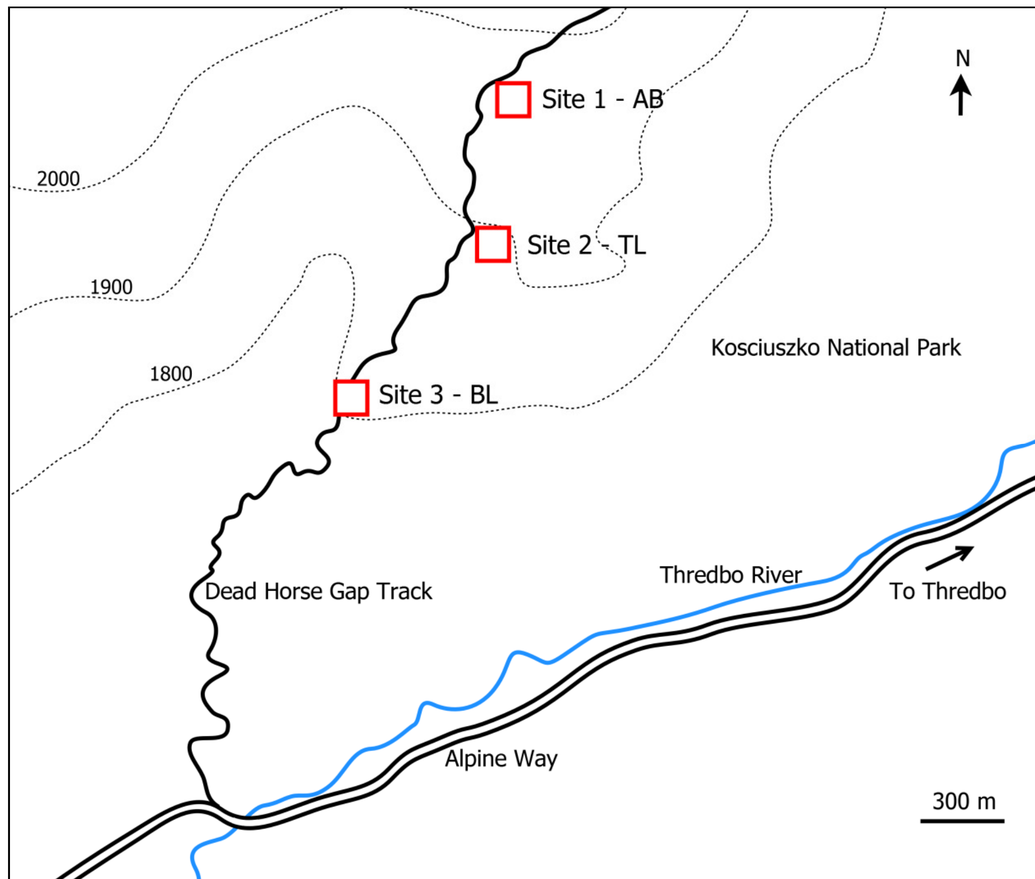
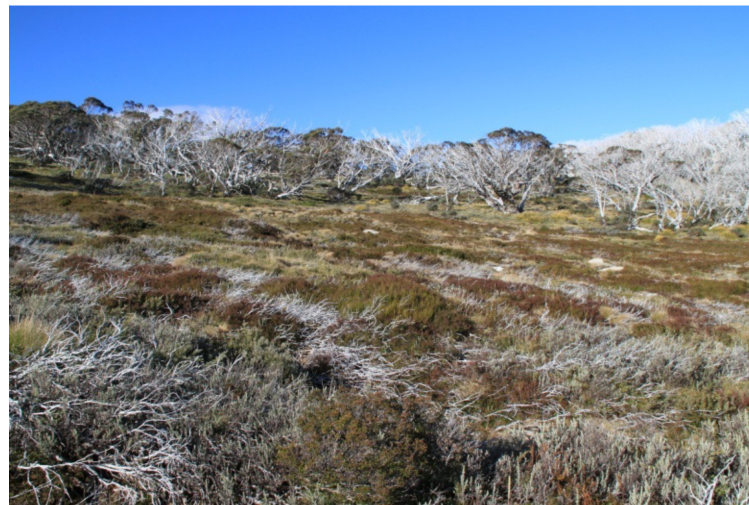


Figure 4.1: Location of sampling sites and their relative altitude along the Dead Horse Gap Trail, Kosciuszko National Park, Australia. Site 1-AB was located above the treeline (alt. 1966 m), Site 2-TL at the treeline (alt. 1890 m), and Site 3-BL below the treeline (alt. 1814 m).

(a)

(b)



(c)

Figure 4.2: Vegetation and landscape characteristic of *(a)* Site 1-AB, above the treeline, *(b)* Site 2-TL, at the treeline, and *(c)* Site 3-BL, below the treeline, in Kosciuszko National Park, Australia.

Over a period of two consecutive days in May, 2012, 41 soil cores were collected, at each of the three sites, within a 100 m quadrat, according to a spatially explicit sampling design (Figure 4.3). The sampling design was based on that of Lister *et al.* (2000), chosen to give a uniform number of point pairs for any given distance class so as to maximise the number of pairwise distances for spatial statistical analysis. This sampling design also best facilitated the analysis of changes in community patterns among the three sites compared to among individual sampling locations within each site, rather than gradual trends over the study extent for which a linear sampling design is better suited. A licence agreement with the NSW National Parks and Wildlife Service permitted the collection of plant and soil material from the selected locations (licence number SL100673).

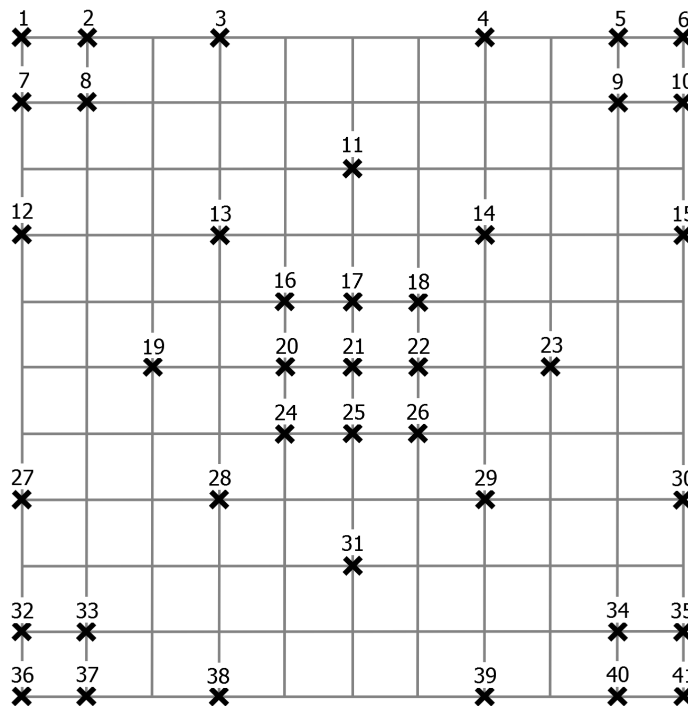


Figure 4.3: Sampling design at each of the three sites along an altitudinal gradient, within Kosciuszko National Park. Samples were taken within a 100 m quadrat. Sampling locations (41 in total) are marked with an '*x*'. Numbers correspond to the identity of each sample used to distinguish samples within sites. Grid size = 10 m.

4.2.2. Sampling

At each sampling location a soil core (10 cm depth; 4 cm diameter) was taken and stored at 4°C. Each core was homogenised, sieved (< 2 mm) and sub sampled so that a portion of each sample was frozen at -80°C prior to molecular analysis, and the remainder was dried at room temperature for environmental analysis. Root material was separated from the soil cores whilst sieving, and was washed, air-dried and weighed to determine total root biomass for each sample.

4.2.3. Environmental analyses and vegetation survey

Soil moisture was measured and recorded (as percentage volumetric water content), before each sample core was taken, using a HydroSense Soil Water Measurement System (12cm probe) (Campbell Scientific Australia Pty. Ltd., Thuringowa Central, Australia). Soil temperature, air temperature and air humidity was also recorded immediately prior to sampling using a LI-COR LI-1400 data logger (5 cm probe) (John Morris Scientific, Chatswood, Australia). Elevation reading at was also recorded at each sampling point, using a Garmin GPSMAP 62S (Garmin, Seven Hills, Australia). While there are limitations of not including measurements of climatic variables over a longer time period, it was deemed appropriate to take readings at each sampling point in order to relate these variables at the spatial and temporal scales at which the soil community was sampled.

Air dried soil was ground using a Mixer Mill MM400 (Retch – MEP Instruments Pty. Ltd., Gladesville, Australia) for determination of total C and N content, and calculation of C:N ratio, using a TruSpec Micro CHN analyser (Leco Corporation, St. Joseph, Michigan, USA) with oatmeal (%N 2.70+/- 0.04, %C 45.85+/-0.40) and synthetic carbon (%C 4.9-5.1) as standards. Soil pH was also measured using a

standard protocol (Thomas 1996) in which distilled water was added to 5 g of air dried soil in a 1:1 w/v ratio, vortexed for 30 sec and allowed to settle for 10 min before taking readings from the supernatant using a CyberScan pH510 digital pH meter (Eutech Instruments Pty. Ltd., Singapore).

Vegetation present within a 0.25 x 0.25 m quadrat placed over where each core was taken was also recorded by photography in the field at each sampling location. Species identifications were later made from the images, using a field guide of Kosciuszko alpine flora by Costin *et al.* (2000), and an estimate of the percentage cover for each species within the quadrat was recorded. For the few species that were unable to be identified, a comprehensive description of the plant was made to distinguish it from other species. Plant species were also grouped into plant functional types (PFTs) based on their morphological characteristics. Plants were classified as tree, shrub, forb, grass, rush, sedge, bryophyte or pteridophyte based on the definitions used by the Circumpolar Arctic Vegetation Mapping Team (Alaska Geobotany Center 2013).

4.2.4. DNA extraction and 454 sequencing

Total DNA was extracted from 0.5 g of soil from each sample using the PowerSoil®-htp 96 Well Soil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, USA), according to the manufacturer's instructions. Briefly, 60 µl of Solution C1 (cell lysis buffer) was added to each sample in the PowerBead tubes, and the samples were homogenised using the TissueLyser (QIAGEN Pty. Ltd., Doncaster, Australia) at 30 Hz for 10 min. The supernatant was separated by centrifugation at 10 000 x *g* for 1 min, then added to a series of inhibitor removal (Solutions C2 and C3) and salt (Solution C4) solutions. The samples were incubated at 4°C for 5 min and centrifuged at 10 000 x *g* for 1 min after each addition. Supernatant was loaded into a spin filter in

three aliquots and centrifuged at 10 000 x *g* for 1 min after each addition. The spin filter membrane was then washed with an ethanol solution (Solution C5) and the DNA was eluted into 50 µl of sterile MilliQ water and stored at -20°C.

A 1:5 dilution was performed on all samples to improve the purity of the DNA samples and the samples were then concentrated to approximately 10 ng/ul using a vacuum centrifuge to optimise DNA concentration for sequencing. DNA was quantified using a QuBit 2.0 Fluorometer (Life Technologies Corporation, Mulgrave, Australia) prior to sequencing. 454 pyrosequencing was performed by the Hawkesbury Institute for the Environment Next Generation Sequencing Facility (Western Sydney University, Australia) using the Roche GS Junior platform (Roche Diagnostics Corporation, Basel, Switzerland).

The amplicon library of the fungal LSU region was prepared using a 50 µl PCR containing: 1µl of DNA; 20 pmol of each primer LR0R and LR3 (Vilgalys and Hester 1990); 200 µM of each of dATP, dCTP, dGTP and dTTP; 10 µl of 5x reaction buffer (Promega Corporation, Sydney, Australia); 2.5 mM $MgCl_2$; 10 µg of bovine serum albumin (BSA) and 2.5 U GoTaq® Flexi DNA polymerase (Promega Corporation, Sydney, Australia). Reactions were performed using a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad Laboratories, Inc., California, USA) with cycling conditions of 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, and a final extension of 72°C for 5 min. A total of 4 sequencing runs were performed with up to 2187 read coverage per sample, using 32 unique multiplex identifiers (MIDs) for each run.

4.2.5. Bioinformatic processing

Bioinformatic processing of the sequence data was conducted using mothur v 1.28.0 (Schloss *et al.* 2009), unless otherwise stated.

Poor quality sequences (quality score ≤20, homopolymer number >10) were trimmed and putative chimeric sequences were identified and removed from the dataset. Chimeric sequences were identified using UCHIME, in which more abundant sequences in the dataset were used as reference sequences (Edgar *et al.* 2011). Homopolymer error correction was performed on the remaining sequences using Acacia (Bragg *et al.* 2012). Post quality check sequences were aligned to a reference alignment of eukaryotic ribosomal LSU DNA sequences (downloaded from SILVA on 25th January 2013). Reads that aligned most closely to non-fungal DNA sequences in the SILVA reference alignment were removed from the dataset and the remaining fungal sequences were then screened to remove those that aligned to regions outside the predicted region (starting before position 69196 or ending after position 77000).

The sequencing depth across all runs varied (367 – 3700 sequences) per sample and so samples were rarefied, using the following resampling procedure. Four sequencing depths of 367, 800, 1000, and 1500 sequences per sample were trialed, in which 123, 114, 99 and 47 of the 123 samples in total were kept, respectively. For each of the four datasets, pairwise distances were calculated among sequences, in which strings of gaps were treated as a single gap and terminal gaps were not penalised. The distances were used to cluster sequences into operational taxonomic units (OTUs) based on a 97% similarity cutoff to their furthest neighbour. Singleton and doubleton OTUs were conserved in the dataset. A unique representative sequence was allocated to each OTU, and a relative abundance matrix for each dataset was exported.

4.2.6. Statistical analysis

All statistical analyses were performed using R v2.14.2 (R Development Core Team 2012), using the 'vegan' package (Oksanen *et al.* 2012), unless otherwise stated.

The relative abundance OTU matrix for each sampling depth was standardised using Hellinger distances, enabling the species data to be used in Euclidean-based ordination methods, while not strongly weighting rare species (Legendre and Gallagher 2001). Preliminary analyses (rarefaction curves and principal components analysis) were performed on each of these four datasets to determine the optimum sequencing depth for subsequent analyses; the depth at which the maximum number of samples that could be retained with a sufficient number of sequences to give an informative representation of the diversity. The dataset standardised to 367 sequences per sample was retained for subsequent analysis (see results). Representative sequences for each OTU in this dataset were compared to the RDPII phylogenetic database (Wang *et al.* 2007) to gain taxonomic affinities, and the total number of sequences and number of unique OTUs corresponding to each taxon were recorded.

The role of spatial separation distance on fungal community structure was first analysed by constructing a Mantel correlogram, in which the spatial variability in the dataset is decomposed into specified distance classes within the extent of the sampling design (Legendre and Legendre 2012). Correction for multiple testing was achieved by Holm's procedure (1979). The Mantel correlogram was complemented by the construction of a series of spatial variables that could be used alongside other explanatory variables in subsequent analyses, by distance-based Moran's eigenvector mapping (dbMEM) analysis (Legendre and Legendre 2012). This method performs a spectral decomposition of the spatial relationships among the sampling sites in a study,

creating a series of variables that correspond to all spatial scales contained within a given sampling design (Borcard and Legendre 2002). The resulting dbMEM eigenvectors show a sine-based pattern relative to the approximate scale to which the spatial variable relates within the extent of the sampling design, and are indexed appropriately from the largest to smallest scales included (Borcard and Legendre 2002; Borcard *et al.* 2004). The constructed dbMEM eigenvectors, together with the edaphic and climatic variables measured at each sampling point, were subjected to forward selection using 999 permutations using the 'packfor' package (Dray 2011). Only significant ($P<0.05$) explanatory variables were included in subsequent analyses.

Predictive co-correspondence analysis (COCA) (Ter Braak and Schaffers 2004) was performed, using the 'cocorresp' package (Simpson 2009), on both the vegetation species matrix and the matrix of plant functional types with the purpose of creating a manageable number of explanatory variables that meaningfully represented the relationship between the plant and the fungal communities. In this analysis, a predictor matrix is related to the response matrix, and a number of ordination axes are produced. Cross validation of each axis tests the goodness of fit of the relationship between the two datasets and the statistical significance of each axis is also tested by permutation. Only axes that are significant and/or show a positive cross validation result are considered to be significant in indicating a relationship between the predictor and the response matrices (in this case the plant and the fungal datasets respectively) (Ter Braak and Schaffers 2004). Significant COCA axes created from the plant functional type dataset were retained for subsequent analysis over those created from the vegetation species matrix, because the COCA produced a smaller number of explanatory variables and similar results were produced using either data type in subsequent analyses.

Variation partitioning was used to disentangle the role of significant environmental (climatic and edaphic characteristics), plant community (plant functional type COCA axes) and spatial (dbMEM eigenvectors) variables in shaping the fungal communities across the entire extent of the study. To complement this, redundancy analysis (RDA) was performed to interpret the importance of each selected variable in shaping the observed structure in the fungal community, across the three sites along the altitudinal gradient, as well as at the various scales included in the sampling design within each site.

As high stochasticity in the community assembly was evident in the results of the current chapter, as was also the case in Chapter 2, neutral models were formulated to estimate the role of deterministic and stochastic processes in shaping the communities along the sampled altitudinal gradient. Neutral diversity (Θ) and immigration (I) parameters were estimated using a recent development of the neutral sampling formula for multiple samples by Etienne (2009). Using the PARI/GP codes given in Etienne (2007), an artificial set of local communities (total of 1000 simulations) of the same size as the observed communities were created, enabling these communities simulated under neutral dynamics to be directly compared to the corresponding observed community. Bray-Curtis dissimilarities were calculated among each pair of observed communities, as well as among the communities in each simulation expected under purely neutral dynamics. The distributions generated by these distances were summarised by the following test statistics: the median observed dissimilarity relative to the average of the median dissimilarity across all of the simulations, and the inter-quartile range (IQR) and inter-decile range (IDR) of the distribution of observed dissimilarities relative to the average of the IQR and IDR across the distributions representing the simulated communities. Bootstrapped 95% confidence intervals were

also calculated for each statistic. As this dataset did not have an even distribution, median, IQR and IDR were calculated rather than mean and standard deviation values, as was done in Chapter 2. These statistics were compared to that calculated under neutrality in order to indicate whether the community assembly is consistent with the prediction under the neutral hypothesis or differ significantly from this prediction, indicating a lack of support for neutrality, and thus suggesting the role of the niche in community assembly (see Chapter 2 for more detail).

4.3. Results

4.3.1. Sequence analysis

A total of 247 739 reads passed quality controls across the entire dataset, ranging from 367 to 3700 sequences per sample. Preliminary analyses indicated little difference in the community patterns observed with increasing sequencing depth (

Figure 4.4), despite rarefaction curves showing that more OTUs were present in samples from which a higher number of sequences were recovered (Figure 4.5). Samples were therefore rarefied to an equal sampling depth of 367 sequences, in order include all samples in subsequent analyses. A total of 45 141 fungal sequences were therefore clustered into 4399 unique OTUs based on 97% similarity.

There was no significant difference in alpha diversity among the three sites along the sampled altitudinal gradient (One-way ANOVA: $F = 0.294$, $P = 0.746$). A mean of 138.76 (± 3.19) OTUs was recorded at Site 1-AB, 135.41 (± 3.61) at Site 2-TL, and 139.32 (±4.71) at Site 3-BL.

The dominant phyla across all samples were Ascomycota (62.5% of OTUs, 63.7% of sequences), followed by Basidiomycota (21.3% of OTUs, 19.3% of sequences); 9.0% of OTUs (6.7% of sequences) could not be be further classified (Table 4.1). Helotiales (17.8% of OTUs, 18.6% of sequences) followed by Agaricales (10.1% of OTUs, 10.9% of sequences) were the most dominant orders recorded across all samples (Table 4.1). The distribution of taxonomic groups was similar across the sampling sites, as no significant differences in the proportion of OTUs for each phylum was observed among the three sites (chi-squared test; $P = 0.858$) (Figure 4.6). However the percentage of sequences in the standardised dataset belonging to the dominant taxonomic groups did differ among sites (chi-squared test; $P < 0.001$). Ascomycotan fungi represented a relatively smaller proportion of the sequences recovered above the treeline, compared to further down the altitudinal gradient. Basidiomycotan and chytrid fungi, on the other hand, represented a greater proportion of the sequences recovered from Site 1-AB, relative to Site 2-TL and Site 3-BL (Table 4.1).

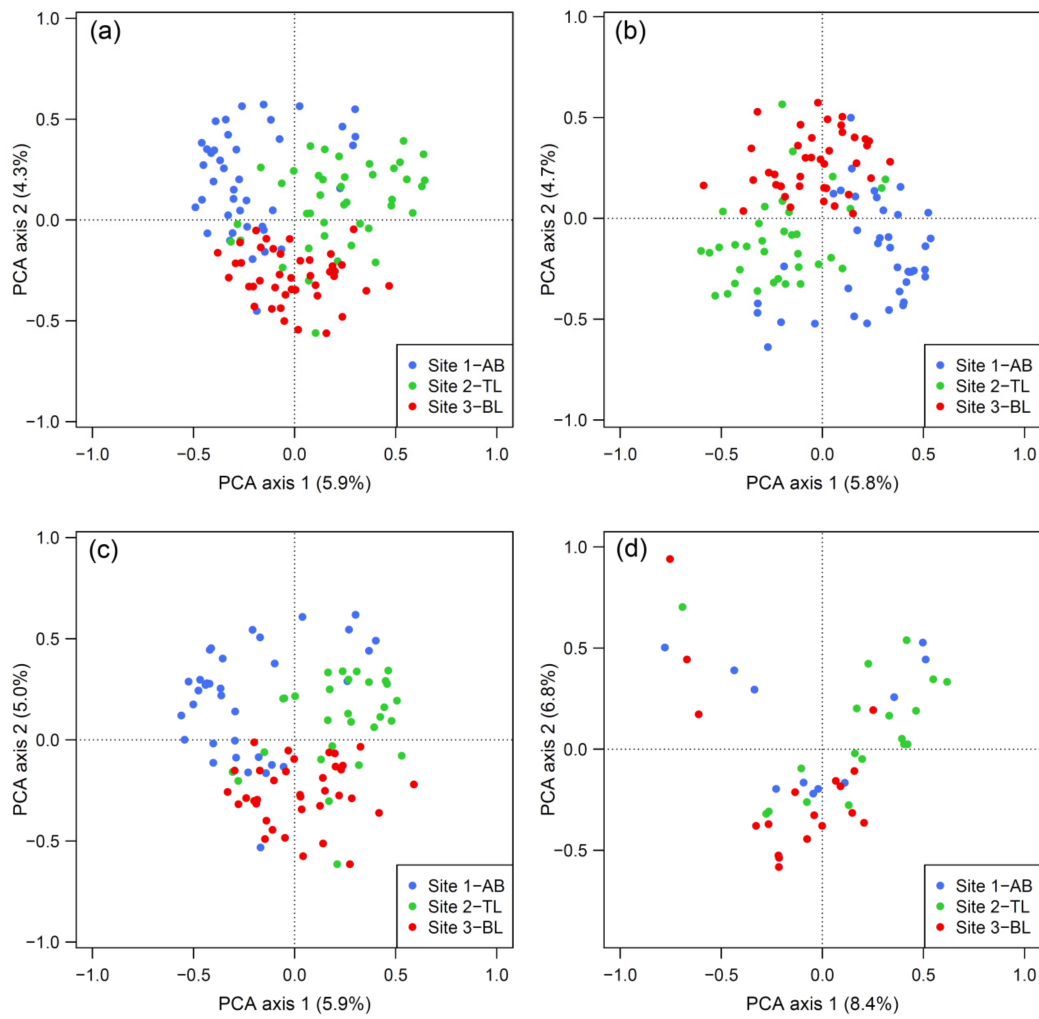


Figure 4.4: Principal components analysis ordination of the fungal community composition standardised at sequencing depths of: (a) 367, (b) 800, (c) 1000 and (d) 1500 sequences, across three sites along the altidunal gradient in Kosciuszko National Park, Australia.

