Application of Ecological Theory to Explain Microbial Regulation of Soil Function

Chanda Trivedi

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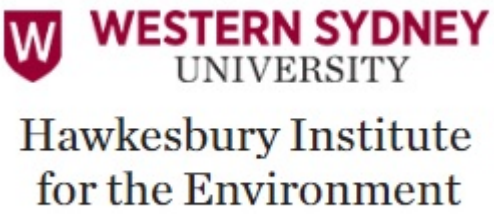

Statement of Authentication

This 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 full or part, for a degree at this or any other institution.

Signature 

Date 27/09/2017

Chanda Trivedi

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List of Abbreviations

° C	degree Celsius
µl	microliter
µm	micrometre
AG	α-Glucosidase
AIC	Akaike information criterion
AICc	akaike information criterion corrected
amo	ammonia monooxygenase
ANOVA	analysis of variance
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
AP	acid phosphatase
BEF	biodiversity-ecosystem functioning relationship
BG	β-glucosidase
Bp	base pairs
C	carbon
CB	β-D-cellubiosidase
C:N	carbon to nitrogen
CH_4	methane
CO_2	carbon dioxide
DNA	deoxyribonucleic acid
GHG	greenhouse gas
HhaI	Haemophilus haemolyticus type I
K	potassium
LAP	leucine aminopeptidase
M	meter
MEq	milliequivalent
Min	minute
Mm	millimetre
MUB	4-Methylumbelliferone
MUC	7-amino-4-methylcoumarin
n	number
N	nitrogen

N_2O	nitrous oxide
NAG	N-acetyl-glucosaminidase
ng	nanogram
NH_4^+	ammonium
NO	nitric oxide
NO_3-	nitrate
*nos*Z	nitrous oxide reductase
NSW	New South Wales
OTU	operational taxonomic unit
P	phosphorus
PCoA	principal coordinates analysis
PCR	polymerase chain reaction
PerMANOVA	permutational multivariate analysis of variance
pH	potential of hydrogen
PHOS	Phosphatase
pmoA	methane monooxygenase
PO_4^{3-}	phosphate
ppm	parts per million
qPCR	quantitative real-time polymerase chain reaction
sp.	Species (singular)
spp.	Species (plural)
T-RFLP	Terminal restriction fragment length polymorphism
TRFs	Terminal restriction fragments
XYL	β-Xylosidase

Definition table

Terms	Definitions	References
Abundance	Number of gene copy number for a given taxa.	Kembel *et al*., (2012)
Broad function	Functions that are carried out by a wide range of organisms or that are measured as a single process but are actually the sum of multiple distinct processes (e.g., soil respiration).	Schimel and Schaeffer, (2012)
Community composition	Relative abundance of phylotypes in a given community assemblage.	Giller *et al.,* (2009)
Copiotrophs	Copiotrophs (r-strategists) are defined as microbes having high growth rates under nutrient-rich conditions and mainly target labile C pools.	Fierer *et al*., (2007); Trivedi *et al*., (2013)
Dryland	Terrestrial ecosystems with an aridity index < 0.65. This definition includes hyper-arid, arid, semi-arid and dry-sub humid ecosystems.	Maestre *et al.,* (2012); Delgado-Baquerizo *et al.,* (2013)
Functional group	A group of phylotypes sharing a given functional attribute e.g. nitrification.	Schimel and Schaeffer, (2012)
Functional redundancy	More than one phylotype carry out the same function. Reductions in diversity does not immediately alter the rates of functioning or the number of functions.	Giller, (2009b)
Microbial functional stability	The ability of a microbial community to minimize dynamic fluctuations of a function (such as respiration rate, enzyme activity or functional potential gene structure) and to resist changes in the community in response to given environmental disturbance.	Allison, (2008)
Narrow function	Functions that involve a specific physiological pathway or which are carried out by a phylogenetically constrained group of organisms.	Schimel and Schaeffer, (2012)
Oligotrophs	Oligotrophs (k-strategists) are slow growing microbes that are dominant in nutrient-poor environments, and mainly target recalcitrant C pools.	Fierer *et al*., (2007); Trivedi *et al*., (2013)
Richness	The number of unique phylotypes present in the sample.	Colwell, (2009)