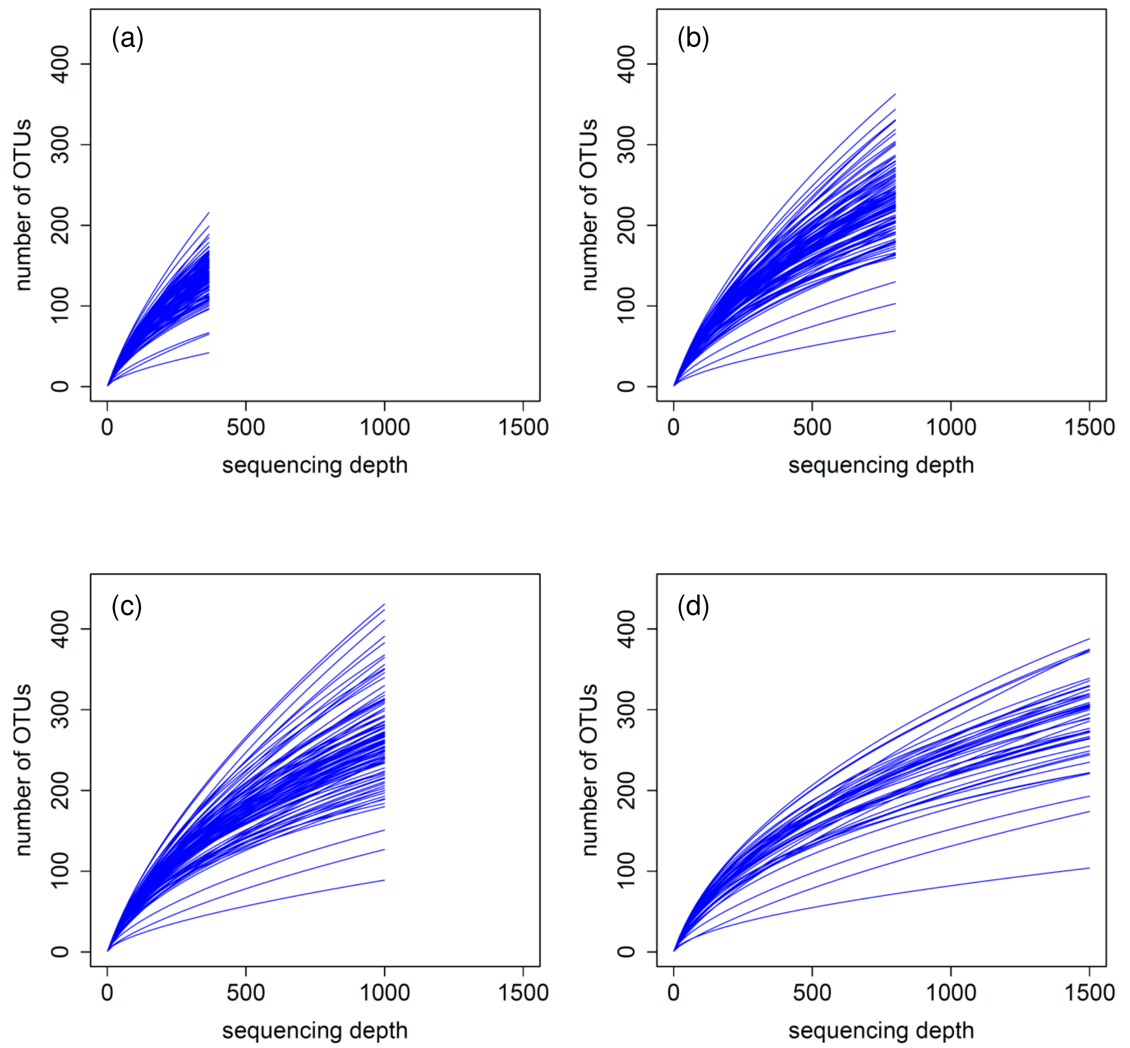


Figure 4.5: Rarefaction curves of four standardised sequencing depths – (a) 367,(b) 800, (c) 1000 and (d) 1500 sequences per sample – of the fungal communities across the altidunal gradient in Kosciuszko National Park, Australia.

Table 4.1: Relative distribution of the component fungal taxa across the altitudinal gradient within Kosciuszko National Park, Australia.

Taxonomic Group	% OTUs Site 1-AB	% sequences Site 1-AB	% OTUs Site 2-TL	% sequences Site 2-TL	% OTUs Site 3-BL	% sequences Site 3-BL	% OTUs total	% sequences total
Ascomycota	62.97	61.57	63.01	64.54	61.96	65.02	62.5	63.71
Helotiales	17.99	16.64	18.13	18.68	17.53	20.54	17.75	18.62
Chaetothyriales	7.11	5.78	7.11	5.54	7.33	5.20	6.82	5.51
Hypocreales	3.38	3.46	3.47	3.10	3.50	4.48	3.36	3.68
Coniochaetales	0.84	1.65	1.08	1.89	1.04	1.75	1.05	1.77
Sordariales	1.64	1.81	1.48	1.80	0.99	1.26	1.41	1.64
Capnodiales	0.84	1.24	0.95	2.76	0.71	0.82	0.82	1.61
Eurotiales	1.84	1.75	1.65	1.86	1.98	0.87	1.91	1.50
Pleosporales	1.79	1.05	1.65	1.79	1.75	1.48	1.64	1.44
Basidiomycota	21.32	20.62	21.17	17.74	21.46	19.66	21.3	19.34
Agaricales	9.99	11.82	9.80	10.12	10.16	10.67	10.07	10.87
Cantharellales	1.44	1.06	1.43	1.23	1.61	0.67	1.48	1.0
Chytridiomycota	5.72	10.33	6.33	9.16	6.05	8.30	5.96	9.26
Spizellomycetales	1.09	5.43	1.21	2.08	0.85	2.59	1.00	3.36
Chytridiales	1.49	1.81	2.17	2.90	2.27	2.75	2.02	2.49
Rhizophydiales	1.34	0.96	1.30	1.86	1.28	1.20	1.23	1.34
Blastocladiomycota	0.55	0.29	0.61	0.41	0.57	0.53	0.55	0.41
Glomeromycota	0.35	0.17	0.09	0.09	0.19	0.07	0.23	0.11
Neocallimastigomycota	0.05	0.007	0	0	0.05	0.007	0.023	0.004
Unclassified Fungi	9.05	7.01	8.76	8.05	9.74	6.41	10.6	7.16

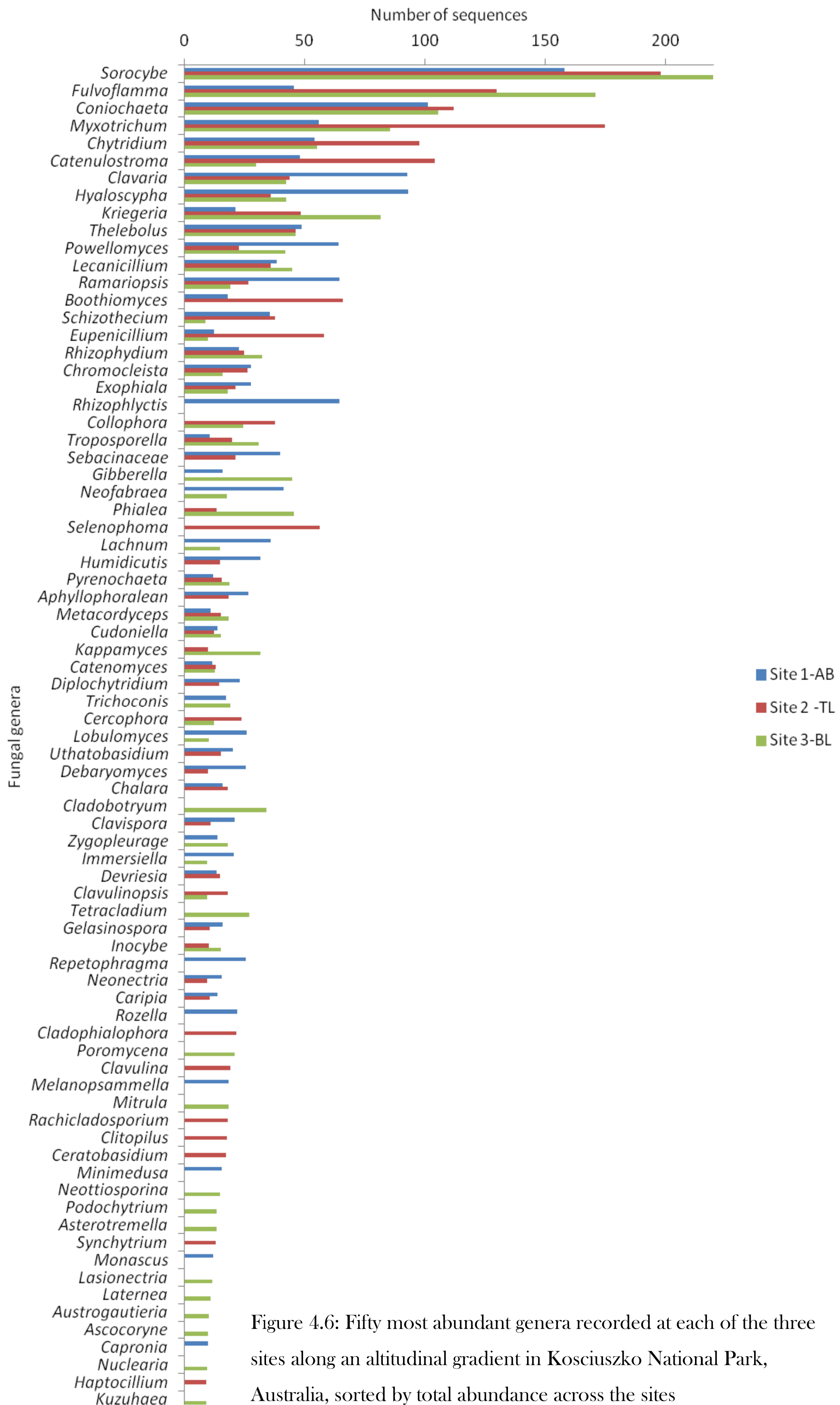


Figure 4.6: Fifty most abundant genera recorded at each of the three sites along an altitudinal gradient in Kosciuszko National Park, Australia, sorted by total abundance across the sites

The fifty most abundant genera recorded at each of the three sites, along the altitudinal gradient are shown in Figure 4.6. *Sorocybe* was most abundant genus at all sites, and was more abundant at, and below the treeline, rather than above the treeline. This distribution trend was also noted for the next four most abundant genera; *Fulvoflamma*, *Coniochaeta*, *Myyxotrichum* and *Chytridium*. Seven, eight and 14 of the genera, above, at and below the treeline, respectively, were unique to their respective sites.

4.3.2. Fungal community patterns across the altitudinal gradient and factors influencing these patterns across sites

Significant positive spatial correlation was evident within distance classes up to approximately 100 m (Figure 4.7). The similarity of samples also decreased with increasing separation distance up to this point. This distance class corresponds to the distances within an individual sampling site, and thus indicates that each sampling site was spatially independent from one another. Forward selection revealed that, of the environmental variables measured across the three sites along the altitudinal gradient, soil moisture, soil C, soil N, C:N ratio, air humidity and temperature, and elevation were significantly related to fungal community structure across sites (Table 4.2; see also Appendix G). Of the 29 dbMEM eigenvectors constructed for the distances incorporated within the entire sampling design, vectors 1, 2, 19, 23-25 were significant, which correspond to spatial structure at the largest scales and a series of vectors corresponding to the finer scales measured within the sampling design (Table 4.2). Predictive co-correspondence analysis (COCA) of plant community data (a summary table of the plant community data is presented in Appendix H) to the fungal community data indicated that the distribution of plant functional types predicted very little of the structure present in the fungal community (6.05% of variance cumulatively

across all axes generated by the response matrix was explained by the predictor matrix). Cross validatory fit testing of the axes produced by the COCA gave negative values (Figure 4.8), indicating that the chosen model matrix (plant functional community) had poor predictive fit over the response matrix (fungal community). However the first of the six axes generated by the analysis was significant ($P = 0.01$) in permutation tests. This axis was conservatively retained in subsequent analysis as the variable describing plant community structure, so as to not prematurely remove any potential sources of variation in the fungal dataset. Interestingly, when COCA was performed using the fungal community as the model matrix and the plant community as the response matrix, cross validatory fit tests were positive (Figure 4.8), indicating that the fungal communities have a more influential role in predicting the plant communities than vice versa.

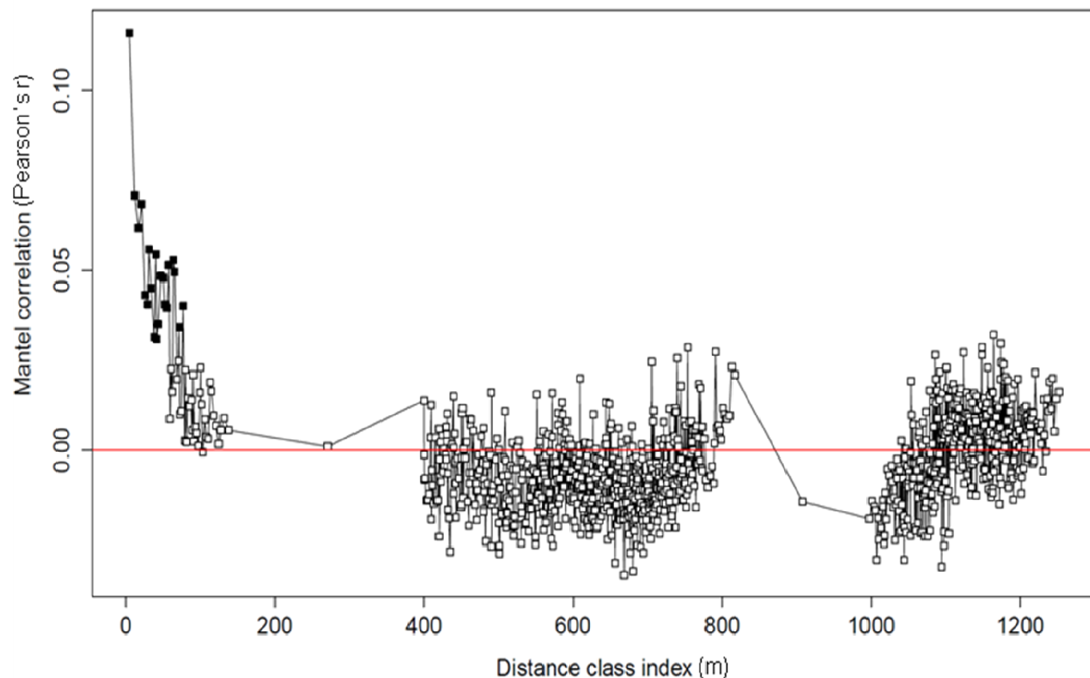


Figure 4.7: Mantel correlogram of the similarity in community composition among samples of increasing separation distances across the sampling gradient. Positive Pearson r values indicate positive spatial autocorrelation at the specified distance class. Significant values are indicated as filled symbols.

Table 4.2: Significance values for the tested explanatory variables, based on their role in shaping fungal community structure across all sampling sites in Kosciuszko National Park, Australia. Variables were forward selected based on 999 permutations, with the exception of the plant community variables derived from the co-correspondence analysis (COCA axes) which were tested using 99 permutations.

Variable	**F statistic**	**P value**
C:N ratio	3.939	0.001
elevation	3.966	0.001
soil moisture	2.530	0.001
C	1.566	0.001
N	1.311	0.005
air temperature	1.281	0.008
air humidity	1.268	0.012
soil temperature	-	ns
pH	-	ns
root biomass	-	ns
dbMEM1	3.589	0.001
dbMEM2	3.589	0.001
dbMEM23	1.971	0.001
dbMEM25	1.340	0.006
dbMEM19	1.228	0.032
dbMEM24	1.197	0.043
dbMEM3 - 18	-	ns
dbMEM20 - 22	-	ns
dbMEM26-29	-	ns
COCA 1	0.012 (F ratio)	0.01
COCA 2-6	-	ns

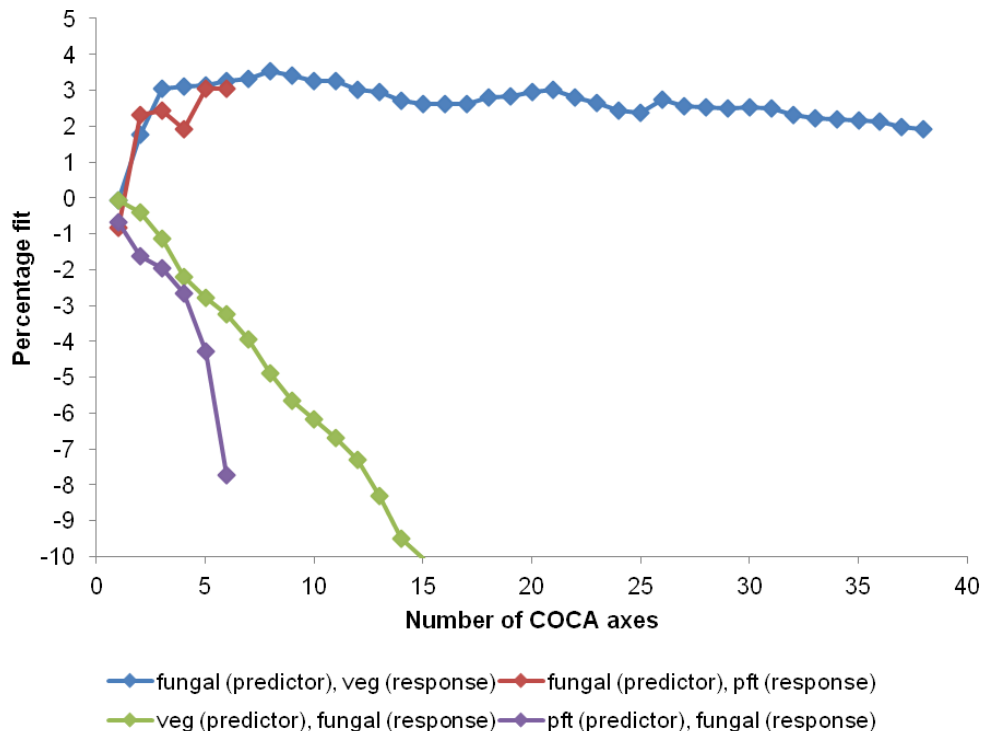


Figure 4.8: Cross validatory testing of the axes produced from the co-correspondence analysis. Tests were performed on the fungal community dataset as the response matrix and the corresponding vegetation species dataset and plant functional type datasets as predictor matrices, as well as on the vegetation species dataset and the plant functional type dataset as response matrices against the fungal community dataset as the predictor matrix. A positive cross validation result (as measured by percentage goodness of fit) indicates the number of COCA axes with a significant role in predicting the relationship between the predictor and the response matrices (Ter Braak and Schaffers 2004).

A total of 9% of the overall variation present in the community dataset across the three sites was explained by the included explanatory variables. The proportions explained by both purely environmental and spatial factors were significant in the overall variation present in the dataset (2%, $P = 0.005$; 1%, $P = 0.005$, respectively). The plant community on its own did not explain a significant amount of the fungal community variation (Figure 4.9). Redundancy analysis of the fungal community gave similar results, with a small proportion of the explained variance represented by the first two axes (3.7% and 2.8% respectively) (Figure 4.10). Some separation in the community composition was however evident among sampling sites, mainly along axis 2. Climatic factors (air temperature, humidity, elevation) were more influential in the separation of Sites 1-AB and 3-BL, along with larger scale spatial variables (dbMEM1), while edaphic characteristics (soil moisture, total N, total C, C:N ratio) and large scale spatial variables (dbMEM2) were influential in the separation of Site 2-TL from 1-AB or 3-BL. Variation among samples within individual sampling sites was also evident, and small scale spatial variables (dbMEM9, 19, 23, 24, 25) were involved in determining this pattern (Figure 4.10).

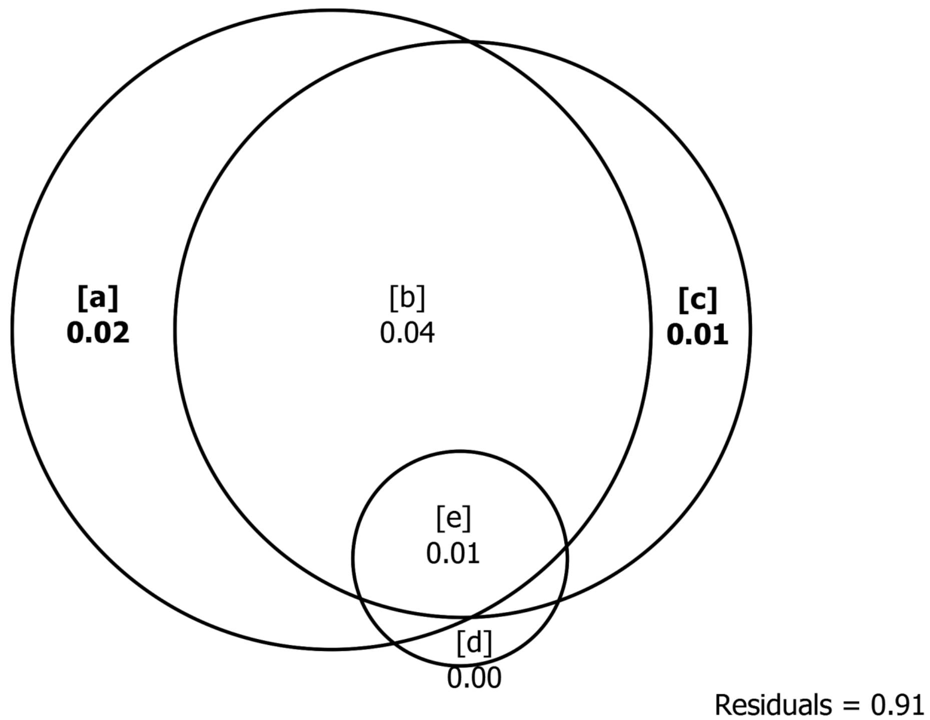


Figure 4.9: Variation partitioning of the proportion of variation in fungal community structure across the sampled altitudinal gradient in Kosciuszko National Park, Australia. The variation is partitioned into that which can be explained by [a] purely abiotic variables, [b] the combination of abiotic and spatial variables that cannot be disentangled from one another, [c] purely spatial variables, [d] purely vegetation characteristics, [e] the combinations of all factors than cannot be disentangled from one another, and the residual unexplained variation. Significant values are indicated by bold type ($P < 0.05$). Note values do not sum to 1 due to rounding.

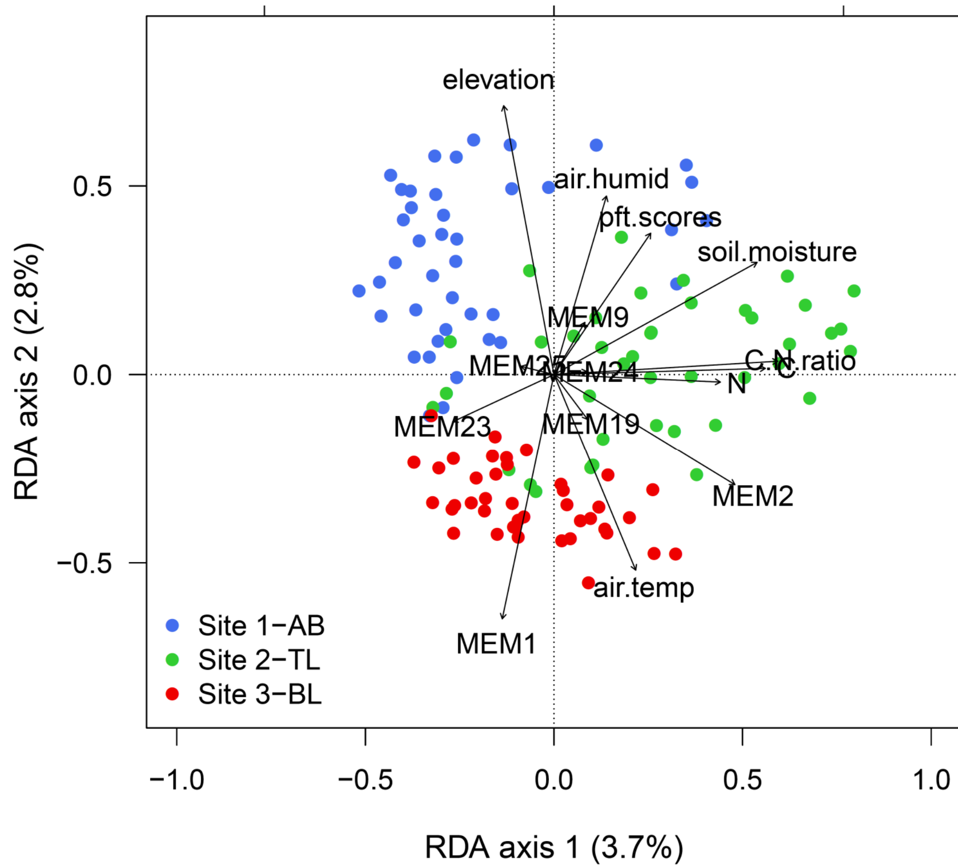


Figure 4.10: Redundancy analysis (RDA) ordination of the fungal communities sampled along an altitudinal gradient in Kosciuszko National Park, Australia, plotted with the significant ($P < 0.05$) abiotic, vegetation (PFT-COCA scores) and spatial (dbMEM eigenvectors) variables responsible for shaping community structure at various scales.

4.3.3. Factors influencing fungal community patterns within individual sites

Redundancy analysis of the fungal communities at each sampling site showed some separation in community composition within Site 1-AB, with little to no obvious separation observed at Site 2-TL and at Site 3-BL (Figure 4.11). A relatively small proportion of variation in the community composition at each site was accounted for in these analyses (Figure 4.11), similarly to that conducted across all three sites (Figure 4.10). Spatial explanatory variables were noted as influential in shaping the community patterns at Sites 1-AB and 2-TL, while vegetation and soil C and N had significant roles across all three sites (Figure 4.11).

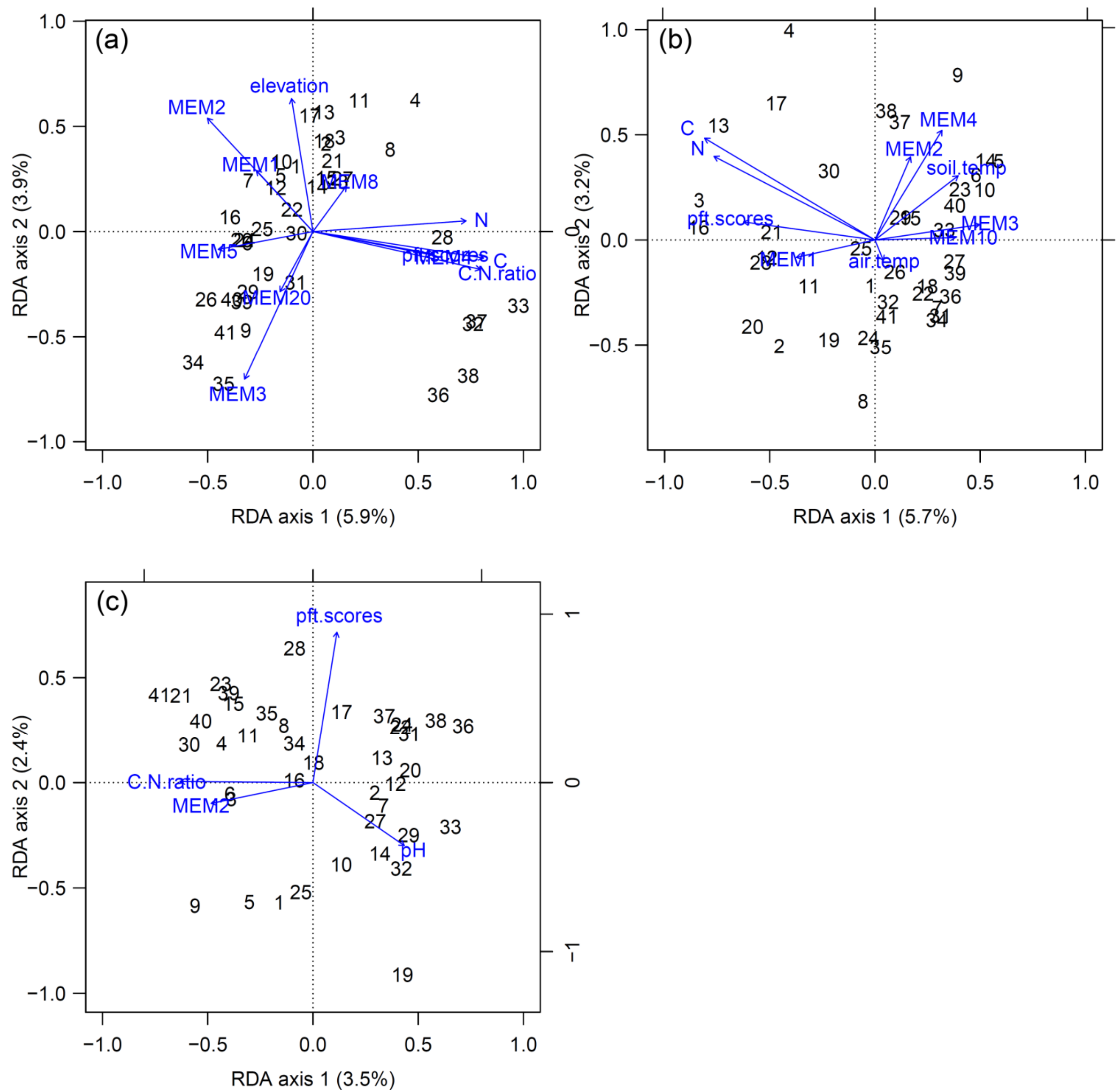


Figure 4.11: Redundancy analysis (RDA) ordination of the fungal communities sampled at (a) Site 1-AB, (b) Site 2-TL and (c) Site 3-BL along an altitudinal gradient within Kosciuszko National Park, Australia, plotted with the significant ($P < 0.05$) abiotic, vegetation (PFT-COCA scores) and spatial (dbMEM eigenvectors) variables responsible for shaping community structure at various scales. Refer to Fig 3 for sample identity at each site.

4.3.4. Neutral modeling of community assembly

The distributions of the observed community dissimilarities at each of the study sites, and across all sites combined, indicated that these communities have a high level of heterogeneity. This was most evident at the treeline (Site 2-TL), while pairwise dissimilarity was smallest above the treeline (Site 1-AB) (Figure 4.12a).

A signal of niche-based assembly was evident in the sampled soil fungal communities, across all sites, and at each site individually, with the exception of Site 3-BL. The median values of community dissimilarity were in each case greater than that of the simulated community under neutral dynamics, indicating divergence among the local communities that is potentially independent of spatial processes (Figure 4.12b). This was most evident at the sites on either side of the treeline (Site 1-AB and Site 3-BL) (Figure 4.12b). The IQR and IDR values were also greater than expected under neutral dynamics, indicating greater dispersion in the distribution of these communities, with the exception of Site 3-BL which showed community dispersion that overlapped with the prediction under purely neutral dynamics (Figure 4.12c). Site 1-AB had the highest IQR and IDR values relative to neutrality (Figure 4.12c), indicating a more varied overall distribution compared with the other sites, as was also evident from its distribution of community dissimilarities (Figure 4.12a).

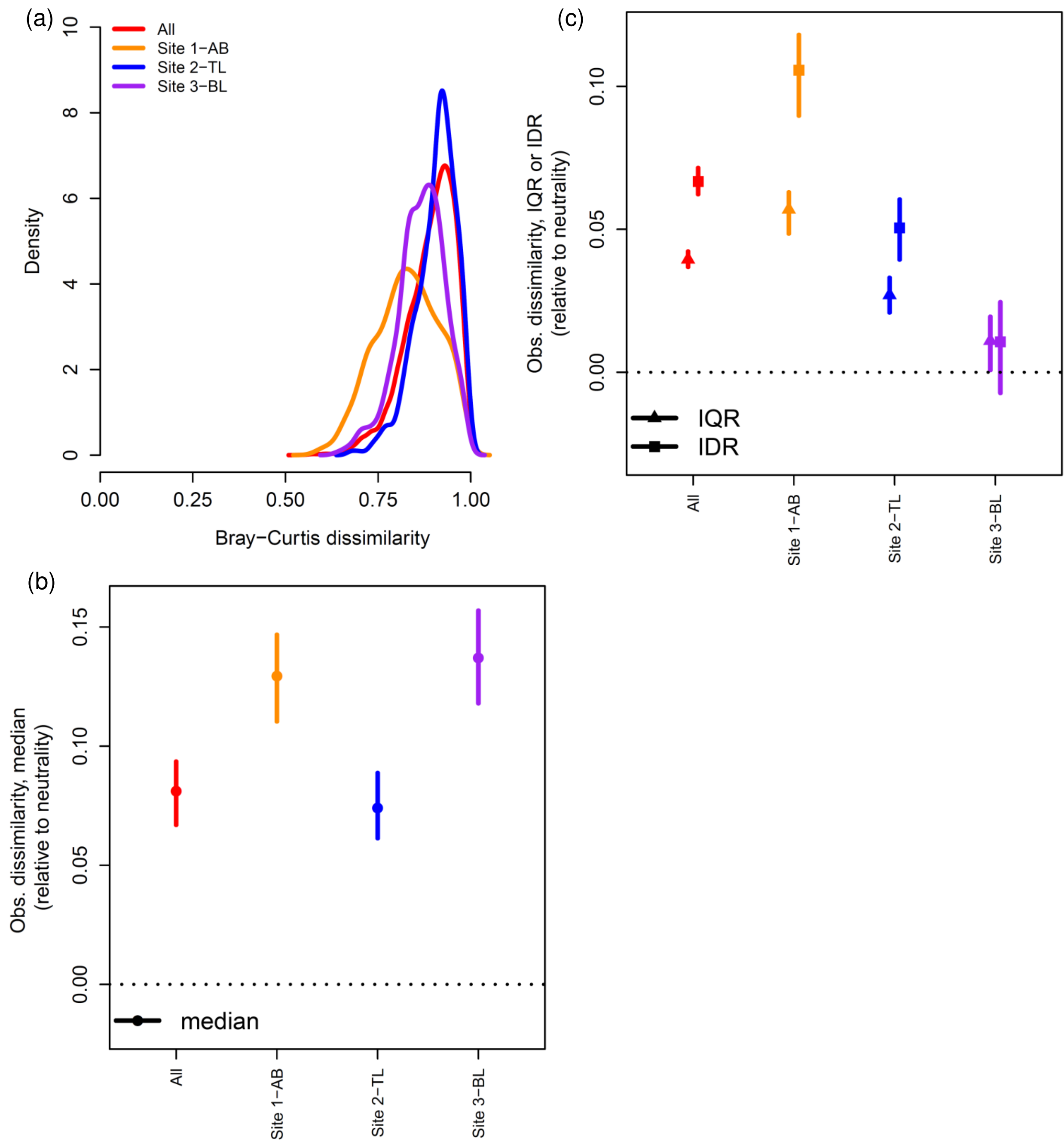


Figure 4.12: Comparison of the observed soil fungal communities along the altitudinal gradient at Kosciuszko National Park to those simulated under purely neutral dynamics. (a) Distribution density curves of the observed fungal community dissimilarity distances (Bray-Curtis). (b) Median dissimilarity values (± 95% confidence intervals) at each site and across all sites relative to that predicted under neutrality (indicated by dotted line). (c) Inter-decile and inter-quartile range values (± 95% confidence intervals) at each site and across all sites relative to those predicted under neutrality (indicated by dotted line).

4.4. Discussion

Fungal alpha diversity was found to remain consistent along the altitudinal gradient. Species richness is noted to decline with increasing altitude for many organism groups, in keeping with the similar concepts of latitudinal gradients of biodiversity (Lomolino 2001). However, in many cases, unimodal patterns have been observed where diversity decreases with increasing elevation overall, with a peak in diversity at intermediate elevations. It is possible that our sampling extent was not large enough to include areas in which species richness would differ significantly from the areas that were sampled. Species richness for ectomycorrhizal fungi has been noted to decline with increasing altitude, for example, in temperate forests of northern Iran (Bahram *et al.* 2012). However, general fungal species richness has also been noted to increase with increasing elevation in the Swiss alpine grasslands (Pellissier *et al.* 2014). Moreover, consistencies in fungal species richness have been reported along altitudinal gradients between the mid-alpine ridge and snowbed environments in Norway (Yao *et al.* 2013), between alpine open meadows and willow understory habitat in the United States (Becklin *et al.* 2012), and among piedmont, montane and montane cloud forests in the Andean Yungas in Argentina (Geml *et al.* 2014). Contrasting patterns to that of species decline with increasing altitude has also been noted in some vascular plants, bryophytes and lichens (e.g. Bhattarai and Vetaas 2003; Bruun *et al.* 2006; Desalegn and Beierkuhnlein 2010). Therefore such inconsistencies in the diversity patterns observed both among and within taxa suggest that extrapolating general patterns of species richness along altitudinal gradients at this stage seems premature.

Moving from above to below the treeline, fungal beta diversity patterns did show some small differences among the three sampling sites. The dominance of taxonomic and/or functional groups shifted along the gradient, with unique taxa being observed at each

site. Basidiomycete fungi were in greater abundance above the treeline, while ascomycete and chytrid fungi were more dominant at and below the treeline. The dominance of saprotrophic fungi was also greater at and below the treeline. For example, *Fulvoflamma spp.*, recorded at much greater abundance at Sites 2-TL and 3-BL, are saprotrophic microfungi often associated with the leaf litter of Eucalypts (Crous et al. 2006). *Myxotrichum spp.* was also much more dominant at the treeline. On the other hand, genera such as *Hyaloscypha*, may be a biotrophic parasite of bryophytes (Baral et al. 2009), and *Clavaria*, a potential ericoid mycorrhizal species (Englander and Hull 1980), were dominant in the fungal community recorded above the treeline. Despite these differences, the overall composition of the fungal communities was not substantially different along the altitudinal gradient. This is considering the small amount of variance explained by the patterns observed and the high degree of variability in the composition of the sampled communities at each site, as indicated by the distribution of their pairwise dissimilarities. High stochasticity in the assembly of fungal communities has been similarly noted previously (Yao *et al.* 2013), with stochastic spore dispersal as well as fine-scale niche partitioning being suggested as possible mechanisms that underlie such patterns. Overall the fungal communities sampled here showed a niche-based assembly, where the communities were more divergent than expected under neutrality and have possibly experienced species sorting or deterministic outcomes following a stochastic colonisation event. Niche-based assembly is also consistent with the soil communities sampled in Chapter 2. Neutral dynamics were however evident below the treeline, indicating that the mechanisms shaping the community assembly along the altitudinal gradient are not necessarily consistent, despite the factors which are influential to the patterns observed in community structure.

The fungal community patterns that were observed across the altitudinal gradient were significantly influenced by both environmental and spatial factors. Climatic conditions were most influential at the larger scales included in the study, in separating Site 1-AB and Site 3-BL in the RDA ordinations, while soil moisture, carbon and nitrogen levels were more influential in fungal community assembly at intermediate spatial scales, in separating Site 2-TL from the sites on either side of the gradient. Climatic indicators such as temperature and precipitation have been noted to be significant drivers along an alpine gradient for ECM fungi (Bahram *et al.* 2012). These were however, mean annual readings of temperature and precipitation in Bahram et al. (2012), rather than snapshot readings at the time-point of sampling. The latter were taken in the current work because mean records were not available for the exact locations and at the scale required to be meaningful for this study. However, now that the scale at which these variables are important is known, it would be of interest to further investigate the effect of climate on fungal community assembly at these scales over longer time periods. Soil pH was interestingly not one of the edaphic factors to have a significant impact on fungal community patterns, despite being a dominant driver across elevation gradients in other studies (Zinger *et al.* 2011; Geml *et al.* 2014; Pellissier *et al.* 2014). This is likely to be because the pH readings were relatively consistent across the sites (pH 4.07 – 5.44; see Appendix G) but may also suggest that the fungi present in this area have a wide optimal pH range for survival (Geml 2014).

Spatial separation distance was important in fungal community structure up to distances of 100 m, indicating that the fungal communities were positively correlated with each other up to this distance. Geographic distance is a factor often not explicitly considered in studies on the drivers of fungal community assembly across elevation gradients (e.g. Timling *et al.* 2012; Yao *et al.* 2013). In those that do consider

geographic distance as a potential driver of fungal community structure, it has been concluded that distance is not important in shaping the community (e.g. Zinger *et al.* 2011; Geml *et al.* 2014; Pellissier *et al.* 2014). However the role of distance, at a range of spatial scales, is often not considered, and in several cases of alpine studies (e.g. Geml *et al.* 2014; Pellissier *et al.* 2014), regional scales are the focus and the sampling is too coarse to detect patterns similar to those described here. In a study conducted at a similar scale to the current work, Zinger *et al.* (2011) reported that geographic distance was not important at scales up to 1000 m, from which it was concluded that either the spatial scale of the study area was too small to observe isolation by distance or that the taxonomic resolution was too coarse to detect the effect of distance. While both of these hypotheses are plausible, the spatial distances between 5 and 100 m were overlooked in their sampling design, and it is at these distances that we found distance to be significant in fungal community assembly. This enforces the importance of including multiple spatial scales in future studies, as the role of different factors on community assembly is often scale dependant (Levin 1992).

Vegetation did not have a strong influence on the assembly of fungal communities along the elevation gradient. The plant community, on the contrary, has been noted to be a primary descriptor of fungal community structure along altitudinal gradients in a number of cases, both for general fungal communities and those more specifically investigating mycorrhizal communities associated with one or more host plant species (Zinger *et al.* 2011; Bahram *et al.* 2012). Host specificity seems to be less important in alpine environments than in temperate forest and woodland systems (Timling *et al.* 2012), and therefore in this case, host specificity may not be very influential in fungal community structure. Moreover, the results of this chapter suggest that the fungal communities have a more influential role in predicting the plant communities across

the sites sampled than vice versa. The importance of the fungal community on plant community structure and productivity has previously been emphasised (e.g. van der Heijden *et al.* 1998; 2008). In alpine environments, the environmental conditions are harsher and the soils are generally poorer than in many other environments, therefore plant-fungal interactions (such as mycorrhizal associations) are thought to be more important in the success of the plant community (Pellissier *et al.* 2013). Therefore, the host specificity of the association would be lower and associations can form irrespective of the particular plant species. The fungal associations that do form, however, are more persistent and colonisation rates are higher in the harsher environments, and this is a significant factor determining how well members of the plant community can survive.

The fungal communities across the altitudinal gradient showed a relatively common composition, however the variability of the communities differed within the individual sites. Above the treeline, clustering of a subset of communities within the site was apparent from the ordination plots, which was less apparent at the treeline and not observed below the treeline. Moreover, the dispersion of the community distributions differed among sites and was greatest above the treeline. The descriptive factors also differed in their importance among the sites, which supports previous claims of the influence of explanatory variables changing with elevation (Kernaghan and Harper 2001). This suggests that, despite a relatively common overall community composition, the processes that are at work to shape these patterns may differ along the gradient. This concept is explored in greater depth in Chapter 5, by taking a more mechanistic approach to the understanding the soil fungal communities along this altitudinal gradient, especially the role of dispersal in community assembly.

Chapter 5 A metacommunity perspective on the assembly of soil fungal communities along an alpine/subalpine altitudinal gradient

5.1. Introduction

The spatial dynamics of an ecological community is closely related to the principle of dispersal. Dispersal, in the context of soil fungal ecology, can be considered as the movement of organisms from one defined local community assemblage to another by means of spores or vegetative structures. Dispersal impacts local community structure by facilitating the colonisation of new species in local communities from which they were previously absent, as well as homogenising differences among local communities. These effects are dependent on the rate and strength of dispersal experienced by species within the community (Leibold 2009). The role of dispersal in community assembly can be considered in greater depth by taking a metacommunity perspective. A metacommunity is defined as a set of local communities that are linked by the dispersal of multiple potentially interacting species (Leibold *et al.* 2004). The metacommunity perspective is a framework for considering how local communities are linked across multiple scales (Leibold *et al.* 2004).

Currently there are four paradigms that form the basis of metacommunity thinking: the 'patch dynamic', 'species sorting', 'mass effects' and 'neutral' perspectives (Leibold and Miller 2004; Holyoak *et al.* 2005). These differ in how they consider the relative

importance of dispersal compared to environmental trait heterogeneity in structuring the communities. The patch dynamics view assumes that dispersal rates are low and local species diversity is impacted by within-patch dispersal in patches that are all homogeneous and capable of hosting populations. The species sorting view emphasises the importance of local environmental gradients, whereby with sufficient dispersal, species will be 'sorted' among heterogeneous patches, using the local environment as a filter. Mass effects (Mouquet and Loreau 2003) come into play when local patch heterogeneity still exists, but dispersal rates are high enough to change population abundances by net emigration or net immigration, while the neutral model (Hubbell 2001a) assumes that differences in species ecological traits within the community are unimportant and that community dynamics are influenced only by stochastic dispersal events and demographic shifts. These perspectives have been developed in relative isolation from one another, however a synthesis of these views is in its infancy (see Leibold *et al.* 2004 for a review).

The metacommunity concept provides an underlying framework that supports many of the spatial patterns of species diversity observed to date, such as species-area relationships, distribution-abundance relationships and species diversity along latitudinal and environmental gradients (Maurer 2009). While many of the above mentioned patterns have now been discovered in fungal communities (e.g. Green *et al.* 2004; Bougoure *et al.* 2007; Peay *et al.* 2007; Tedersoo and Nara 2010), applying the metacommunity framework to soil fungi has received relatively little attention. The value of a metacommunity perspective is a focus of some recent studies of fungal community spatial dynamics (e.g. Hovatter *et al.* 2011; Feinstein and Blackwood 2013), however any metacommunity characteristics are only inferred from the observed spatial patterns of community structure, rather than explicitly considering the

role of dispersal in the assembly of natural communities. One of the few direct observations of the dispersal process in soil fungal communities has been undertaken by Peay *et al.* (2012), in which the spore dispersal mechanisms thought to drive the isolation effect of island biogeography in ectomycorrhizal fungi were tested. Dispersal rates of ectomycorrhizal propagules and host colonisation were measured in a pine 'tree island' experiment. While this study utilised a natural system, experimental manipulations were undertaken in order to measure spore dispersal, and therefore it is unknown how applicable these findings are for understanding metacommunity extent and dispersal limitation in other natural systems.

In Chapter 4, the assembly and structure of soil fungal communities was examined along an altitudinal gradient spanning across an alpine to subalpine ecotone. It was demonstrated that significant spatial correlation existed over the sampled locations up to distances of 100m, suggesting that dispersal was an active mechanism in this system both above and below the treeline. It was unclear from these results, however, to what extent the local communities were actually connected to each other. Therefore the work presented in the current chapter utilises a metacommunity approach to understand the role of dispersal more explicitly in this system. This builds on the previous chapter by empirically constructing representations of the metacommunities that exist at the three study sites along the altitudinal gradient.

Little empirical work has, to date, focussed explicitly on measuring the characteristics of a metacommunity. Maurer *et al.* (2013) presented a pioneering method to address this, whereby estimates of metacommunity extent are calculated using the premise that every local community that exists interacts with a distinct metacommunity that contributes immigrants to that community. Geographic distance, environmental distance and phylogenetic relatedness are used as criteria to construct such estimates,

in order to reflect the relative importance of dispersal distance, environmental filtering and shared biogeographic histories, respectively, on community assembly. Measuring metacommunities in this way enables the exploration of the more complex spatial dynamics of the metacommunity that are beyond the strict hierarchy of organisation understood under the current framework of metacommunity thinking. From this it is also possible to make empirical hypotheses about the spatial context in which community dynamics play out and interact across scales in natural systems (Maurer *et al.* 2013).

In the current chapter, the above method was applied to construct representations of the metacommunities that are involved in maintaining species diversity in soil fungal communities along the altitudinal gradient sampled in Chapter 4. The work presented in this chapter provides advancement both in the study of metacommunities as a general ecological concept, having an empirical focus, and also more specifically in the area of fungal community ecology, providing a perspective to community assembly that has received very little attention to date. Two main research questions were considered: *(i)* how do the characteristics of the metacommunity change across the alpine to subalpine ecotone explored in the sampling design, and *(ii)*, what are the fundamental processes structuring the metacommunities at each site along the altitudinal gradient?

5.2. Methods

Metacommunity estimates were constructed using a method developed by Maurer *et al.* (2013). Three datasets were assembled for use in the metacommunity calculations: *(i)* surveys of abundance obtained for a large collection of local communities located across a large geographic extent, *(ii)* samples of environmental variables thought to influence species occurrence within the ecosystems within which the surveys were conducted, and *(iii)* an estimate of the phylogenetic relationships among species found in these surveys. These datasets were derived from the sampling outlined in Chapter 4, though are described as appropriate for the focus of the current chapter below.

5.2.1. Community relative abundance surveys

Species relative abundance data for the fungal communities was generated by 454 pyrosequencing of the DNA from soil samples collected using a spatially explicit sampling design (see Chapter 4 for further details on sampling design). A total of 123 samples were collected across three sites within the Australian alpine region, in Kosciuszko National Park, corresponding to locations above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the biological treeline; further details are described in Chapter 4. Relative abundance data for the entire dataset was divided into three separate matrices, one for each sampling site.

A geographic distance matrix was also established for each site, using the x,y coordinates recorded for each sampling location within the overall extent of the study (see Chapter 4).

5.2.2. Environmental variables

Soil pH, moisture, temperature, total N, total C, C:N ratio, air humidity and temperature, and elevation were measured at the corresponding location of each sample. These variables were chosen as they were thought to either be important in driving soil fungal community composition or were indicative of the local climatic conditions experienced at each location. Forward selection of these variables revealed that C:N ratio, elevation, soil moisture, C, N, air temperature and air humidity were significantly associated with fungal community composition (see Chapter 4) and thus these variables were used as the environmental dataset.

Vegetation community composition was also accounted for by co-correspondence analysis of the plant functional types present at each sampling location for each site. Significant axes were also included in the environmental dataset to represent the variability explained by the plant community (see Chapter 4).

5.2.3. Phylogenetic relationships

The phylogenetic relatedness between any two sampling locations was measured as the mean phylogenetic distance separating the species from each of the two samples. Representative sequences (one per fungal OTU) were selected from the rarefied data using the get.oturep function in mother v 1.28.0 (Schloss *et al.* 2009) and exported for use in subsequent steps. These sequences were aligned using MAFFT (Katoh and Toh 2010) and evolutionary history reconstructed using BEAST (Drummond *et al.* 2012); both applications were run on the CIPRES Science Gateway (Miller *et al.* 2010). Sequences were aligned using the FFT-NS-1 algorithm. Phylogenetic reconstruction and estimation of branch lengths assumed a Yule speciation process, GTR+G substitution model, and uncorrelated relaxed clock model. The MCMC

chain was run for 40 million generations and sampled every 10 000 generations. The median node heights were mapped onto the tree with maximum clade credibility in the posterior sample, constrained to the final 1000 trees sampled from the MCMC chain. Cophenetic distances among tips in this ultrametric tree were calculated using the 'ape' package (Paradis *et al.* 2004) in R v2.14.2 (R Development Core Team 2012), which were used to calculate mean phylogenetic distances for each sampling location using the 'picante' package (Kembel *et al.* 2010). The function 'mpd' was used to calculate the mean pairwise distance between all species in each community, giving an indication of how closely related the average pair of species or individuals is in each community (see Figure 5.1). Estimates were not weighted by species abundance.

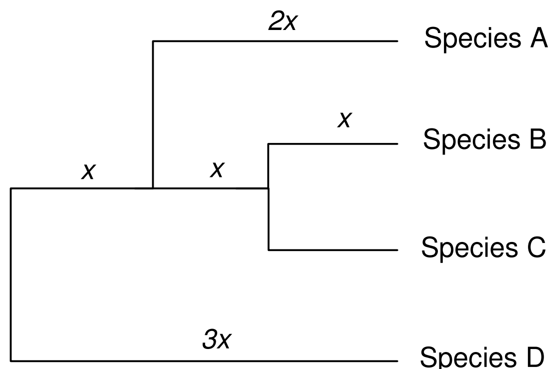


Figure 5.1: An example of how mean pairwise distances are calculated in order to establish a measure of phylogenetic relatedness within each of the sampled communities. The mean phylogenetic distance of all species within this community is (2x + 2x + 3x + x + 3x + 3x)/ 4 = 14x/4 = 3.5x

An additional analysis of whether the communities showed phylogenetic overdispersion or clustering compared with expectations under a null (random) model was also conducted. The mean nearest taxon distance and its standardised effect size

was calculated for each site using the 'picante' package (Kembel *et al.* 2010) in R v2.14.2 (R Development Core Team 2012). Values above zero suggest phylogenetic overdispersion in the community with species within communities being more distantly related than expected, while negative values indicate clustering and that the species within communities are more closely related to one another than expected under random assembly conditions.

5.2.4. Calculations of metacommunity extent

The details of the calculations used to construct metacommunity estimates using geographic, environmental or phylogenetic datasets (referred to as the predictor matrices herein) are presented by Maurer *et al.* (2013). The concepts of these calculations are however briefly described as follows (see Appendix I for R script of the basis of these calculations). In this work, each sample (the fungal species abundance data recorded from an individual soil core) is referred to as a local community, and the *focal community* is defined as the particular local community for which a metacommunity estimate is being constructed.

The basis of the metacommunity estimate is calculating the similarity of the focal community to a series of groups of surrounding local communities. These groups are subsets of the surrounding local communities that are of increasing distance from the focal community. The metacommunity for any particular focal community is the group of surrounding local communities that are collectively the most similar to the focal community; the metacommunity extent is the distance at which this occurs (Figure 5.2). These distances can be calculated in geographic, environmental or phylogenetic space (Figure 5.2). The similarity of the local communities that make up the metacommunity, as well as the extent of the metacommunity, is likely be different

for any one focal community, depending on the type of distance matrix used in the calculations (Figure 5.2). Therefore, the calculations were performed using each of the three predictor matrices (geographic distances, environmental distances and phylogenetic relatedness), and repeated for each focal community within each of the three study sites.

The similarity of each focal community to its group of surrounding local communities (referred to as psim by Maurer *et al.* (2013) and in the R script) was calculated as the sum of the minimum relative abundance value of each species either; *(i)* combined across the group of local communities under consideration, or, *(ii)* present in the focal community (Table 5.1). This similarity calculation was performed between each focal community and a series of subsets of the surrounding local communities. The surrounding local communities were divided into subsets according to their distance from the focal community (Figure 5.3). Euclidean distance matrices were calculated for each predictor matrix (geographic location, environmental characteristics, and community mean phylogenetic distances at individual sampling points). The local communities with a corresponding Euclidean distance that was less than a specified distance from the focal community were included in that particular subset; each subsequent subset consisted of additional local communities existing at increasing Euclidean distances from the focal community (Figure 5.3).

The sequence of distance classes was calculated by Maurer *et al.* (2013) using 2*nd (where nd = total number of local communities), based on a linear one dimensional sampling design. In the work presented in this chapter, it was more appropriate to use 4*nd to calculate the sequence of distance classes, as a two dimensional sampling design was used. This ensured that each subsequent distance class included, on average, one additional local community than the previous distance class.

Metacommunity estimates were calculated for geographic distance with both a sequence of distance classes generated using 2*nd and a sequence generated using 4*nd, and were compared for accuracy to metacommunity estimates calculated using a sequence of distance classes that included exactly one additional local community for each subsequent distance class (see Appendix J).

The subset of local communities for which psim is the greatest is considered to be the group of local communities that make up the metacommunity of the chosen focal community. Therefore, this psim value, referred to by Maurer *et al.* (2013) as the metacommunity distance function (optdist), is the relative similarity of the metacommunity to the chosen focal community, and the corresponding distance class for the subset is the distance to which the metacommunity extends (Figure 5.3). The number of local communities and the identity of the communities included in the metacommunity estimate were also identified. Thus a total of three metacommunity estimates (the similarity - optdist, the distance extent, and the number and identity of the included local communities) for each local community at each study site were generated.

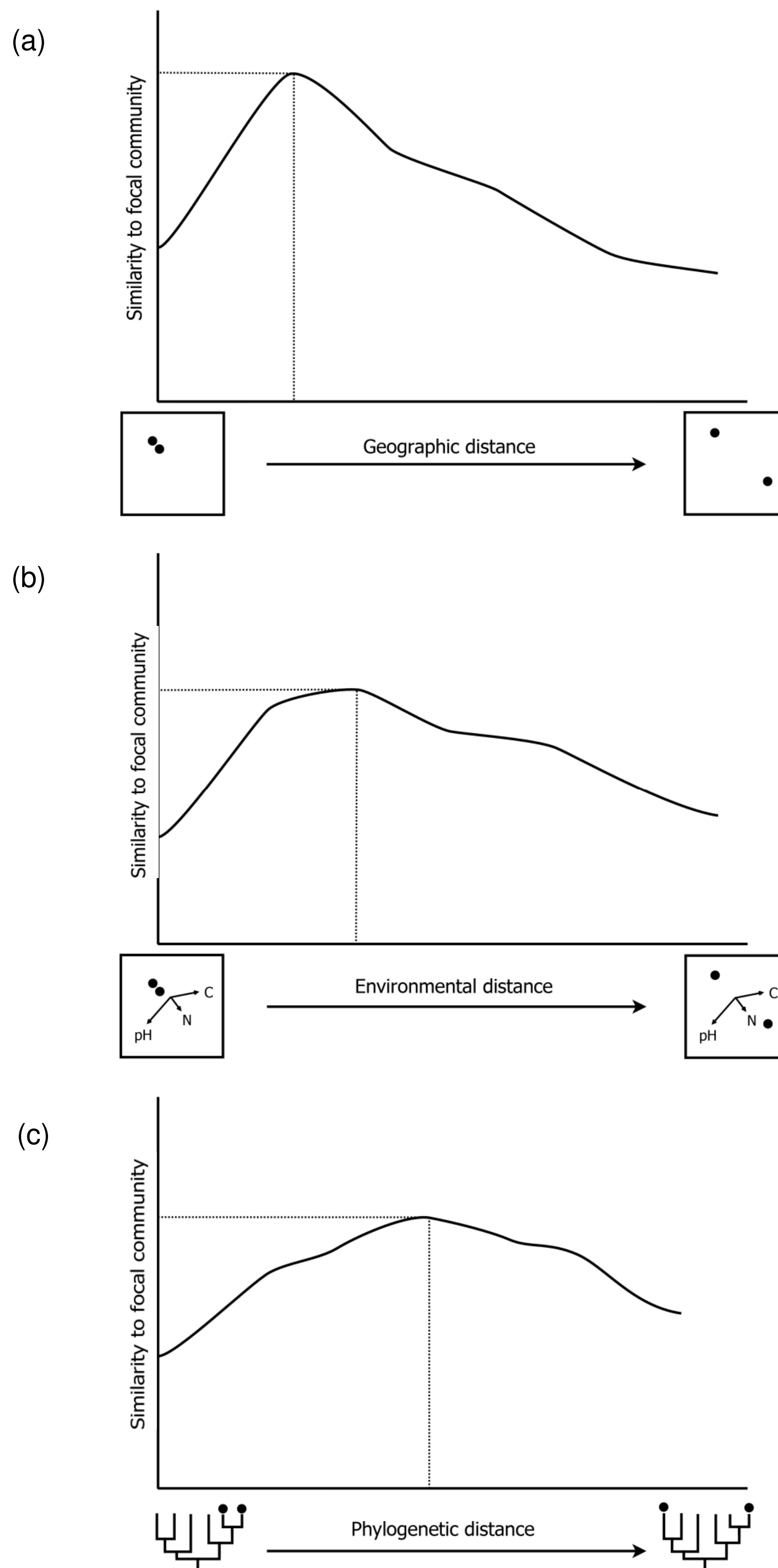
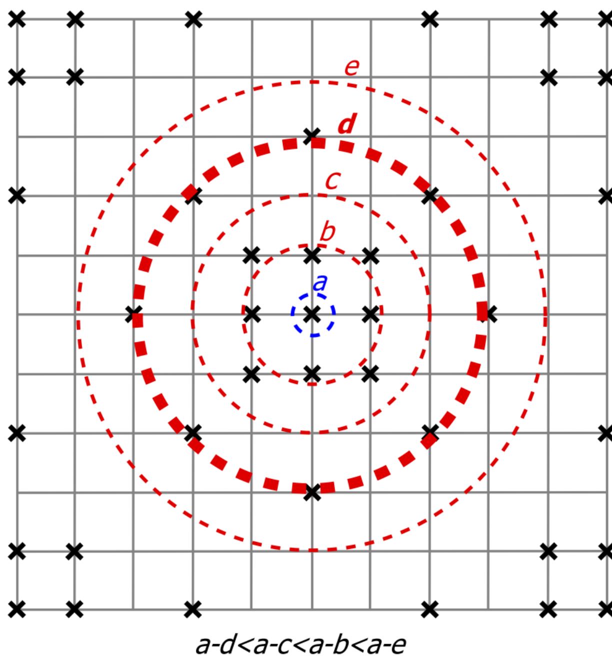


Figure 5.2: Conceptual diagram of the similarity of the focal community to its surrounding local communities with increasing distance in (a) geographic, (b) environmental, or (c) phylogenetic space. The similarity of the focal community to the surrounding group of local communities changes as more local communities are included at increasing distance from the focal community. The point at which the similarity of the focal community to the group of surrounding local communities is the greatest is considered the metacommunity for that focal community, and the distance at which this occurs is the metacommunity extent.

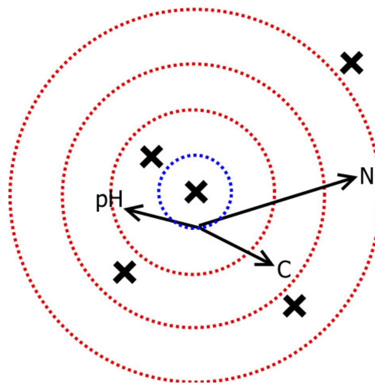
Table 5.1: Example of how the similarity of the combined local communities within each subset relative to the focal community is calculated. The relative abundance of each species in the focal community is compared to the relative abundance of the corresponding species within a particular subset of local communities combined. Blue shading indicates the minimum relative abundance value for each species included in the calculation of psim for subset 1, yellow shading represents the same for those used in the calculation for subset 2. Green shading indicates a relative abundance value that was the minimum for that species in both cases, and therefore included in both calculations. In this example, the calculated psim value for the communities represented by subset 1 is the greatest (indicated in bold), and therefore this subset would be considered as the metacommunity estimate for this focal community.

	relative abundance			psim value relative to focal community
	species A	species B	species C	
focal community	0.1	0.7	0.2	
combined local communities within subset 1	0.4	0.5	0.1	**0.1 + 0.5 + 0.1 = 0.7**
combined local communities within subset 2	0.2	0.3	0.5	0.1 + 0.3 + 0.2 = 0.6

(a) geographic distance



(b) environmental distance



(c) phylogenetic distance

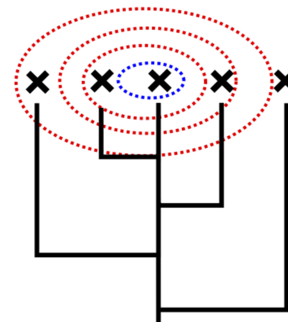


Figure 5.3: Conceptual diagram of how the estimates of metacommunity extent are calculated per focal community. Each sampling point, or local community, is signified by an x positioned in (a) geographic, (b) environmental or (c) phylogenetic space. The focal community in each case is indicated with a blue ring. The red rings represent a series of distance classes, with boundaries of increasing distance from the focal community. Each distance class therefore contains a subset of local communities that are less than the specified distance from the focal community. The similarity of the combined community within each subset is compared to the focal community. The distance class for which the combined set of local communities is most similar to the focal community is considered to be the distance to which the metacommunity extends for that particular focal community. In the example using geographic distances (a) the focal community is labelled *a*, and the subsets of local communities within a series of distance classes are labelled *b* to *e*. In this instance, the difference between the communities of *a* and *d* is less than that of *a* and *b*, *a* and *c*, and *a* and *e* (i.e. *a* and *d* are most similar). Therefore the boundary of *d* (indicated in bold) is the estimated extent of the metacommunity.

5.2.5. Statistical analysis

Summary statistics were calculated, including the mean, median, minimum and maximum values for the similarity, size and extent of the metacommunities constructed, using each predictor matrix. Metacommunity estimate data was then analysed by either comparing; *(i)* the estimates calculated using the various predictor matrices for an individual sampling site, in order to see how using different predictor matrices influences how the metacommunities are constructed, or *(ii)* by comparing the estimates calculated using each predictor matrix across the three sampling sites, to see how the characteristics of the metacommunity (the similarity, size and extent) varied across the altitudinal gradient of the study design. The metacommunity similarities for each focal community, calculated using each predictor matrix, were plotted and lines of regression were fitted in order to see the relative importance of each predictor matrix in shaping the constructed metacommunities.

5.3. Results

The characteristics of the constructed metacommunities varied among sampling sites along the altitudinal gradient, and while differences were observed among the estimates using different predictor matrices, general patterns across all three criteria (i.e. geographic, environmental and phylogenetic distances) were apparent from the calculated mean values of the metacommunity estimates. The minimum and maximum similarity values, while not as useful for making ecological inferences, give context to the other results. The maximum similarity values across all sites, using each criteria ranged from 0.269 to 0.431 (Table 5.2). The minimum similarity values at Site 2-TL was much lower for each criteria than at the other sites, indicating a more

variable community composition at the treeline (Table 5.2). The mean similarity values indicated that the constructed metacommunities were most similar to their respective focal communities above the treeline (Site 1-AB). This similarity dropped substantially at the treeline (Site 2-TL) and was also lower below the treeline (Site 3-BL; Figure 5.4). This pattern was consistent for the three criteria (i.e. geographic, environmental and phylogenetic distances) used to construct the metacommunities in each case. Metacommunity size, i.e. the number of local communities included in the metacommunity estimate, was smallest at Site 1-AB using all three criteria, and generally increased with decreasing altitude (Figure 5.5). However a plateau in metacommunity size was observed at Site 2-TL and Site 3-BL in the estimates constructed using phylogenetic distances (Figure 5.5c). The metacommunity similarity estimate constructed using phylogenetic relationships was lower than those constructed using geographic and environmental distances across all sites (Table 5.2). The metacommunity size was also greater using phylogenetic distance than the other two criteria (Table 5.2).

By comparing the relative similarities for the metacommunities constructed across the three sites using the different criteria, it was possible to assess the relative role of each of the criteria in shaping the metacommunities. A slope of one indicates that the two criteria are equivalent in their metacommunity estimates across all sampling sites and suggests that the criteria used to estimate the metacommunity are of equal importance in metacommunity assembly, while a slope less or greater than one indicates that the criteria plotted on the x or y axis, respectively, has a relatively stronger role in the metacommunity assembly processes (see Table 5.3). A relatively strong correlation existed between the geographic and environmental estimates of the metacommunity above (Site 1-AB: slope = 0.956 ± 0.074) and at (Site 2-TL: slope = 0.923 ± 0.085) the

treeline (Table 5.3, Figure 5.6). Geographic distance was the strongest predictor of the metacommunity below the treeline (Site 3-BL; Figure 5.6), while phylogenetic distance was a weaker predictor of the metacommunity across all sites, when compared to both the geographic and environmental estimates. The influence of phylogenetic distance was, however, stronger at Site 1-AB compared to the other sites and was the least influential at Site 2-TL (Figure 5.6b,c). Further analysis of the strength of phylogenetic relatedness in the communities showed that the standardised effect size of the mean nearest taxon distance was significantly less than zero at the majority of sampling locations across all three sites ($P = 0.01$) indicating that individuals within communities were more closely related than expected by chance. This pattern was strongest above the treeline (Site 1-AB) and weaker, but still significant at Site 2-TL and Site 3-BL, which were similar (Figure 5.7).

Table 5.2: Descriptive statistics for the similarity, size and extent of the predicted metacommunities for each local community, generated using geographic, environmental and phylogenetic distances above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the treeline along an altitudinal gradient in Kosciuszko National Park, Australia. Estimates for metacommunity similarity are based OTU relative abundances and calculated as described in Table 1; 0 = complete dissimilarity, 1 = complete similarity. Units for the metacommunity extent estimates are metres (for geographic; minimum = 10, maximum =122.189), inertia (for environmental; min. = 0, max. = 5.878), and branch length (for phylogenetic; min. = 0, max. = 0.127)

		Geographic			Environmental			Phylogenetic		
		Site 1-AB	Site 2-TL	Site 3-BL	Site 1-AB	Site 2-TL	Site 3-BL	Site 1-AB	Site 2-TL	Site 3-BL
Metacommunity similarity	Mean	0.305	0.198	0.225	0.303	0.209	0.226	0.289	0.183	0.217
	Median	0.299	0.198	0.229	0.313	0.208	0.231	0.301	0.182	0.219
	Minimum	0.143	0.043	0.096	0.143	0.047	0.102	0.106	0.042	0.096
	Maximum	0.431	0.305	0.329	0.422	0.312	0.288	0.396	0.269	0.292
Number of local communities included in metacommunity	Mean	10.756	15.415	18.561	11.293	12.707	17.488	16.488	20.805	20.390
	Median	7	9	17	7	8	14	17	21	17
	Minimum	1	2	2	1	1	1	1	1	1
	Maximum	40	39	40	36	40	40	40	40	40
Metacommunity extent	Mean	39.298	47.390	54.719	2.787	2.983	3.523	0.031	0.033	0.027
	Median	31.636	42.855	54.074	2.427	2.811	3.702	0.024	0.021	0.027
	Minimum	10	10	10	1.510	1.193	0.772	0.002	0.002	0.0006
	Maximum	122.189	120.586	114.176	5.179	5.430	5.878	0.117	0.127	0.070

Table 5.3: Slope ± standard error of fitted regression lines between the metacommunity similarities, calculated using each criteria (geographic distance, environmental distance and phylogenetic distance), for each focal community sampled above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the treeline along an altitudinal gradient in Kosciuszko National Park, Australia. A slope of one indicates that the two criteria are equivalent in their metacommunity estimates across samples and suggests that these criteria used to estimate the metacommunity are of equal importance in metacommunity assembly. A slope of less than one indicates that the criterion plotted along the x axis is of stronger influence in metacommunity assembly than that plotted along the y axis (see Figure 5.6). 95% confidence intervals are presented in parentheses.

	geographic (x axis) vs environmental (y axis)	geographic (x axis) vs phylogenetic (y axis)	environmental (x axis) vs phylogenetic (y axis)
Site 1-AB	0.956 ± 0.074 (0.807, 1.105)	0.910 ± 0.093 (0.722, 1.096)	0.847 ± 0.090 (0.666, 1.028)
Site 2-TL	0.923 ± 0.085 (0.752, 1.095)	0.755 ± 0.065 (0.627, 0.887)	0.656 ± 0.075 (0.504, 0.808)
Site 3-BL	0.739 ± 0.101 (0.535, 0.943)	0.765 ± 0.080 (0.604, 0.926)	0.802 ± 0.078 (0.644, 0.961)

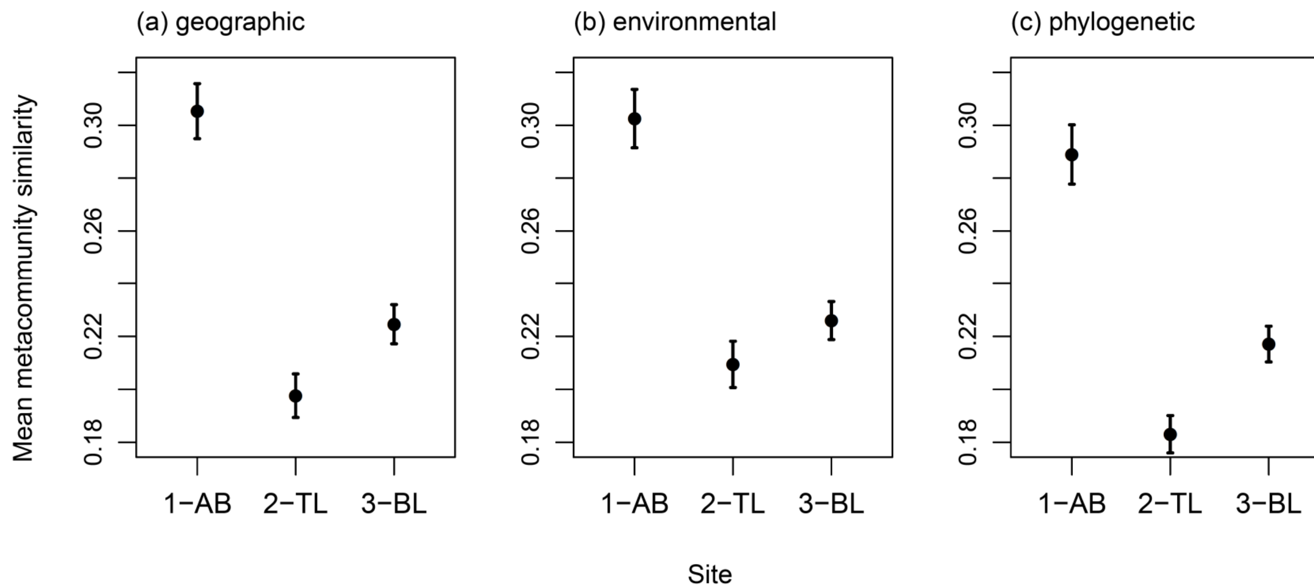


Figure 5.4: Mean similarity of the focal community to its calculated metacommunity (± standard error) at each site above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the treeline, along an altitudinal gradient in Kosciuszko National Park, Australia. The metacommunity estimates in each case were constructed using (a) geographic, (b) environmental, and (c) phylogenetic distances respectively.

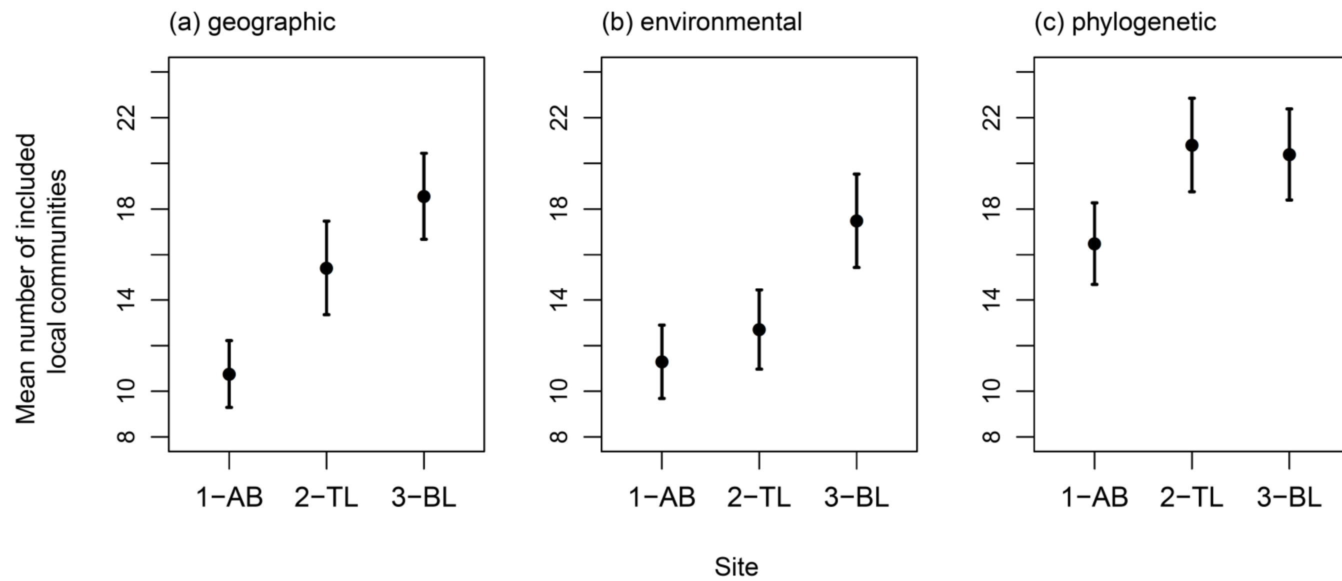


Figure 5.5: Mean metacommunity size, or the number of surrounding local communities included in each metacommunity estimate, (± standard error) at each site above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the treeline, along an altitudinal gradient in Kosciuszko National Park, Australia. The metacommunity estimates in each case were constructed using (a) geographic, (b) environmental, and (c) phylogenetic distances respectively.

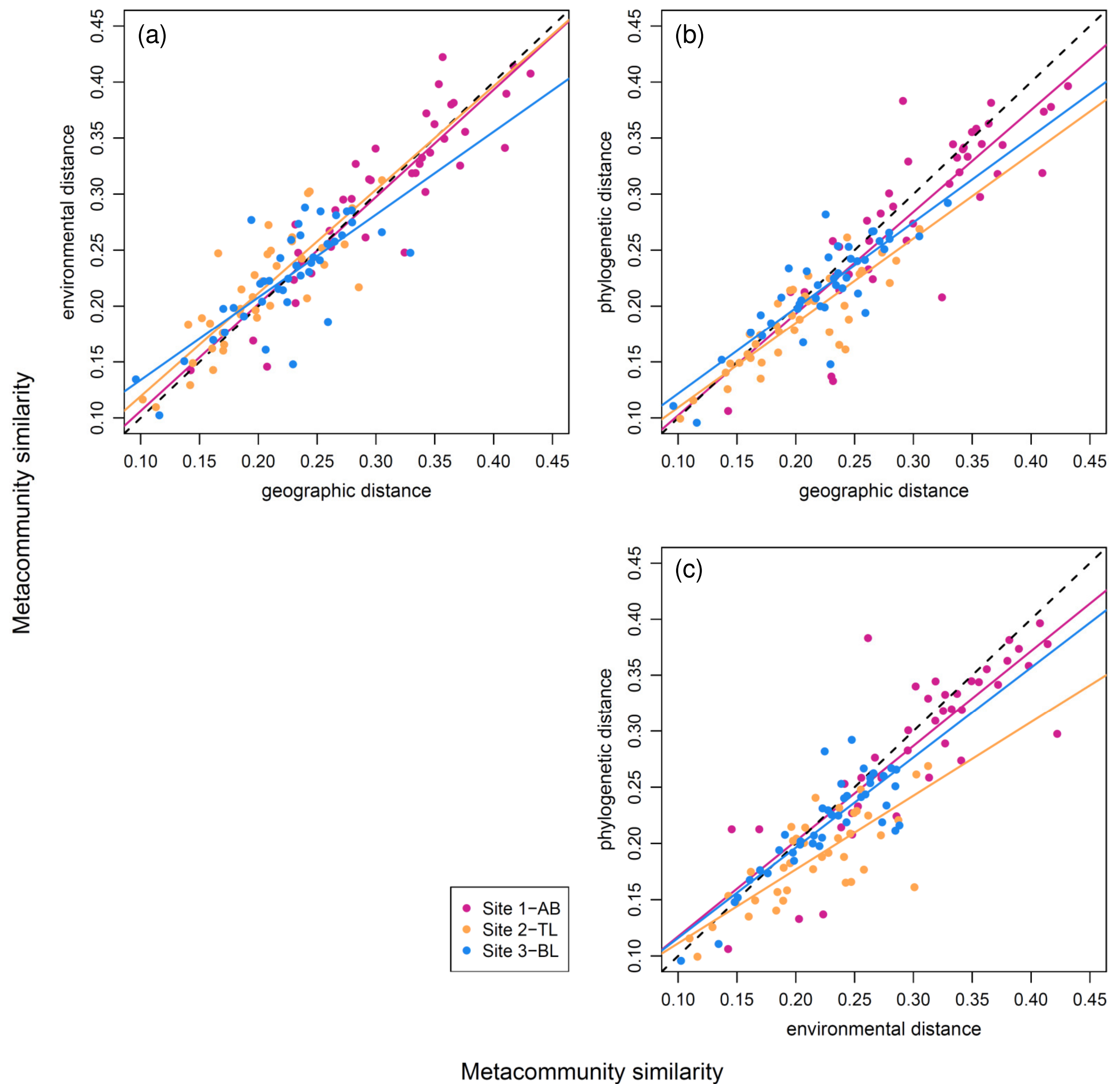


Figure 5.6: Similarities between focal communities and their respective metacommunities for each local community sampled within three sites along an altitudinal gradient each site above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the treeline, along an altitudinal gradient in Kosciuszko National Park, Australia; (a) metacommunity similarities calculated based on geographic distances are plotted against the corresponding similarities based on environmental distances; (b) similarities calculated based on geographic distances plotted against the corresponding similarities based on phylogenetic distances, (c) similarities calculated based on environmental distances plotted against the corresponding similarities based on phylogenetic distances. The dashed line represents a line with a slope of one and an intercept of zero; the coloured lines represent lines of regression fitted to the points of their respective sampling site.

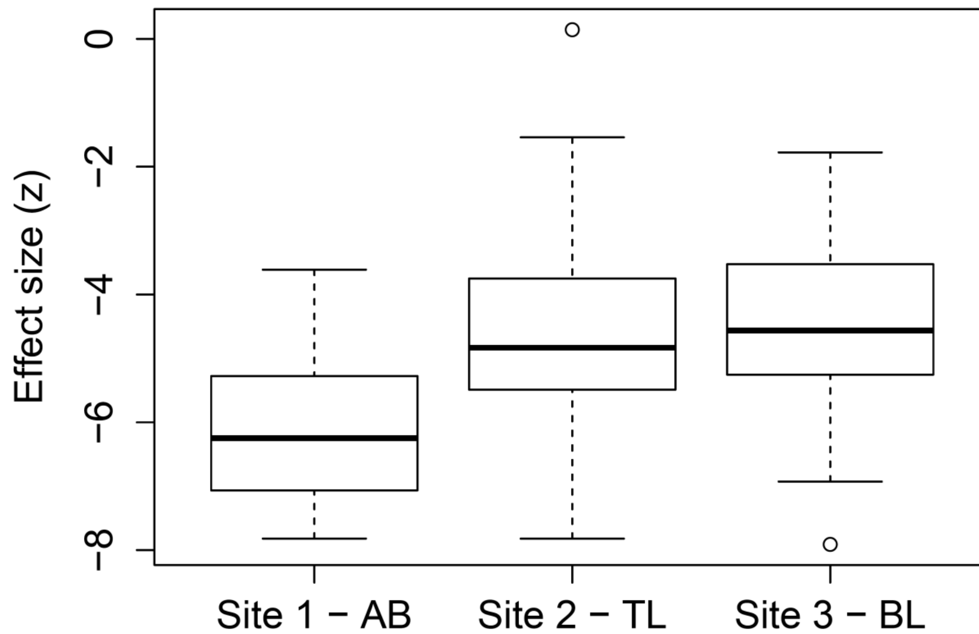


Figure 5.7: Box and whisker plot of the effect size of mean nearest taxon distances in the fungal communities sampled above (Site 1-AB), at (Site 2-TL) and below (Site 3-BL) the treeline, along an altitudinal gradient in Kosciuszko National Park, Australia. Values less than zero indicate the relatedness of the communities is greater than that expected by chance, based on comparisons to the corresponding null community. Boxes represent the interquartile range of the observed values; whiskers represent the range of observed values or (when an outlier - the open circle outside the whiskers - is present) 1.5x the interquartile range.

5.4. Discussion

The general trend observed across all criteria used to calculate metacommunity estimates along the altitudinal gradient was that metacommunities above the treeline consisted of fewer local communities that were less dispersed than the metacommunities at and below the treeline. This is in keeping with the results of the neutral modelling in Chapter 4, in which communities expressed a higher level of pairwise similarity, on average, and a greater range of similarities above the treeline than at the other two sites, suggesting a greater degree of clustering of local communities. This trend also suggests that in the alpine environment above the treeline, species mixing is occurring to a much greater extent among fewer communities in order to make up the local community assemblages, while in the subalpine environment below the treeline, fewer species may be recruited from individual local communities and species mixing is occurring among local communities that are more 'distant' from one another, whether measured in geographic, environmental or phylogenetic space (Figure 5.8).

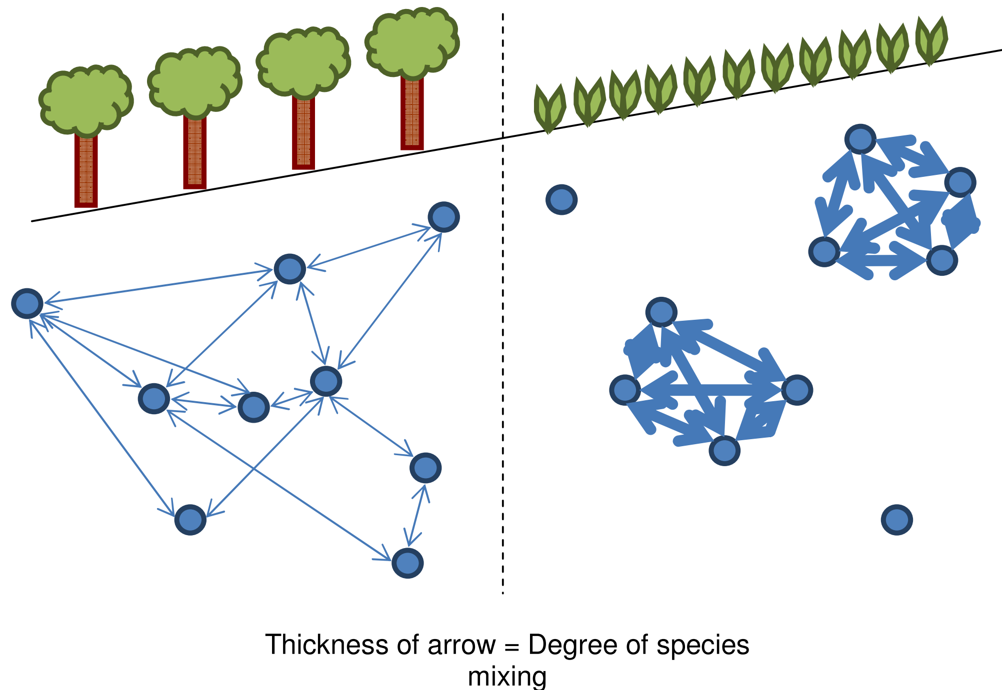


Figure 5.8: Schematic diagram of the metacommunity characteristics along the altitudinal gradient in Kosciuszko National Park, Australia. The blue circles represent local communities and the arrows represent the connections among local communities within a metacommunity, by dispersal. The thickness of the arrows indicates the degree of species mixing that is occurring among local communities.

These evident characteristics of metacommunities along the altitudinal gradient therefore indicate that dispersal limitations are likely to be greater in the alpine environment, above the treeline. This is a surprising result assuming that wind-based movement of spores would result in reduced dispersal limitation in a more exposed environment. Vegetative dispersal, however, has been noted as a more successful method of dispersal than spore dispersal for ECM fungi (Peay *et al.* 2010b). If this is also the case for other fungal groups, vegetative dispersal, by means of mycelial colonisation, would be greater in systems with an abundance of tree roots, as would be the case in the subalpine environment, compared to the alpine environment.

Moreover, greater dispersal below the treeline may be influenced by the presence of animal vectors both above and below-ground. Soil food webs, are suggested to be an important method for fungal dispersal (Lilleskov and Bruns 2005) and soil invertebrate communities, often with fungi as a main food source, have been noted to have greater diversity and are more active at lower altitudes (Powers *et al.* 1998). Animal and bird diversity and activity above-ground have also been noted to be greater in subalpine environments, with more extreme conditions and longer snow cover hampering foraging within shrubs and on the ground at higher elevations (Osborne and Green 1992). A combination of the activity of above and below-ground vectors may therefore promote more extensive fungal dispersal below the treeline, compared to at higher elevations.

While, the above inferences about the differences in metacommunity extent along the altitudinal gradient are somewhat limited by the focus of the sampling design, differences in the metacommunity estimates at each site, constructed using each of the three criteria, were also evident, and more strongly suggest differences exist in the mechanisms that shape the sampled metacommunities. It is also noteworthy that biased metacommunity extent estimates were potentially calculated for some estimates (those where the number of local communities included in the metacommunity estimate reflected the number of samples taken at each site), and thus metacommunity sizes calculated using each criteria may absolutely include more local communities than were sampled. This indicates that, overall, the factors shaping metacommunity extent are also operating over broader scales than were investigated at each site. In saying this however, comparisons among the sites (rather than absolute quantification), for each criteria used, are still possible and show meaningful differences in the dominant factors shaping the sampled metacommunities.

The metacommunity estimates derived using geographic and environmental distances resulted in largely similar patterns among the three sites. This possibly indicates that the spatial processes that organise these metacommunities are directly related to the spatial variation in environmental conditions (Maurer *et al.* 2103). The role of geographic distance was, however, stronger than the environment below the treeline (the slope of the relationship between metacommunity similarity calculated using geographic and environmental distances was less than one), indicating that spatial processes are likely more influential below the treeline. Dispersal rates are thought to be influential to the relative importance of proximity versus environment in drawing organisms from the metacommunity for local community assembly. Slow dispersers result in slower local community establishment and therefore the assembly is more stochastic and influenced more by proximity over environmental conditions (Leibold 2009). It is therefore possible that, while dispersal is more extensive below the treeline, local community establishment is also occurring over longer time scales in subalpine soils than in the alpine environment. The subalpine environment is in general a more favourable environment for a greater proportion of the year (e.g. shorter periods of snow cover, slightly warmer temperatures), and therefore dispersal as a process can occur over longer time periods. The alpine environment is by contrast harsher, and conditions which are suitable for dispersal exist for a smaller portion of the year. Therefore dispersal may only be possible in these short time periods when conditions are favourable and so there may be selection on traits that allow for rapid, short range dispersal to compensate for this.

Evidence of phylogenetic clustering within local communities was also observed, suggesting that the constituents of the species pool from which local communities were established are more closely related than expected by chance (Kraft *et al.* 2008;

Cavender-Bares *et al.* 2009). Phylogenetic relationships were, however, a slightly weaker predictor of the metacommunity characteristics across all sites (the slope of relationship between metacommunity similarity calculated using phylogenetic relatedness was always less than one). Moreover, the extent of the phylogenetic metacommunities was larger than those constructed using the other criteria. It is therefore likely that evolutionary processes do not constrain community assembly as strongly as spatial processes or environmental variation, and that the species clades in which similar ecological traits are grouped, are relatively not as important in shaping the composition of the metacommunity. This does not mean that ecological traits are not important for shaping the metacommunity through environmental filtering or competitive elimination of co-occuring species, but rather indicates that such traits associated with environmental filtering may be only weakly constrained during evolution. This is also in keeping with the result of niche-based assembly concluded in Chapter 4. More information is required on these traits that are involved in the local community and metacommunity assembly processes, and how their expression in different evolutionary lineages impacts on these processes, in order to more fully understand the role of trait and functional diversity in fungal community assembly (Aguilar-Trigueros *et al.* 2014; Aguilar-Trigueros *et al.* 2015).

Overall, the observed fungal community composition across the altitudinal gradient was concluded to be relatively similar, as was presented in Chapter 4. However, the current chapter provides evidence that despite this, biological differences exist in the mechanisms that are involved in assembling these communities. The metacommunity perspective presented in this chapter shows that the role of dispersal may be of equal importance in overall community structure, however the rate and the degree of species mixing differs depending on the environmental context. The ecological variation that

exists in these communities is in keeping with niche-based community assembly, as suggested in Chapter 4, however the importance of dispersal is also evident, indicating that mass-effects metacommunity dynamics are likely operating in the assembly of these communities.

Chapter 6 General Discussion

The research presented in this thesis sought to investigate the spatial structure of below-ground fungal communities and the processes that drive their assembly. Spatial patterns of community assembly were investigated across a range of spatial scales, from separation distances of 20 cm to 500 km, and the roles of spatial distance, habitat type, edaphic characteristics, climatic conditions, vegetation type and the diversity of the plant community, as well as phylogenetic relatedness, in shaping the observed fungal communities were considered. Additionally, the functional processes that shape community assembly were considered by looking at the role of niche and neutral dynamics as well as understanding community assembly from the perspective of the metacommunity.

6.1. Spatial patterns in fungal community assembly

Large scale fungal community patterns were found, spanning distances of between 100 and 500 km (Chapter 2), as well as at smaller scales of 20 cm to approximately 100 m (Chapters 3 and 4). From separation distances of approximately 100 m up to over 1 km (Chapter 4), and even up to 100 km (Chapter 2), no significant spatial patterns were detected, suggesting that a distinct lack of spatial patterning exists for the sampled below-ground fungal communities at regional or intermediate scales. High local diversity, followed by only moderate regional diversity, is fitting with the triphasic

model of the species-area relationship, in which species diversity levels off at intermediate scales and increases again at continental scales (O'Dwyer and Green 2010). The scales at which microbial diversity demonstrates these levels of spatial structure are thought to be greater than for larger organisms such as plants and animals, with factors such as broader taxonomic resolution and lower habitat specificity providing possible explanations for the moderate regional diversity of micro-organisms (Green *et al.* 2004; Horner-Devine *et al.* 2004).

6.2. Factors influencing fungal community patterns

At the smaller scales examined, spatial separation distance appeared to be the most influential factor in the spatial patterns of the fungal community. Geographic distance was the most important explanatory variable at scales of 20 cm to 100 m (Chapter 3), and was also important in fungal community structure in Chapter 4, up to distances of 100 m. Spatial distance was also found to be a significant factor in fungal community assembly at the largest scales examined (100 – 500 km; Chapter 2).

The role of environmental factors, specifically the edaphic characteristics that were measured (e.g. pH, C, N, C:N ratio, soil moisture), did not play a dominant role in shaping the fungal community, independently of other measured variables, at any of the scales at which spatial patterns were detected. The role of space and the environment was largely intertwined at largest scales, with the measured edaphic characteristics being of little influence without also considering the role of space (Chapter 2). Edaphic characteristics did not have a significant role in fungal community assembly, and strong correlations between fungal beta diversity and the dispersion of edaphic variables were not evident at the smaller scales (Chapter 3). The

strength of the relationship between fungal community patterns and environmental drivers, both edaphic conditions and climatic conditions (Chapter 4), seem to be more apparent at larger spatial scales, as a greater distinction in the changes to environmental conditions and the responses to these conditions, is greater at broader scales (Prober *et al.* 2015). Spatial distance, however, remained an underlying factor in describing the role of environmental factors in shaping fungal community assembly.

The role of the surrounding vegetation in structuring the soil fungal community was additionally examined in Chapters 3 and 4, and was found to have little influence in their assembly and spatial patterns, at least with regard to how the vegetation was characterised. Vegetation type (i.e. presence of a shrub layer or tree canopy) was not a significant descriptor of the fungal community patterns at scales of 20 cm to 200 m, sampled in both disturbed, open plant community and an established plant community with a canopy layer (Chapter 3). Vegetation, when grouped into plant functional types, also had little predictive power over the fungal community across the subalpine/alpine ecotone studied in Chapter 4, even though a sharp shift in the plant community was observed above versus below the treeline. Correlations between plant beta diversity and fungal beta diversity, were evident at the scales investigated in Chapter 3, however it is unclear whether this was a direct relationship, as increased plant diversity can indirectly influence microbial diversity through increased plant production associated with greater diversity rather than diversity itself (Zak *et al.* 2003). It is likely that the strength and specificity of any relationship between plant and fungal community assembly, in these cases, depends on the diversity of the above and below-ground components, as well as the plant community type (e.g. temperate or alpine).

6.3. Mechanistic perspective of fungal community assembly

Across the three experimental setups, a high proportion of the variation in each community dataset remained unexplained. This is despite including more explanatory variables and focusing the sampling design at different scales over the three subsequent experiments. High levels of community dissimilarity have been observed previously for soil fungal communities, across various spatial scales (e.g. Dumbrell *et al.* 2010; Powell *et al.* 2015), which is in keeping with the current findings. In such cases, variation in community structure is attributed to stochastic mechanisms, whereby the influence of priority effects and succession are of potential importance (Dini-Andreote *et al.* 2015; Powell *et al.* 2015). While this is not entirely ruled out in the current work, neutrality modeling results indicate that in some instances, deterministic, or niche-based dynamics, remained as important assembly mechanisms (Chapters 2, 3 and 4). Therefore niche-based mechanisms may still be contributing to below-ground fungal community assembly in many cases where stochastic mechanisms appear to dominate. The ability to link community patterns with environmental variables, even when niche-based mechanisms are thought to be driving their assembly, could be hampered by using a taxonomic based approach to characterise the fungal community. A promising alternative approach to characterising community patterns is the use of functional traits, as coexistence under niche-based assembly relies on the display of favourable phenotypic traits to gain a competitive advantage, rather than strictly genetic similarity (Chave 2013; Aguilar-Trigueros *et al.* 2014; Crowther *et al.* 2014). Taking a functional approach to the study of biodiversity has been the recommendation of a number of recent reviews (e.g. McGill *et al.* 2006) including several on microbes and fungi (e.g. Gamper *et al.* 2010; Parrent *et al.* 2010; Martiny *et al.* 2015; Treseder and Lennon 2015). A functional trait approach advantageously enables the same traits in

communities with different constituent taxa to be compared; thus facilitating the ability to generalise or contrast the ecological processes acting in distinct sampling locations or environments (Parrent *et al.* 2010). However, empirical studies using such an approach are lagging. This research would complement the use of neutrality modeling to gain a holistic perspective on the functional role of soil fungal communities in ecosystem processes.

As alluded to previously, niche-based mechanisms were found to drive the assembly of soil fungal communities sampled at the large spatial scales in Chapter 2, as well as above and at the treeline sampled in Chapter 4. In contrast, neutral dynamics dominated the assembly of the soil fungal communities at smaller spatial scales sampled in Chapter 3, below the treeline sampled in Chapter 4, and also dominated the assembly of root associated communities over the same large scale sampling design (Chapter 2). These results support the continuum hypothesis (Gravel *et al.* 2006), and indicate that the role of stochastic or deterministic assembly mechanisms in driving community structure depends on the strength of dispersal and the degree of niche overlap experienced by the community. In Chapter 2, the root-associated fungi did not have a strong habitat preference, compared to the corresponding soil-associated community, and thus had a high niche overlap. Likewise, the soil fungal community sampled below the treeline in Chapter 4, showed greater dispersal and species mixing among individual communities (Chapter 5), than those sampled at or above the treeline. In both cases, the community patterns exhibited by the communities being compared were not vastly different from one another, however the processes governing the patterns were different. Niche and neutral mechanisms can generate similar community patterns if the mechanisms that are active contribute to both the spatial and environmental components of variation within the community (Smith and

Lundholm 2010). This work supports the point that community patterns do not equate to processes, and to gain a thorough understanding of the role of fungal communities in ecosystem functioning, a mechanistic perspective is essential.

6.4. Ecologically relevant scales of pattern and process

It is evident from the results of this thesis that the factors that are important in fungal community patterns vary depending on the scale of observation. The importance of scale in studying spatial ecological dynamics has been previously emphasised, as the influence of factors on community assembly is not necessarily consistent across all scales (Levin 1992). Thus it is necessary to include multiple scales in spatial ecological studies, as was a central aim of this thesis. Many spatial ecological studies are focused at 'local' or 'regional' scales, in order to classify the scale at which patterns and processes are meaningful (e.g. Drenovsky *et al.* 2010; Bahram *et al.* 2012). It is implied that local spatial dynamics are in reference to those influencing individual organisms, whereas regional dynamics are those influencing groups of organisms and the interactions among them (Holyoak *et al.* 2005). Focusing studies at one scale then becomes problematic, however, because processes at different scales can interact, by means of dispersal, in order to generate community patterns. Therefore when designing studies to examine local or regional spatial dynamics, it may be more meaningful to simply consider distance as a continuous variable, and examine the mechanisms driving community patterns at a range of separation distances, and thus across multiple scales, as was achieved across Chapters 2, 3 and 4.

Multiple scales can also refer not only to distances, but also to levels of community organisation. By studying fungal community dynamics across individual communities

(e.g. Chapter 4) as well as their metacommunities (Chapter 5), the role of dispersal was made explicit, which shows that differences in the characteristics of the metacommunity influence the processes by which local communities are structured, or vice versa. This is further evidence to support the fact that processes do not act at the same scale at which patterns are evident. A major setback to the progress of understanding the implications of environmental change on community dynamics of organisms, especially fungi and other microbes, and their role in ecosystem functioning, is that assembly processes are likely occurring at much finer scales than the scale of their role in environmental change. Thus there is a need to scale up our knowledge of the patterns and processes of community assembly. The role of dispersal is key in this, as it effectively links community dynamics operating at various scales (Chave 2013). In order to press forward in understanding this area, further attention needs to be focused on the explicit role of dispersal, and its implications for metacommunity dynamics, in cryptic systems, including below-ground fungal communities.

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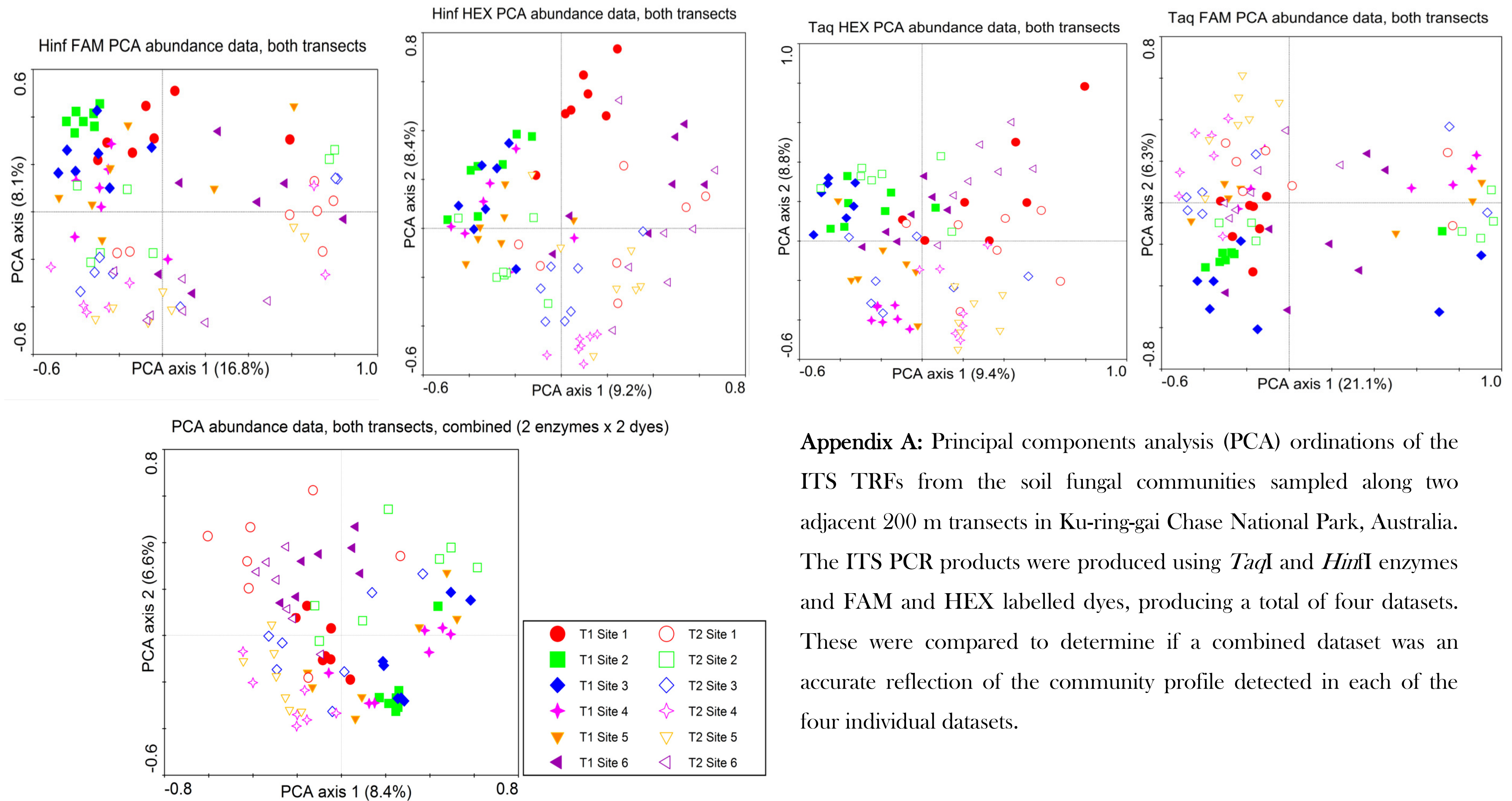
Appendices



Appendix A: Principal components analysis (PCA) ordinations of the ITS TRFs from the soil fungal communities sampled along two adjacent 200 m transects in Ku-ring-gai Chase National Park, Australia. The ITS PCR products were produced using *Taq*I and *Hin*fI enzymes and FAM and HEX labelled dyes, producing a total of four datasets. These were compared to determine if a combined dataset was an accurate reflection of the community profile detected in each of the four individual datasets.

Appendix B: Mean number of OTUs (± standard error), calculated as the mean of the HEX labelled ITS TRFs, for each of the eight spatially separated study sites along coastal NSW, Australia.

Site	Mean number of OTUs in the root communities (± SE)	Mean number of OTUs in the soil communities (± SE)
1	30.1 ± 4.11	40.0 ± 2.21
2	30.7 ± 3.19	41.6 ± 2.83
3	29.0 ± 2.76	38.2 ± 5.90
4	34.3 ± 3.58	34.7 ± 3.14
5	27.7 ± 3.46	31.7 ± 3.04
6	30.3 ± 4.84	24.4 ± 4.90
7	26.8 ± 2.96	31.7 ± 2.49
8	31.8 ± 4.14	34.8 ± 5.55
Total	1203.5 ± 0.195	1385.5 ± 0.241

Appendix C: Summary table of the measured edaphic variables collected from eight spatially separated study sites along coastal NSW, Australia.

		pH	N (%)	C (%)	C:N ratio
Site 1	mean	4.562	0.223	1.260	6.115
	min	4.280	0.157	0.933	3.576
	max	4.820	0.267	1.694	9.049
	st dev	0.234	0.048	0.298	2.687
Site 2	mean	4.812	0.156	0.920	6.600
	min	4.570	0.081	0.582	4.184
	max	5.100	0.245	1.487	12.559
	st dev	0.257	0.064	0.357	3.556
Site 3	mean	5.892	0.048	0.745	16.727
	min	4.700	0.025	0.508	11.034
	max	6.830	0.079	0.913	20.079
	st dev	0.784	0.021	0.189	3.422
Site 4	mean	6.042	0.034	0.527	19.464
	min	5.780	0.014	0.360	8.710
	max	6.280	0.061	0.636	39.879
	st dev	0.204	0.018	0.102	11.881
Site 5	mean	4.546	0.740	0.752	1.226
	min	4.480	0.407	0.642	0.660
	max	4.590	0.972	0.878	2.095
	st dev	0.052	0.278	0.107	0.700
Site 6	mean	4.960	0.047	0.581	21.230
	min	4.820	0.012	0.380	3.528
	max	5.090	0.108	0.874	41.639
	st dev	0.102	0.038	0.184	15.191
Site 7	mean	7.364	0.016	0.352	69.043
	min	7.090	0.002	0.256	10.737
	max	7.960	0.040	0.425	223.813
	st dev	0.354	0.015	0.072	89.963

Site 8	mean	6.354	0.018	0.403	36.315
	min	5.990	0.006	0.142	12.083
	max	6.610	0.054	0.861	73.682
	st dev	0.248	0.020	0.294	29.322
Total	mean	5.567	0.160	0.692	22.090
	min	4.280	0.002	0.142	0.660
	max	7.960	0.972	1.487	223.813
	st dev	0.995	0.261	0.279	38.590

Appendix D: Mean number of OTUs (± standard error), calculated as the mean number of total ITS TRFs from the soil fungal communities sampled along two adjacent 200 m transects in Ku-ring-gai Chase National Park, Australia

	Transect 1	Transect 2
Site 1	25.93 ± 0.501	24.32 ± 0.613
Site 2	26.07 ± 0.657	19.71 ± 0.416
Site 3	19.93 ± 0.481	21.25 ± 0.329
Site 4	22.96 ± 0.384	17.86 ± 0.515
Site 5	22.964 ± 0.513	21.46 ± 0.883
Site 6	24.29 ± 0.536	22.71 ± 0.524
Total	23.85 ± 0.095	21.22 ± 0.102

Appendix E: Summary table of the measured edaphic variables collected across two adjacent transects from Ku-ring-gai Chase National Park, NSW, Australia.

Transect 1		soil temperature (°C)	soil moisture (% VWC)	C (%)	N (%)	C:N ratio	pH	elevation (m)	root biomass (g)
Site 1	mean	20.89	8.57	1.66	0.06	27.75	4.90	198.00	2.84
	min	20.47	7.00	0.98	0.04	16.04	4.55	198.00	1.82
	max	21.39	11.00	2.85	0.08	35.61	5.27	198.00	4.31
	st dev	0.31	1.27	0.74	0.02	6.00	0.22	0.00	0.83
Site 2	mean	21.02	19.86	1.94	0.06	30.40	4.70	197.00	2.15
	min	20.96	17.00	1.40	0.05	28.71	4.60	197.00	1.14
	max	21.10	22.00	2.56	0.08	31.78	4.83	197.00	3.35
	st dev	0.06	1.68	0.46	0.01	1.23	0.08	0.00	0.66
Site 3	mean	20.66	34.29	1.41	0.06	24.47	4.77	195.00	3.61
	min	20.21	32.00	1.16	0.05	16.59	4.56	195.00	0.72
	max	21.19	39.00	1.92	0.09	30.02	4.89	195.00	10.60
	st dev	0.37	2.36	0.27	0.02	4.05	0.11	0.00	3.80
Site 4	mean	20.43	18.57	2.29	0.07	31.41	4.24	194.00	1.68
	min	20.14	15.00	1.65	0.06	27.61	3.92	194.00	0.44
	max	20.82	22.00	3.20	0.10	33.83	4.84	194.00	4.04
	st dev	0.26	2.44	0.67	0.02	2.24	0.31	0.00	1.28
Site 5	mean	19.44	31.00	1.76	0.07	25.88	4.60	194.00	2.68
	min	19.17	28.00	1.17	0.05	19.68	4.39	194.00	1.00
	max	19.88	33.00	2.59	0.08	30.91	4.94	194.00	6.45
	st dev	0.22	2.16	0.57	0.01	3.97	0.21	0.00	1.77
Site 6	mean	19.87	10.14	1.93	0.09	20.56	5.09	194.14	2.12
	min	19.56	6.00	0.90	0.05	14.19	4.82	194.00	0.25

	max	20.60	14.00	3.57	0.18	27.21	5.39	195.00	9.08
	st dev	0.36	2.79	0.94	0.04	4.51	0.18	0.38	3.29
T1 total	mean	20.38	20.40	1.83	0.07	26.75	4.72	195.36	2.51
	min	19.17	6.00	0.90	0.04	14.19	3.92	194.00	0.25
	max	21.39	39.00	3.57	0.18	35.61	5.39	198.00	10.60
	st dev	0.63	9.94	0.66	0.02	5.26	0.33	1.61	2.23

Transect 2		soil temperature (°C)	soil moisture (% VWC)	C (%)	N (%)	C:N ratio	pH	elevation (m)	root biomass (g)
Site 1	mean	19.65	11.14	1.49	0.08	17.89	4.98	197.71	1.14
	min	19.50	10.00	1.00	0.06	14.23	4.83	197.00	0.32
	max	19.76	12.00	2.49	0.11	22.77	5.17	198.00	3.73
	st dev	0.08	0.90	0.53	0.02	3.71	0.11	0.49	1.17
Site 2	mean	19.51	40.14	1.73	0.08	22.58	4.88	194.29	3.50
	min	19.42	18.00	1.47	0.06	19.89	4.82	194.00	1.73
	max	19.67	49.00	2.49	0.09	26.68	4.96	195.00	10.90
	st dev	0.09	10.19	0.35	0.01	2.28	0.05	0.49	3.29
Site 3	mean	19.76	15.57	1.48	0.05	29.52	4.84	191.00	1.53
	min	19.56	13.00	1.07	0.04	21.93	4.64	191.00	0.93
	max	19.97	21.00	2.67	0.06	47.30	4.94	191.00	2.24
	st dev	0.17	3.26	0.55	0.01	9.36	0.10	0.00	0.48
Site 4	mean	19.56	14.29	2.70	0.07	38.44	4.62	191.00	1.75
	min	19.30	11.00	2.06	0.05	32.07	4.45	191.00	0.84
	max	19.97	18.00	3.70	0.12	46.65	4.74	191.00	4.33
	st dev	0.25	2.50	0.66	0.02	5.34	0.12	0.00	1.20
Site 5	mean	19.48	11.43	2.40	0.06	38.98	4.49	192.43	1.29
	min	19.40	11.00	1.83	0.04	32.49	4.39	192.00	0.92
	max	19.56	12.00	3.67	0.10	50.73	4.58	193.00	1.84
	st dev	0.05	0.53	0.63	0.02	6.05	0.07	0.53	0.28
Site 6	mean	19.82	10.57	1.10	0.03	39.63	5.22	193.00	1.31
	min	19.57	9.00	0.83	0.02	27.34	4.96	193.00	0.47
	max	19.96	12.00	1.47	0.04	54.11	5.40	193.00	2.45
	st dev	0.16	1.13	0.25	0.01	9.27	0.18	0.00	0.92
T2 total	mean	19.63	17.19	1.82	0.06	31.17	4.84	193.24	1.75
	min	19.30	9.00	0.83	0.02	14.23	4.39	191.00	0.32
	max	19.97	49.00	3.70	0.12	54.11	5.40	198.00	10.90
	st dev	0.19	11.65	0.75	0.02	10.31	0.27	2.02	1.72
Total	mean	20.01	18.80	1.82	0.07	28.96	4.78	194.30	2.13
	min	19.17	6.00	0.83	0.02	14.19	3.92	191.00	0.25
	max	21.39	49.00	3.70	0.18	54.11	5.40	198.00	10.90
	st dev	0.60	10.73	0.70	0.02	8.61	0.30	2.27	2.00

Appendix F: Summary table of the vegetation survey conducted along two adjacent 200 m transects in Ku-ring-gai Chase National Park, Australia. Plants were identified to species level, or given a consistent label based on their description if identification was uncertain.

Scientific name (or description)	Total count scores - Transect 1					
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
? pea	0	10	0	0	0	0
?Pultenaea	0	0	6	0	0	0
?red unknown	0	1	0	0	0	0
Acacia longifolia	0	0	0	0	0	1
Acacia myrtifolia	8	0	0	0	0	1
Acacia suaveolens	2	2	2	2	0	0
Actinotus minor	46	11	6	9	2	0
Allocasuarina littoralis	5	3	0	2	2	1
Angophora hispida	0	0	0	1	3	1
Aristida warburgii	0	0	0	0	0	2
Baeckea brevifolia	0	0	2	0	0	0
Banksia ericifolia	95	54	118	30	12	10
Banksia serrata	6	0	0	1	0	0
Bossiaea ensata	1	0	0	0	0	0
Bossiaea heterophylla	1	0	0	0	0	0
Bossiaea scolopendria	1	0	2	0	5	3
Brachyloma daphnoides	0	0	0	0	17	0
Calytrix tetragona	0	30	0	0	0	0
Cassytha sp.	0	0	0	0	0	1
Caustis flexuosa	0	3	0	21	0	3
Ceratopetalum gummiferum	0	0	0	0	0	1
Conospermum ericifolium	1	0	0	0	2	0
Cymbopogon refractus	0	0	0	0	1	2
Dampiera stricta	1	1	1	1	1	1

Dianella caerulea	1	1	1	2	0	1
Dichondra repens	0	0	0	0	0	2
Dillwynia retorta	21	3	0	2	0	1
Drosera peltata	0	0	0	0	1	0
Entolasia marginata	0	0	1	0	0	5
Epacris microphylla	0	2	51	0	0	0
Epacris pulchella	0	10	0	19	12	2
Eragrostis curvula	0	0	0	0	0	3
Eucalyptus sp. seedling	1	1	0	0	0	1
Gompholobium grandiflorum	18	2	0	2	0	1
Goodenia bellidifolia	1	0	0	0	0	0
Grevillea buxifolia	7	15	6	8	1	0
Grevillea sericea	17	39	48	24	14	9
Hakea sericea	0	3	5	0	10	0
Hemigenea purpurea	1	1	0	1	1	0
Hibbertia aspera	2	1	20	8	0	0
Hibbertia bracteata	2	0	0	0	0	0
Hovea linearis	0	0	0	0	0	1
Hypolaena fastigata	0	0	0	1	0	0
Joycea pallida	0	0	0	0	0	1
Kunzea ambigua	1	15	8	0	0	5
Kunzea capitata	0	2	6	0	2	0
Lambertia formosa	5	0	0	0	5	0
Lepidosperma urophorum	0	0	3	0	0	0
Leptocarpus tenax	0	0	1	0	0	0
Leptospermum squarrosum	0	0	0	0	4	0
Leptospermum trinervium	4	5	4	6	6	0
Lepyrodia scariosa	0	6	20	0	4	0
Leucopogon microphyllus	39	38	21	6	9	1
Lindsaea linearis	0	0	0	0	1	0
Lomandra glauca	5	2	9	0	1	3
Lomandra obliqua	0	0	0	3	0	0
Micrantheum ericoides	1	0	0	1	0	0

Oxalis perennans	0	0	0	0	1	0
Patersonia longifolia	3	11	5	7	7	4
Persoonia levis	0	0	0	0	2	0
Persoonia pinifolia	0	0	0	0	1	1
Petrophile pulchella	62	25	35	40	30	0
Phyllanthus hirtellus	0	0	0	0	0	6
Phyllota phylicoides	0	0	0	0	0	1
Platysace linearifolia	0	1	0	0	0	0
Pultenaea ferruginea	0	0	5	0	0	7
Scaevola ramosissima	0	4	0	0	0	2
Schoenus ericetorum	0	0	0	3	0	0
Stylidium graminifolium	1	2	0	1	0	0
Tetratheca ericifolia	0	1	0	0	0	0
Woollsia pungens	3	74	61	36	0	0
Xanthorrhoaea media	1	0	1	3	5	0
Xyris bracteata	0	4	7	0	2	0
Total plant abundance	363	383	455	240	164	84

Appendix F cont.

Scientific name (or description)	Total count scores - Transect 2					
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
? small tufts	0	4	8	0	0	0
Acacia myrtifolia	10	0	0	0	1	0
Acacia suaveolens	2	1	0	0	0	2
Actinotus minor	99	54	31	74	38	45
Allocasuarina littoralis	1	2	192	2	0	12
Angophora hispida	0	0	0	1	0	0
Aotus ericoides	6	2	0	0	0	0
Baeckea brevifolia	0	37	5	0	0	0
Banksia ericifolia	14	48	37	29	11	9
Banksia oblongifolia	0	23	0	0	0	0
Banksia serrata	22	0	3	4	1	1
Banksia spinulosa	0	0	0	5	0	1
Boronia pinnata	0	0	0	0	5	0
Bossiaea ensata	5	0	11	0	2	0
Bossiaea heterophylla	4	0	0	4	0	0
Bossiaea scolopendria	0	0	0	21	1	0
Burdiardia umbrellata	0	2	0	0	0	0
Calochilus paludosus	1	0	0	0	0	0
Cassytha glabella	35	11	7	3	0	0
Caustis flexuosa	7	2	0	0	44	0
Caustis pentandra	8	0	0	0	0	0
Ceratopetalum gummiferum	0	0	0	1	0	0
Comesperma ericinum	0	0	0	11	1	0
Conospermum ericifolium	0	8	0	0	2	0
Conospermum longifolium	2	3	0	0	3	0
Corymbia gummifera	5	0	1	2	2	1
Cymbopogon refractus	2	1	0	2	0	0
Dampiera stricta	0	10	0	1	0	17
Daviesia alata	2	0	0	0	0	0
Dillwynia retorta	1	0	0	14	17	0

Drosera peltata	0	67	0	0	0	0
Entolasia marginata	0	0	0	0	0	1
Epacris microphylla	3	347	1	39	0	1
Epacris pulchella	2	0	6	1	0	4
Eucalyptus seedling	2	0	3	4	4	2
Gompholobium glabratum	0	0	1	1	0	0
Gompholobium grandiflorum	14	0	5	12	13	8
Goodlenia hederacea	0	0	2	0	0	0
Grevillea buxifolia	10	3	0	0	2	4
Grevillea sericea	13	9	6	11	16	12
Hakea dactyloides	0	0	0	0	0	2
Hakea teretifolia	0	43	4	0	0	0
Hemigenea purpurea	15	0	6	2	0	0
Hibbertia bracteata	2	2	0	7	7	0
Hovea linearis	2	0	0	0	2	0
Hypolaena fastigata	0	0	7	6	0	2
Kunzea capitata	0	37	18	0	0	0
Lambertia formosa	0	1	6	2	2	4
Lepidosperma laterale	0	0	0	11	3	4
Lepidosperma urophorum	1	0	0	0	0	0
Leptocarpus tenax	0	230	0	0	0	0
Leptosperma laterale	0	0	4	0	0	0
Leptospermum arachinoides	0	0	2	0	0	0
Leptospermum squarrosum	0	24	0	0	0	0
Leptospermum trinervium	6	0	15	28	5	20
Lepyrodia scariosa	0	7	23	0	0	12
Leucopogon microphyllus	20	10	74	4	4	17
Lindsaea linearis	0	52	0	0	22	0
Lomandra brevis	0	0	5	5	12	20
Lomandra glauca	60	0	5	50	29	10
Lomandra longifolia	4	5	3	2	6	0
Lomandra multiflora	0	0	3	1	0	0
Lomandra obliqua	3	0	0	3	0	1

Lomatia silaifolia	4	0	0	0	0	0
Micrantheum ericoides	9	0	0	0	0	0
Microlaena stipoides	0	0	1	0	0	0
Mitrasacme paludosa	0	1	6	5	0	0
Oxalis perennans	1	0	0	0	0	0
Patersonia longifolia	6	1	1	1	5	5
Persoonia levis	0	0	0	0	1	0
Persoonia pinifolia	5	5	5	8	8	4
Petrophile pulchella	10	65	3	11	0	0
Phyllanthus hirtellus	9	0	0	37	14	10
Pimelea linifolia	0	0	0	1	0	5
Platysace linearifolia	0	0	2	14	24	8
Pultenaea ferruginea	16	12	1	0	27	23
Pultenaea tuberculata	11	0	0	0	3	2
Schoenus apogon	0	0	0	0	0	109
Schoenus ericetorum	2	0	2	0	0	0
Stipa pubescens	6	24	0	0	0	2
Stylidium graminifolium	0	0	0	0	0	1
Tetratheca ericifolia	0	0	0	6	4	1
Tetratheca glandulosa	0	0	0	0	1	0
Woollsia pungens	9	0	0	0	0	0
Xanthorrhoaea media	4	5	1	6	5	10
Xanthosia tridentata	8	0	0	1	28	6
Xyris bracteata	0	1	1	0	0	0
Total plant abundance	483	1159	517	453	375	398

Appendix G: Summary table of the measured edaphic variables collected above, at and below the treeline, across the sampled altitudinal gradient in Kosciuszko National Park, Australia

		soil temp. (°C)	soil moisture (%VWC)	elevation (m)	air temp. (°C)	relative humidity (%)	pH	root biomass (g)	C (%)	N (%)	C:N ratio
Site 1 - AB	mean	5.49	29.27	1967.78	7.73	70.89	4.69	1.68	13.65	0.78	16.96
	min	4.20	10.00	1962.00	6.02	60.00	4.07	0.30	6.98	0.47	13.18
	max	6.81	91.00	1977.00	9.65	82.00	5.10	4.83	35.58	1.58	29.26
	st dev	0.67	18.95	4.14	0.99	4.82	0.22	1.01	6.78	0.24	3.16
Site 2 - TL	mean	5.21	50.66	1892.56	10.80	70.41	4.85	1.29	20.09	0.89	20.42
	min	3.87	8.00	1882.00	7.60	45.00	4.12	0.07	4.41	0.26	12.10
	max	7.40	93.00	1902.00	14.71	88.30	5.44	3.78	52.68	1.92	35.66
	st dev	0.85	26.06	5.64	1.66	9.70	0.30	0.78	15.64	0.50	5.84
Site 3-BL	mean	5.63	18.39	1802.44	11.15	54.88	4.78	0.95	15.42	0.87	17.52
	min	4.40	8.00	1780.00	9.00	41.50	4.33	0.12	9.35	0.57	13.75
	max	7.96	32.00	1817.00	14.76	80.00	5.17	5.70	28.12	1.28	26.24
	st dev	0.75	4.95	9.69	1.51	8.94	0.22	0.97	4.18	0.16	2.45
Total	mean	5.44	32.77	1887.59	9.89	65.39	4.78	1.30	16.39	0.85	18.30
	min	3.87	8.00	1780.00	6.02	41.50	4.07	0.07	4.41	0.26	12.10
	max	7.96	93.00	1977.00	14.76	88.30	5.44	5.70	52.68	1.92	35.66
	st dev	0.78	23.01	68.21	2.09	10.97	0.25	0.97	10.41	0.34	4.33

Appendix H: Summary table of the mean vegetation relative percentage cover scores above, at and below the treeline across the sampled altitudinal gradient in Kosciuszko National Park, Australia. Plants were identified to species level where possible, or given an identifying description. Percentage cover scores were recorded within each 0.25 m x 0.25 m quadrat over each sampling location.

	Mean percentage cover score			
Identification (common species name)	Site 1-AB	Site 2-TL	Site 3-BL	Overall
Astelia psychrocharis (Kosciuszko pineapple grass)	0.00	1.71	0.00	0.57
Astelia alpina var*. novae-hollandiae* (Silver Astelia)	0.00	1.73	0.00	0.58
Baeckea utilis var*. utilis* (Mountain Baeckea)	4.98	6.10	0.00	3.69
Celmisia pugioniformis (Dagger-leaf Celmsia) or *C. costiniana* (Herbfield Celmisia)	9.51	0.00	0.00	3.17
Clemsia tomentella (Bog Celmisia)	0.00	0.49	0.00	0.16
Chionochloa frigida (Ribbon Grass)	0.49	0.49	0.00	0.33
Cyperaceae narrow	0.00	1.95	0.73	0.89
Cyperaceae thin (? woodrush)	0.00	1.95	0.00	0.65
Cyperaceae wide	0.00	0.00	1.46	0.49
dark green/silver elongated leaf, shiny	0.00	0.00	1.95	0.65
Empodisma minus (Spreading Rope-bush)	10.07	8.05	0.00	6.04
Epacris glacialis (Bog Heath)	17.59	0.00	0.00	5.86
Epacris paludosa (Swamp heath))	2.68	5.49	0.00	2.72
Eucalyptus black sally seedling	0.00	0.00	0.98	0.33
Exocarpos nanus (Alpine Ballart)	0.02	0.00	0.00	0.01
Gingidia algens (Kosciuszko Aniseed)	0.00	0.49	0.00	0.16
Goodenia hederaceae subsp. *alpestris* (Ivy Goodneia)	2.46	2.56	1.83	2.28
Grevillea australis (Alpine Grevilea)	7.73	1.56	0.00	3.10
Helichrysum scorpioides (Button Everlasting)	0.00	0.12	0.00	0.04
Hovea montana (Alpine Hovea)	0.00	0.00	2.20	0.73
little fern like	0.15	0.00	0.00	0.05
long daisy like leaf	0.00	0.00	0.73	0.24

Luzula australasica subsp. *dura* (Feldmark Woodrush)	0.49	0.00	0.00	0.16
Moss	0.00	0.24	0.00	0.08
Olearia phlogopappa var. *flavescens* (larger Dusty daisy bush)	0.00	0.00	1.46	0.49
Olearia phlogopappa var. *subrepanda* (Dusty daisy bush)	0.24	3.39	4.39	2.67
Oreomyrrhis eriopoda (Australian Caraway)	0.00	0.61	1.71	0.77
Oxylobium ellipticum (Common shaggy pea)	0.10	0.98	3.54	1.54
Phebalium ovatifolium	0.00	0.00	6.95	2.32
Poaceae	18.78	35.37	55.37	36.50
Prostanthera cuneata (Alpine mint-bush)	1.46	4.44	0.00	1.97
Richea continentis (Candle heath)	0.49	2.32	0.00	0.93
Rytidosperma nivicola (Snow Wallaby-grass)	12.61	1.22	0.00	4.61
Scoenus calyptratus (Alpine Bog Rush)	0.00	0.37	0.00	0.12
Trisetum spicatum subsp. *Australiense* (Bristle grass)	0.00	0.85	0.00	0.28
Uncinia sinclairii (Sinclair's hooked sedge)	0.00	0.24	0.00	0.08
Unknown similar to swamp heath, broader leaf	0.00	0.00	4.63	1.54
Unknown creeper	0.00	0.73	0.00	0.24
Unknown maple shape leaf	0.00	0.00	0.12	0.04

Appendix I: R script for calculating metacommunity and extent, based on Maurer *et al.* (2013). The analysis was performed on relative abundance data for the three study sites outlined in Chapter 4, using geographic, environmental and phylogenetic distance matrices for the calculations

```
library(vegan)

# writing the spatial analysis function

spatial_analyse <- function(abun, dist_data, my.dist,
outprefix) {

  # assigning variables
  S = ncol(abun)
  N = nrow(abun)
  metgeo = matrix(0,N,S)
  k = N
  nd=length(my.dist) # number of distances
  optdist=matrix(0,k,3)

  colnames(optdist) <- c("Similarity", "Distance", "Communities")

  all_scores <- matrix(0, N, nd)
  colnames(all_scores) <- my.dist

  par(mfrow=c(5,4),mar=c(1,1,1,1))

  which <- as.list(numeric(N*nd))
  dim(which) <- c(N,nd)

  whichout <- as.list(numeric(N))
  dim(whichout) <- c(1,N)
```

```
# this loop constructs a set of metacommunities defined as
# all local communities less than distance cd in
# standardized euclidean distance space from focal community i

for (i in 1:k) {
  metco=matrix(0,nd,S+1)
  psim=matrix(0,nd,3)
  metrab=matrix(0,1,S)
  ncom=matrix(0,nd,1)

  # look at each specified distance, l = 1 to nd
  # comparing all the communities less than the current
  # distance from the focal community
  for (l in 1:nd) {
    used <- NULL
    cd=my.dist[l]
    metenv=rep(0,S)
    for (j in 1:N) {
      if (i != j) {
        indexi = min(i,j)
        indexj = max(i,j)
        if (gdistm[indexi,indexj] <= cd) {
          ncom[l]=ncom[l]+1
          metenv = metenv + abun[j,]
          used <- append(used,j)
        }
      }
    }

    if (is.null(used)) {
      used <- c(0)
    }

    which[[i,l]] <- used

    # metco is the meta community at each distance for the
    # current focal community
    metco[l,1]= cd
    metco[l,2:(S+1)]= metenv[1:S]
  }

# psim is metacommunity distance function for distance cd
# psim has a column for every distance
# column 1 is the distances
psim[,1]=metco[,1]
# column 3 is the number of communities that were included
psim[,3]=ncom
# fcom is the abundances for the current focal community
fcom=abun[i,]
# relative abundances for the current focal community
fcomrab=fcom/sum(fcom)
```

```
# for each distance
    for (ik in 1:nd) {
      factor <- sum(metco[ik,2:(S+1)])
      metrab <- metco[ik,2:(S+1)]/factor
      # look at each species
      for (jk in 1:S) {
        # meta community relative abundance for the current
        # species
        psim[ik,2]=psim[ik,2] + min(fcomrab[jk],metrab[jk])
        # if less than 1 (changed from 100 due to sampling
        # design)
        # communities are included then don't use it
        if(psim[ik,3] < 1) {
          psim[ik,2]=0
        }
      }
    }

    plot(psim[,1], psim[,2], main=i, xlab='Distance (m)',
ylab='Similarity',
         type='o')

    sdist=sort(psim[,2],decreasing=TRUE,index.return=TRUE)
    optdist[i,1]=sdist$x[1]
    ind=sdist$ix[1]
    optdist[i,2]=psim[ind,1]
    optdist[i,3]=psim[ind,3]

    all_scores[i,] = t(psim[,2])

    whichout[[i]] <- which[[i,
findInterval(psim[sdist$ix[1],1], my.dist)]]
  }

  # opdist gives the maximum of the metacommunity distance
  # function for each local community
  # optdist contains, for every single community - number of
  # communities included in the metacommunity, actual distance
  # value and the optimum distance function value

  write.table(optdist, file=paste(outprefix, "optdist.txt",
sep=''), row.names=FALSE, sep='\t')
  write.table(all_scores, file=paste(outprefix,
"similarities.txt", sep=''), row.names=FALSE, sep='\t')
  write.table(whichout, file=paste(outprefix, "which.txt",
sep=''), row.names=FALSE, sep='\t')
}
```

```
# abun is the matrix of abundances of S species on N sites.
# abundance data was imported for each of the 3 study
# locations as abun1, abun2, abun3
# dist_data is the distance matrix of geographic,
# environmental or phylogenetic distances
# my.dist is the sequence of distance intervals used to break
# the geographic, environmental or phylogenetic distances into
# subsets

# geographic dataset

# importing geographic data as a distance matrix- the same
# dataset was used for each site
geo<-read.table('gdist_matrix.txt', header=T, sep='\t')
row.names(geo) <- geo[,1]
geo<-geo[,2:ncol(geo)]

# calculating the sequence of distance intervals for
# geographic data using range/4*nd
my.dist <- seq(min(gdist),max(gdist), (max(gdist)-
min(gdist))/(4*41))

# run spatial analysis function for geographic data
spatial_analyse(abun1, geo, my.dist, "site1_geo4_41_")
spatial_analyse(abun2, geo, my.dist, "site2_geo4_41_")
spatial_analyse(abun3, geo, my.dist, "site3_geo4_41_")

# environmental dataset

# import environmental matrix (dist data) for all samples
# Split up into 3 individual sites
env<-read.table("sig_environ_meta.txt", header=T)
env1<-env[1:41,1:ncol(env)]
env2<-env[42:82,1:ncol(env)]
env3<-env[83:123,1:ncol(env)]

# standardise env matrix to zero mean and unit variance
env1.st<-decostand(env1,"standardize")
env2.st<-decostand(env2,"standardize")
env3.st<-decostand(env3,"standardize")

# distance matrix for standardised env matrix
env1.dist<-vegdist(env1.st, method = "euclidean")
env2.dist<-vegdist(env2.st, method = "euclidean")
env3.dist<-vegdist(env3.st, method = "euclidean")

# calculating the sequence of distance intervals for
# environmental data using range/4*nd
my.dist.env1<-
seq(min(env1.dist),max(env1.dist),(max(env1.dist)-
min(env1.dist))/(4*41))
my.dist.env2<-
seq(min(env2.dist),max(env2.dist),(max(env2.dist)-
min(env2.dist))/(4*41))
```

```
my.dist.env3<-
seq(min(env3.dist),max(env3.dist),(max(env3.dist)-
min(env3.dist))/(4*41))

#run spatial analysis function for environmental data
spatial_analyse(abun1, env1.st, my.dist.env1, "site1_env_")
spatial_analyse(abun2, env2.st, my.dist.env2, "site2_env_")
spatial_analyse(abun3, env3.st, my.dist.env3, "site3_env_")

# phylogenetic datasets

# import phylogenetic datasets for all samples
# Split up into 3 individual sites
mpd1<-all.samples.mpd[1:41]
mpd2<-all.samples.mpd[42:82]
mpd3<-all.samples.mpd[83:123]

# create distance matrix for mpd matrix
phyl1.dist<-vegdist(mpd1, method = "euclidean")
unique.phyl1.dist<-c(sort(unique(phyl1.dist)))
phyl2.dist<-vegdist(mpd2, method = "euclidean")
unique.phyl2.dist<-c(sort(unique(phyl2.dist)))
phyl3.dist<-vegdist(mpd3, method = "euclidean")
unique.phyl3.dist<-c(sort(unique(phyl3.dist)))

# calculating the sequence of distance intervals for
phylogenetic data using range/4*nd
my.dist.phyl1<-
seq(min(phyl1.dist),max(phyl1.dist),(max(phyl1.dist)-
min(phyl1.dist))/(4*41))
my.dist.phyl2<-
seq(min(phyl2.dist),max(phyl2.dist),(max(phyl2.dist)-
min(phyl2.dist))/(4*41))
my.dist.phyl3<-
seq(min(phyl3.dist),max(phyl3.dist),(max(phyl3.dist)-
min(phyl3.dist))/(4*41))

# run spatial analysis function for phylogenetic data
spatial_analyse(abun1, mpd1, my.dist.phyl1, "site1_phyl_")
spatial_analyse(abun2, mpd2, my.dist.phyl2, "site2_phyl_")
spatial_analyse(abun3, mpd3, my.dist.phyl3, "site3_phyl_")
```

Appendix J: Metacommunity output data for each focal community, calculated using geographic, environmental and phylogenetic datasets

Table B-1: Metacommunity calculation output for Site 1-AB using geographic distances

	Similarit y	Distance	Number of Communitie s	Communities included in metacommunit y
1	0.29972 7	10	2	2, 7
2	0.26180 6	60.4850 3	12	1, 3, 4, 7, 8, 11, 12, 13, 16, 17, 19, 20
3	0.26547 1	40.4512 9	7	1, 2, 4, 7, 8, 11, 13
4	0.27220 7	45.2593 9	10	3, 5, 6, 9, 10, 11, 14, 15, 17, 18
5	0.36397 2	72.5052 8	19	3, 4, 6, 9, 10, 11, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 25, 26, 30
6	0.26248 7	85.3268 7	21	3, 4, 5, 9, 10, 11, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 29, 30
7	0.34968 1	78.1147 3	21	1, 2, 3, 4, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28
8	0.29121	61.2863 8	16	1, 2, 3, 4, 7, 11, 12, 13, 16, 17, 18, 19, 20, 21, 24, 27
9	0.26067 3	14.8081	3	5, 6, 10
10	0.34286 9	60.4850 3	12	4, 5, 6, 9, 11, 14, 15, 17, 18,

				22, 23, 30
11	0.33923 6	31.6364 4	10	3, 4, 13, 14, 16, 17, 18, 20, 21, 22
12	0.41075 9	41.2526 4	8	1, 2, 7, 8, 13, 16, 19, 27
13	0.34208 5	31.6364 4	11	3, 8, 11, 12, 16, 17, 18, 19, 20, 21, 24
14	0.23401 1	40.4512 9	17	4, 5, 9, 10, 11, 13, 15, 16, 17, 18, 20, 21, 22, 23, 25, 26, 29
15	0.37573 3	61.2863 8	19	4, 5, 6, 9, 10, 11, 14, 16, 17, 18, 21, 22, 23, 25, 26, 29, 30, 34, 35
16	0.35669 8	28.4310 4	11	11, 13, 17, 18, 19, 20, 21, 22, 24, 25, 26
17	0.33061	10	3	16, 18, 21
18	0.41689 2	20.4175 5	6	14, 16, 17, 21, 22, 26
19	0.23154	94.9430 7	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
20	0.29564	45.2593 9	18	11, 12, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31
21	0.36614 2	14.8081	8	16, 17, 18, 20, 22, 24, 25, 26
22	0.33709 4	20.4175 5	7	17, 18, 20, 21, 23, 25, 26

23	0.245231	20.41755	1	22
24	0.358185	28.43104	11	16, 17, 18, 19, 20, 21, 22, 25, 26, 28, 31
25	0.431422	10	3	21, 24, 26
26	0.333784	10	2	22, 25
27	0.282763	122.189	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
28	0.27936	42.85534	19	13, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 29, 31, 32, 33, 36, 37, 38
29	0.237059	22.8216	5	22, 23, 25, 26, 31
30	0.353444	81.32013	28	4, 5, 6, 9, 10, 11, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 28, 29, 31, 34, 35, 38, 39, 40, 41
31	0.237057	22.8216	5	24, 25, 26, 28, 29
32	0.230245	36.44454	6	27, 28, 33, 36, 37, 38
33•0.207538•28.43104•6•27, 28, 32, 36, 37, 38 0.207538	28.43104	6	27, 28, 32, 36, 37, 38	8
34	0.409622	14.8081	3	35, 40, 41
35	0.32425	14.8081	3	34, 40, 41

36	0.142599	42.85534	6	27, 28, 32, 33, 37, 38
37	0.231607	14.8081	3	32, 33, 36
38	0.195731	31.63644	6	28, 31, 32, 33, 36, 37
39	0.294277	22.8216	2	34, 40
40	0.371659	31.63644	5	30, 34, 35, 39, 41
41	0.346045	42.85534	6	29, 30, 34, 35, 39, 40

Table B-2: Metacommunity calculation output for Site 2-TL using geographic distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.170303	10	2	2, 7
2	0.28542	50.06749	8	1, 3, 7, 8, 11, 12, 13, 16
3	0.22858	42.85534	9	1, 2, 4, 7, 8, 11, 12, 13, 16
4	0.140556	50.06749	12	3, 5, 6, 9, 10, 11, 13, 14, 15, 16, 17, 18
5	0.273387	14.8081	3	6, 9, 10
6	0.237058	10	2	5, 10
7	0.208797	114.1755	35	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 36, 37, 38, 39
8	0.305178	10	2	2, 7
9	0.142143	28.43104	6	4, 5, 6, 10, 14, 15
10	0.228883	36.44454	6	4, 5, 6, 9, 14, 15
11	0.18576	51.67019	23	2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26
12	0.241417	45.25939	10	1, 2, 3, 7, 8, 13, 16, 19, 20, 27
13	0.242203	30.03374	9	3, 8, 11, 12, 16, 17, 19, 20, 21
14	0.196912	36.44454	15	4, 5, 9, 10, 11, 15, 16, 17, 18, 20, 21, 22, 23, 25, 26
15	0.197763	61.28638	19	4, 5, 6, 9, 10, 11, 14, 16, 17, 18, 21, 22, 23, 25, 26, 29, 30, 34, 35
16	0.279971	14.8081	4	13, 17, 20, 21
17	0.210718	10	3	16, 18, 21
18	0.195276	64.49178	30	2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 39
19	0.210149	30.03374	8	12, 13, 16, 20, 21, 24, 27, 28
20	0.203451	10	3	16, 21, 24
21	0.2156	54.07424	24	3, 4, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 38, 39
22	0.161671	10	3	18, 21, 26

23	0.152044	22.8216	5	14, 18, 22, 26, 29
24	0.169776	58.88233	26	8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 31, 32, 33, 34, 36, 37, 38, 39
25	0.160764	63.69043	30	3, 4, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 38, 39, 40
26	0.184867	78.11473	39	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
27	0.158946	31.63644	6	19, 28, 32, 33, 36, 37
28	0.171116	22.8216	5	19, 20, 24, 25, 31
29	0.101908	70.10123	30	4, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 33, 34, 35, 37, 38, 39, 40, 41
30	0.144342	108.566	37	3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
31	0.16591	54.07424	27	13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
32	0.19891	10	2	33, 36
33	0.18368	120.5863	39	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41
34	0.243765	120.5863	39	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41
35	0.256133	10	2	34, 41
36	0.20785	100.5525	32	1, 2, 7, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 37, 38, 39, 40, 41
37	0.11308	117.3809	36	1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 39, 40, 41
38	0.042887	70.10123	23	13, 16, 17, 18, 19, 20, 21, 22, 24, 25,

				26, 27, 28, 29, 31, 32, 33, 34, 36, 37, 39, 40, 41
39	0.245228	31.63644	6	29, 31, 34, 35, 40, 41
40	0.184984	51.67019	9	23, 26, 29, 30, 31, 34, 35, 39, 41
41	0.254315	14.8081	3	34, 35, 40

Table B-3: Metacommunity calculation output for Site 3-BL using geographic distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.224531	10	2	2, 7
2	0.234214	70.90258	18	1, 3, 4, 7, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 24, 27
3	0.161611	114.1755	38	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39
4	0.236056	51.67019	14	3, 5, 6, 9, 10, 11, 13, 14, 15, 16, 17, 18, 22, 23
5	0.21874	102.1552	34	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 39, 40, 41
6	0.204579	10	2	5, 10
7	0.271117	14.8081	3	1, 2, 8
8	0.20313	113.3741	37	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39
9	0.11589	64.49178	20	3, 4, 5, 6, 10, 11, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 25, 26, 29, 30
10	0.225359	90.13497	27	3, 4, 5, 6, 8, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 31, 34, 35, 41
11	0.246661	51.67019	23	2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26
12	0.252253	94.94307	31	1, 2, 3, 4, 5, 7, 8, 9, 11, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 36, 37, 38
13	0.201533	40.45129	17	2, 3, 7, 8, 11, 12, 14, 16, 17, 18, 19, 20, 21, 22, 24, 25, 28
14	0.266141	70.10123	30	2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 39
15	0.18777	81.32013	28	3, 4, 5, 6, 9, 10, 11, 13, 14, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 39, 40, 41

16	0.264811	10	2	17, 20
17	0.304981	14.8081	5	16, 18, 20, 21, 22
18	0.227875	85.32687	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
19	0.095851	54.07424	27	1, 2, 3, 7, 8, 11, 12, 13, 14, 16, 17, 18, 20, 21, 22, 24, 25, 26, 27, 28, 29, 31, 32, 33, 36, 37, 38
20	0.27943	58.88233	28	2, 3, 4, 7, 8, 11, 12, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 37, 38, 39
21	0.193985	54.07424	24	3, 4, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 38, 39
22	0.23972	36.44454	15	11, 13, 14, 16, 17, 18, 20, 21, 23, 24, 25, 26, 28, 29, 31
23	0.258684	28.43104	7	14, 15, 18, 22, 26, 29, 30
24	0.232347	41.25264	17	11, 13, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 31, 38
25	0.137033	72.50528	38	2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
26	0.170156	70.90258	35	3, 4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 38, 39, 40, 41
27	0.243081	51.67019	14	12, 13, 16, 19, 20, 24, 25, 28, 31, 32, 33, 36, 37, 38
28	0.235679	81.32013	36	1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
29	0.209346	67.69718	28	9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 31, 33, 34, 35, 37, 38, 39, 40, 41
30	0.259281	40.45129	7	15, 23, 29, 34, 35, 40, 41
31	0.245036	41.25264	15	16, 17, 18, 20, 21, 22, 24, 25, 26, 28, 29, 33, 34, 38, 39
32	0.178959	70.90258	18	12, 13, 16, 17, 19, 20, 21, 24, 25, 26,

				27, 28, 31, 33, 36, 37, 38, 39
33	0.329232	41.25264	8	19, 27, 28, 31, 32, 36, 37, 38
34	0.206345	10	2	35, 40
35	0.22942	10	2	34, 41
36	0.275238	30.03374	5	27, 32, 33, 37, 38
37	0.279666	50.06749	8	24, 27, 28, 31, 32, 33, 36, 38
38	0.252796	41.25264	8	24, 28, 31, 32, 33, 36, 37, 39
39	0.220812	58.88233	16	20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 38, 40, 41
40	0.171471	70.90258	18	15, 18, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 34, 35, 38, 39, 41
41	0.217081	70.90258	14	15, 21, 22, 23, 25, 26, 29, 30, 31, 34, 35, 38, 39, 40

B-4: Metacommunity calculation output for Site 1-AB using environmental distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.340599	1.830728	1	10
2	0.252951	2.335223	6	1, 3, 4, 5, 7, 15
3	0.285712	2.610402	7	2, 4, 8, 10, 15, 29, 31
4	0.295187	2.472813	6	2, 3, 5, 8, 10, 15
5	0.379865	3.206624	17	1, 2, 3, 4, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 25, 26
6	0.255676	5.13288	30	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 30, 31, 34, 38, 39, 40
7	0.362396	3.023172	23	1, 2, 3, 4, 5, 8, 9, 10, 12, 13, 14, 15, 16, 17, 20, 24, 25, 26, 29, 30, 31, 34, 41
8	0.261331	4.857701	33	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 23, 24, 25, 26, 28, 29, 30, 31, 33, 34, 35, 38, 39, 40, 41
9	0.267333	3.069035	9	1, 2, 5, 7, 10, 12, 19, 26, 34
10	0.371932	1.830728	2	1, 5
11	0.332422	2.472813	10	5, 13, 14, 16, 17, 18, 21, 22, 25, 33
12	0.389642	1.784864	6	17, 19, 24, 25, 26, 34
13	0.302021	3.023172	19	7, 11, 12, 14, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 30, 33, 35, 38
14	0.247745	2.564539	13	1, 2, 5, 10, 11, 12, 13, 16, 17, 19, 21, 25, 26
15	0.355583	1.784864	4	2, 4, 7, 16
16	0.422336	1.509685	1	25
17	0.318599	3.114898	27	1, 2, 4, 5, 7, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 30, 33, 34, 38, 39
18	0.414167	2.243497	7	11, 16, 21, 22, 25, 28, 33
19	0.27287	2.28936	7	12, 14, 17, 20, 24, 26, 34
20	0.3125	3.940436	32	1, 2, 5, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35,

				38, 39, 41
21	0.381469	1.784864	4	17, 18, 25, 33
22	0.326973	1.509685	1	27
23	0.229261	5.178743	36	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 38, 39, 40, 41
24	0.34929	3.298351	21	1, 7, 9, 12, 13, 14, 16, 17, 19, 20, 21, 22, 25, 26, 27, 30, 34, 35, 38, 39, 41
25	0.407595	2.42695	17	7, 11, 12, 13, 14, 16, 17, 18, 20, 21, 22, 24, 26, 30, 33, 35, 38
26	0.318798	1.647275	6	12, 20, 24, 25, 30, 34
27	0.326973	1.509685	1	22
28	0.295637	2.42695	4	17, 18, 21, 33
29	0.241486	3.986299	24	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 14, 15, 16, 17, 20, 23, 24, 25, 26, 30, 31, 34, 39, 41
30	0.398121	2.060044	9	7, 15, 16, 20, 21, 24, 25, 26, 38
31	0.238693	2.381086	5	2, 3, 23, 29, 41
32	0.223434	4.857701	5	18, 28, 33, 36, 37
33	0.145625	2.42695	9	11, 16, 17, 18, 21, 22, 25, 28, 38
34	0.341278	1.784864	4	12, 24, 26, 39
35	0.247956	1.693138	1	16
36	0.142599	3.71112	3	28, 32, 37
37	0.202725	4.674248	5	18, 28, 32, 33, 36
38	0.168937	1.876591	4	20, 25, 30, 33
39	0.31335	1.784864	1	34
40	0.325402	4.903564	26	1, 3, 6, 7, 8, 11, 12, 13, 15, 16, 17, 20, 22, 23, 24, 25, 26, 29, 30, 31, 33, 34, 35, 38, 39, 41
41	0.33707	3.252488	17	1, 2, 3, 7, 8, 12, 15, 16, 20, 24, 25, 26, 29, 30, 31, 34, 39

B-5: Metacommunity calculation output for Site 2-TL using environmental distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.160084	1.732023	4	2, 6, 7, 14
2	0.216838	4.621088	38	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
3	0.257946	2.772086	3	4, 13, 16
4	0.183473	2.579482	3	3, 13, 16
5	0.255221	1.423856	3	6, 7, 9
6	0.242507	1.192731	1	5
7	0.272479	1.269773	2	10, 34
8	0.312441	1.500898	3	34, 39, 41
9	0.129232	1.847586	7	1, 2, 5, 6, 7, 10, 14
10	0.26158	1.269773	1	7
11	0.214869	2.348357	7	1, 2, 10, 12, 18, 19, 20
12	0.207084	2.810607	6	1, 2, 10, 11, 20, 22
13	0.300816	2.810607	5	3, 4, 16, 17, 21
14	0.227892	2.001669	11	1, 2, 5, 6, 7, 9, 10, 23, 26, 34, 35
15	0.196351	4.929255	33	1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 14, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41
16	0.287313	3.38842	9	3, 4, 11, 12, 13, 17, 20, 28, 30
17	0.249591	3.465462	10	3, 4, 11, 13, 16, 20, 21, 28, 30, 37
18	0.208175	3.426941	20	1, 2, 5, 6, 7, 9, 10, 11, 12, 14, 15, 19, 22, 23, 24, 25, 26, 27, 34, 35
19	0.20052	3.157295	22	1, 2, 7, 8, 11, 12, 14, 18, 20, 21, 23, 24, 25, 26, 27, 33, 34, 35, 36, 37, 39, 41
20	0.222311	3.542504	17	1, 2, 3, 4, 11, 12, 13, 16, 17, 19, 21, 25, 28, 30, 32, 33, 37
21	0.235936	3.889191	17	1, 2, 3, 4, 11, 12, 13, 16, 17, 19, 20, 30, 32, 33, 37, 38, 39
22	0.14268	3.658066	11	1, 7, 10, 11, 12, 14, 18, 23, 24, 25, 26

23	0.189374	1.462377	2	14, 26
24	0.175834	4.967776	32	1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 30, 33, 34, 35, 36, 37, 39, 40, 41
25	0.161872	4.852213	32	1, 2, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 30, 33, 34, 35, 36, 37, 39
26	0.197741	4.158837	28	1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 18, 19, 22, 23, 24, 25, 27, 31, 33, 34, 35, 36, 37, 39, 40, 41
27	0.184448	2.772086	13	1, 2, 7, 8, 19, 23, 26, 33, 34, 36, 39, 40, 41
28	0.165433	3.619545	7	3, 4, 12, 13, 16, 17, 20
29	0.116486	3.735108	8	8, 27, 31, 32, 38, 39, 40, 41
30	0.148957	3.619545	15	1, 2, 3, 4, 6, 11, 13, 16, 17, 19, 20, 21, 25, 33, 37
31	0.247275	2.232794	4	8, 39, 40, 41
32	0.189715	3.889191	16	8, 11, 12, 13, 17, 19, 20, 21, 27, 29, 31, 33, 36, 38, 39, 41
33	0.195277	4.197358	33	1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 14, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41
34	0.302453	1.500898	2	7:08
35	0.237059	2.04019	4	2, 7, 14, 34
36	0.246398	2.540961	7	8, 19, 23, 27, 33, 34, 41
37	0.109674	5.430026	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 39, 40, 41
38	0.047412	3.927712	15	8, 13, 17, 19, 20, 21, 27, 29, 31, 32, 33, 36, 39, 40, 41
39	0.241142	2.50244	8	7, 8, 15, 27, 31, 34, 40, 41
40	0.192552	2.772086	15	1, 2, 5, 6, 7, 8, 9, 10, 14, 23, 27, 31, 34, 39, 41
41	0.251849	2.425399	7	7, 8, 27, 31, 34, 39, 40

B-6: Metacommunity calculation output for Site 3-BL using environmental distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.203583	3.911166	19	2, 3, 5, 7, 9, 11, 12, 13, 15, 20, 25, 30, 31, 32, 34, 36, 38, 39, 40
2	0.273483	0.939568	1	20
3	0.16959	5.124917	25	1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 20, 23, 25, 26, 27, 30, 32, 33, 34, 35, 39, 40, 41
4	0.227415	4.706382	37	1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
5	0.243008	2.237026	6	4, 6, 8, 9, 15, 16
6	0.22232	2.822975	7	5, 7, 8, 9, 13, 16, 35
7	0.263164	2.906682	11	2, 5, 6, 8, 13, 27, 32, 34, 39, 40, 41
8	0.204005	4.245994	26	2, 4, 5, 6, 7, 9, 10, 13, 15, 16, 19, 23, 24, 27, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
9	0.102394	0.772154	1	5
10	0.224547	3.701898	18	2, 5, 6, 9, 15, 16, 19, 20, 22, 25, 27, 28, 30, 33, 34, 35, 38, 41
11	0.243606	4.413408	22	1, 2, 7, 12, 13, 14, 15, 17, 18, 20, 25, 27, 30, 32, 33, 34, 35, 36, 38, 39, 40, 41
12	0.241109	5.794573	39	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
13	0.220148	0.897715	1	7
14	0.281138	5.04121	25	1, 2, 9, 10, 11, 15, 16, 17, 18, 19, 20, 22, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38
15	0.190853	3.408924	28	1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 16, 20, 22, 23, 25, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 39, 40, 41
16	0.257672	5.292331	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40,

				41
17	0.266022	3.953019	14	11, 14, 16, 18, 20, 22, 24, 29, 31, 32, 33, 36, 37, 38
18	0.259237	3.074096	5	14, 17, 33, 36, 37
19	0.134305	3.743752	11	5, 6, 8, 9, 10, 16, 27, 29, 33, 35, 38
20	0.285505	1.902198	5	2, 25, 30, 36, 38
21	0.276999	3.074096	2	23, 41
22	0.288121	3.24151	7	2, 14, 16, 20, 28, 36, 38
23	0.25561	3.701898	9	4, 15, 16, 21, 28, 33, 39, 40, 41
24	0.236212	5.417891	32	1, 2, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38
25	0.150707	3.618191	23	1, 2, 5, 7, 9, 10, 11, 12, 13, 15, 16, 20, 27, 28, 30, 32, 33, 34, 36, 38, 39, 40, 41
26	0.197573	4.036726	7	3, 7, 21, 27, 39, 40, 41
27	0.230582	3.743752	23	2, 6, 7, 8, 10, 11, 12, 13, 15, 16, 18, 19, 20, 25, 26, 32, 33, 34, 35, 36, 38, 40, 41
28	0.263205	2.864829	3	16, 22, 23
29	0.2227	4.120433	25	2, 4, 5, 8, 9, 10, 13, 14, 15, 16, 17, 19, 20, 22, 24, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38
30	0.185823	5.87828	39	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
31	0.238647	4.162287	29	1, 2, 4, 5, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 20, 22, 23, 24, 25, 28, 29, 30, 32, 33, 34, 36, 37, 38, 39
32	0.198453	2.655561	11	2, 7, 13, 20, 25, 33, 34, 36, 37, 38, 39
33	0.247582	3.869312	34	2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 28, 29, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41
34	0.160876	4.580822	35	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 25, 27, 28, 29, 30, 31, 32, 33, 35, 36,

				37, 38, 39, 40, 41
35	0.147956	5.124917	39	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41
36	0.284664	1.190689	1	38
37	0.274715	3.11595	8	13, 18, 29, 31, 32, 34, 36, 38
38	0.284664	1.190689	1	36
39	0.21439	4.20414	30	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 16, 20, 21, 23, 25, 26, 27, 28, 30, 31, 32, 33, 34, 36, 37, 38, 40, 41
40	0.176222	2.571854	5	7, 13, 26, 39, 41
41	0.215492	3.199657	13	6, 7, 13, 15, 16, 21, 23, 25, 26, 27, 33, 39, 40

B-7: Metacommunity calculation output for Site 1-AB using phylogenetic distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.27369	0.031099	9	4, 8, 10, 11, 13, 17, 18, 21, 28
2	0.232818	0.062001	36	1, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 39, 40, 41
3	0.224249	0.011104	10	2, 5, 16, 20, 22, 26, 27, 29, 31, 35
4	0.282698	0.024737	8	1, 8, 11, 13, 17, 18, 21, 28
5	0.362851	0.00656	6	2, 16, 22, 23, 26, 29
6	0.258309	0.07109	25	2, 3, 5, 7, 9, 12, 15, 16, 19, 20, 22, 23, 24, 26, 27, 29, 30, 31, 34, 35, 36, 37, 39, 40, 41
7	0.355434	0.020193	18	5, 10, 11, 12, 14, 15, 16, 17, 22, 23, 24, 25, 26, 29, 30, 32, 36, 40
8	0.383104	0.016557	5	4, 13, 18, 21, 28
9	0.276372	0.032917	21	2, 3, 5, 6, 15, 16, 19, 20, 22, 23, 26, 27, 29, 30, 31, 34, 35, 37, 39, 40, 41
10	0.341459	0.025646	19	2, 5, 7, 11, 12, 14, 15, 16, 17, 22, 23, 24, 25, 26, 29, 30, 32, 36, 40
11	0.319405	0.03019	18	1, 4, 7, 10, 12, 13, 14, 15, 17, 21, 23, 24, 25, 28, 30, 32, 36, 40
12	0.373437	0.022011	19	2, 5, 7, 10, 11, 14, 15, 16, 17, 22, 23, 24, 25, 26, 29, 30, 32, 36, 40
13	0.339916	0.028373	8	1, 4, 8, 11, 17, 18, 21, 28
14	0.227018	0.023828	19	2, 5, 7, 10, 11, 12, 15, 16, 17, 22, 23, 24, 25, 26, 29, 30, 32, 36, 40
15	0.34374	0.07927	39	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41
16	0.297404	0.032008	27	2, 3, 5, 7, 9, 10, 12, 14, 15, 17, 20, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 34, 35, 36, 37, 40, 41
17	0.309152	0.032917	24	1, 2, 4, 5, 7, 10, 11, 12, 13, 14, 15, 16, 21, 22, 23, 24, 25, 26, 28, 29, 30, 32, 36, 40

18	0.377836	0.01474	3	8, 13, 21
19	0.258055	0.049277	17	2, 3, 5, 6, 9, 16, 20, 22, 26, 27, 29, 31, 34, 35, 37, 39, 41
20	0.329155	0.021102	15	2, 3, 5, 9, 16, 22, 23, 26, 27, 29, 30, 31, 34, 35, 37
21	0.38147	0.03019	8	1, 4, 8, 11, 13, 17, 18, 28
22	0.332424	0.002015	3	2, 16, 26
23	0.228463	0.08745	39	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41
24	0.344756	0.019284	19	2, 5, 7, 10, 11, 12, 14, 15, 16, 17, 22, 23, 25, 26, 29, 30, 32, 36, 40
25	0.396455	0.023828	18	5, 7, 10, 11, 12, 14, 15, 16, 17, 22, 23, 24, 26, 29, 30, 32, 36, 40
26	0.344411	0.003833	5	2, 5, 16, 22, 29
27	0.288826	0.047459	31	2, 3, 5, 6, 7, 9, 10, 11, 12, 14, 15, 16, 17, 19, 20, 22, 23, 24, 25, 26, 29, 30, 31, 32, 34, 35, 36, 37, 39, 40, 41
28	0.300715	0.032917	11	1, 4, 8, 10, 11, 13, 17, 18, 21, 25, 32
29	0.253103	0.010195	9	2, 3, 5, 16, 22, 23, 26, 30, 31
30	0.35831	0.012013	14	5, 7, 12, 14, 15, 16, 22, 23, 24, 25, 26, 29, 36, 40
31	0.214351	0.009286	9	2, 3, 16, 20, 22, 26, 27, 29, 35
32	0.136921	0.075634	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41
33	0.212534	0.005651	1	38
34	0.318798	0.007469	1	41
35	0.207952	0.027464	22	2, 3, 5, 7, 9, 12, 15, 16, 20, 22, 23, 24, 26, 27, 29, 30, 31, 34, 36, 37, 40, 41
36	0.106268	0.005651	8	7, 12, 14, 15, 24, 25, 32, 40
37	0.132834	0.117442	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,

				31, 32, 33, 34, 35, 36, 38, 39, 40, 41
38	0.212534	0.005651	1	33
39	0.258583	0.055639	20	2, 3, 5, 6, 9, 16, 19, 20, 22, 23, 26, 27, 29, 30, 31, 34, 35, 37, 40, 41
40	0.317892	0.00656	6	7, 12, 15, 24, 30, 36
41	0.333403	0.053821	25	2, 3, 5, 6, 7, 9, 12, 15, 16, 19, 20, 22, 23, 24, 26, 27, 29, 30, 31, 34, 35, 36, 37, 39, 40

B-8: Metacommunity calculation output for Site 2-TL using phylogenetic distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.135023	0.0602	38	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41
2	0.240535	0.033751	29	1, 3, 4, 7, 8, 11, 12, 13, 14, 15, 16, 18, 19, 21, 22, 23, 25, 26, 27, 28, 29, 31, 32, 34, 35, 36, 39, 40, 41
3	0.176723	0.036487	21	2, 4, 7, 8, 11, 12, 13, 14, 18, 19, 22, 23, 25, 26, 27, 28, 34, 35, 36, 38, 41
4	0.140327	0.010037	10	8, 11, 12, 13, 14, 18, 22, 28, 35, 41
5	0.247955	0.014598	5	6, 9, 10, 30, 33
6	0.165124	0.009125	5	5, 17, 30, 33, 37
7	0.207357	0.083914	40	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
8	0.268846	0.016422	21	1, 2, 4, 11, 12, 13, 14, 15, 18, 19, 21, 22, 28, 29, 31, 32, 35, 36, 39, 40, 41
9	0.125729	0.018246	7	5, 6, 10, 17, 30, 33, 37
10	0.224796	0.014598	2	5, 9
11	0.177188	0.041959	36	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41
12	0.2005	0.018246	24	1, 2, 4, 8, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 28, 29, 31, 32, 35, 36, 39, 40, 41
13	0.161011	0.014598	22	1, 4, 8, 11, 12, 14, 15, 16, 17, 18, 20, 21, 22, 28, 29, 31, 32, 35, 37, 39, 40, 41
14	0.191668	0.050168	38	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41
15	0.214685	0.010037	19	1, 11, 12, 13, 14, 16, 17, 18, 20,

				21, 22, 28, 29, 31, 32, 37, 39, 40, 41
16	0.220707	0.001829	1	20
17	0.227066	0.002741	3	16, 20, 37
18	0.214	0.010037	13	4, 8, 11, 12, 13, 14, 15, 22, 28, 31, 35, 40, 41
19	0.20436	0.001829	2	2, 25
20	0.18801	0.044695	33	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 25, 28, 29, 30, 31, 32, 33, 35, 36, 37, 39, 40, 41
21	0.204773	0.041047	33	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 25, 28, 29, 30, 31, 32, 33, 35, 36, 37, 39, 40, 41
22	0.153497	0.003653	3	14, 18, 28
23	0.149222	0.029191	17	2, 3, 4, 7, 8, 12, 14, 18, 19, 25, 26, 27, 34, 35, 36, 38, 41
24	0.173947	0.115836	31	1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 15, 16, 18, 19, 21, 22, 23, 25, 26, 27, 28, 29, 31, 32, 34, 35, 36, 38, 39, 40, 41
25	0.174777	0.029191	21	2, 3, 4, 7, 8, 11, 12, 13, 14, 15, 18, 19, 22, 23, 26, 27, 28, 34, 35, 36, 41
26	0.202276	0.049256	17	2, 3, 4, 7, 8, 12, 14, 18, 19, 23, 25, 27, 34, 35, 36, 38, 41
27	0.156871	0.062024	35	1, 2, 3, 4, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
28	0.149444	0.020982	26	1, 2, 4, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 29, 31, 32, 35, 36, 37, 39, 40, 41
29	0.099342	0.018246	24	1, 4, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 28, 30, 31, 32, 33, 37, 39, 40, 41
30	0.148462	0.059288	33	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 28, 29, 31, 32, 33, 35, 36, 37, 39, 40, 41
31	0.165886	0.020982	25	1, 4, 6, 8, 11, 12, 13, 14, 15, 16,

				17, 18, 20, 21, 22, 28, 29, 32, 33, 35, 36, 37, 39, 40, 41
32	0.178404	0.062024	38	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 39, 40, 41
33	0.182426	0.12678	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41
34	0.261353	0.031015	12	2, 3, 7, 8, 19, 23, 25, 26, 27, 35, 36, 38
35	0.231608	0.004565	1	8
36	0.20872	0.009125	5	2, 8, 19, 25, 35
37	0.115532	0.010949	15	1, 6, 11, 13, 15, 16, 17, 20, 21, 29, 31, 32, 33, 39, 40
38	0.041636	0.065673	25	2, 3, 4, 7, 8, 11, 12, 13, 14, 15, 18, 19, 22, 23, 24, 25, 26, 27, 28, 31, 34, 35, 36, 40, 41
39	0.188009	0.003653	8	1, 15, 16, 21, 29, 31, 32, 40
40	0.15826	0.044695	37	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 41
41	0.228954	0.05108	38	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40

B-9: Metacommunity calculation output for Site 3-BL using phylogenetic distances

	Similarity	Distance	Number of Communities	Communities included in metacommunity
1	0.19885	0.019648	10	2, 11, 13, 14, 21, 23, 25, 26, 40, 41
2	0.218839	0.041814	33	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 32, 34, 35, 37, 38, 39, 40, 41
3	0.176203	0.008882	12	4, 6, 8, 9, 10, 18, 19, 22, 24, 34, 38, 39
4	0.229585	0.028514	30	3, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40
5	0.21877	0.051947	37	1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
6	0.20506	0.036114	35	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
7	0.258109	0.004449	8	4, 5, 8, 17, 24, 29, 34, 38
8	0.202034	0.021548	22	3, 4, 5, 6, 7, 9, 10, 15, 16, 17, 18, 19, 20, 22, 24, 29, 32, 33, 34, 37, 38, 39
9	0.095679	0.047514	38	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
10	0.281855	0.006349	6	3, 6, 9, 18, 19, 22
11	0.242216	0.026615	13	1, 2, 12, 13, 14, 20, 21, 23, 25, 26, 35, 40, 41
12	0.240272	0.067146	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
13	0.197671	0.027881	26	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 18, 19, 20, 22, 23, 24, 25, 26, 32, 34, 35, 38, 39, 40, 41

14	0.266816	0.068412	34	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 32, 33, 34, 35, 37, 38, 39, 40, 41
15	0.207776	0.005716	3	28, 30, 36
16	0.266617	0.026615	23	3, 4, 5, 6, 7, 8, 9, 10, 15, 17, 18, 19, 22, 24, 28, 29, 30, 33, 34, 36, 37, 38, 39
17	0.262426	0.006349	7	4, 5, 7, 16, 24, 29, 37
18	0.243618	0.015848	19	3, 4, 6, 7, 8, 9, 10, 12, 13, 19, 20, 22, 24, 32, 34, 35, 38, 39, 40
19	0.110649	0.010149	14	3, 4, 5, 6, 7, 8, 10, 17, 18, 22, 24, 29, 34, 38
20	0.265547	0.034847	33	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 22, 23, 24, 25, 26, 29, 32, 33, 34, 35, 37, 38, 39, 40, 41
21	0.233824	0.032948	6	1, 2, 11, 14, 25, 41
22	0.216114	0.04498	37	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 24, 25, 26, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
23	0.241267	0.05448	38	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41
24	0.22479	0.029148	30	3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40
25	0.15183	0.015215	12	2, 9, 12, 13, 20, 23, 26, 32, 35, 39, 40, 41
26	0.191856	0.022181	19	1, 2, 3, 6, 9, 10, 11, 12, 13, 18, 20, 22, 23, 25, 32, 35, 39, 40, 41
27	0.225361	0.070312	35	2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40
28	0.253608	0.012682	5	15, 16, 30, 33, 36
29	0.231174	0.010149	12	4, 5, 7, 8, 16, 17, 19, 24, 33, 34, 37, 38

30	0.194089	0.004449	3	15, 28, 36
31	0.25304	0.04308	17	4, 5, 7, 8, 15, 16, 17, 24, 27, 28, 29, 30, 33, 34, 36, 37, 38
32	0.184601	0.000649	1	20
33	0.292074	0.017748	16	4, 5, 7, 8, 15, 16, 17, 19, 24, 28, 29, 30, 34, 36, 37, 38
34	0.167558	0.010782	15	3, 4, 5, 6, 7, 8, 10, 17, 18, 19, 22, 24, 29, 37, 38
35	0.147904	0.067146	40	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41
36	0.250886	0.027248	17	4, 5, 7, 8, 15, 16, 17, 24, 27, 28, 29, 30, 31, 33, 34, 37, 38
37	0.260135	0.008882	11	4, 5, 7, 8, 16, 17, 24, 29, 33, 34, 38
38	0.211296	0.027881	30	3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 28, 29, 30, 32, 33, 34, 35, 36, 37, 39, 40
39	0.199978	0.046247	38	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 40, 41
40	0.173515	0.001916	2	13, 23
41	0.207167	0.018382	9	1, 2, 11, 14, 21, 23, 25, 26, 40