Soil resistance	The ability of a given soil attribute not to be affected by an environmental disturbance.	Andersson, (2004)
Stress	A deviation from optimal conditions that leads to a reduced growth rate or a cellular damage in result of environmental or internal changes.	Zhou, (2011)

Abstract

A growing body of experimental and observational studies have indicated that plant and animal diversity drives ecosystem function and stability in terrestrial ecosystems. Therefore, it is of paramount importance to identify the consequences of biodiversity loss to assess long-term sustainability of ecosystem functions and services (e.g. climate regulation and nutrient cycling). Soil microbial communities represent one of the most diverse and complex natural communities and are responsible for many ecologically and economically important processes. For example, soil microbes play critical roles in regulating nutrient cycling, decomposition of organic matter, and gas emission. These ecological processes are fundamental for human wellbeing. Despite their importance and complexity, soil microbes receive little attention in the ongoing debate regarding global biodiversity loss, global change and conservation issues, primarily due to the perceived functional redundancy and the large diversity of the soil microbial community. This is no accident; there is a lack of theoretical and experimental protocols which demonstrate microbial regulation of soil ecosystem processes. Consequently, much less is known of the role of microbial diversity in controlling ecosystem functioning. This is critical knowledge gap which hinders inclusion of microbial community response in simulation models or management and policy decisions. Therefore, this research investigated the relationship between microbial diversity and ecosystem functioning (BEF) and resistance to better understand the consequences of microbial diversity loss on ecosystem function and sustainability. To achieve this, my project was divided into experimental (Chapters 2-4) and observational (Chapters 3 and 5) studies. Each chapter in this thesis will highlight the importance of microbial diversity in ecosystem function.

In chapter two, I investigated the relationship between microbial diversity and ecosystem functions (broad; specialised; and multiple functions together - multifunctionality). Soil microbial diversity was manipulated by using a serial dilution approach. Samples from microcosms under different levels of diversity were analysed to determine the impact of diversity loss on general, specialised and multifunction in two different soil types. Bacterial and fungal communities were analysed using Illumina MiSeq sequencing and terminal restriction fragment length polymorphism (T-RFLP) analysis for *amoA* (for ammonia oxidisers only for ammonia oxidising archaea), *nosZ* (for denitrifiers) and *pmoA* (for methanotrophs). A total of 17 functions

including gas emission, enzyme activity, micro respiration related to carbon (C), nitrogen (N) and phosphorous (P) cycles were measured including three specialised [nitrate (NO_3) production, nitrous oxide (N_2O) consumption and methane (CH_4) consumption] and broad functions [carbon di oxide (CO_2) production, basal respiration)]. Results from this chapter suggested that any reduction in functional and taxonomic microbial diversity led to proportional declines in the rate of key processes (CH_4 consumption) and multiple ecosystem functions relate to C and N cycle simultaneously (multifunctionality). Statistical modelling provided evidence for a lack of redundancy in the soil BEF relationship.

In Chapter3, a regional field survey and a microcosm experiment manipulating the diversity of bacteria in two soils were used to identify the role and relative importance of microbial richness in predicting multifunctionality. Two independent approaches (i.e. experimental and observational), and statistical approaches were used to identify the role and relative importance of bacterial richness and community composition in driving multifunctionality (here defined as seven measures of respiration and enzyme activities). In our experimental approach, the richness treatment consisted of one, two, four and six bacterial taxa per microcosm, for each of these richness levels, all the possible equally distributed taxa combinations were prepared (see methodological details in Chapter 3). For our observational approach, we collected samples from an environmental gradient in Eastern Australia. Results indicate that microbial richness was positively related to multifunctionality in both the observational and experimental approaches. Moreover, results from the two different approaches, provided evidence that microbial richness is as important as community composition in driving multifunctionality, and that it is an independent driver of multiple ecosystem functions.

Given the importance of microbial diversity for ecosystem functioning, I investigated the consequences of microbial diversity loss to the resistance of broad (CO_2) and specific (CH_4) function (Chapter 4). I used three simulated disturbances, including elevated temperature, N deposition and wetting and drying, to assess the impact of these recognised global climate drivers on soil microbial microcosm. I specifically assessed the interaction of microbial relative abundance, alpha diversity (Richness and Shannon diversity), as well as microbial community composition (beta

diversity) on microbial and soil functional resistance. I used the Illumina MiSeq platform to assess microbial relative abundance, alpha and beta diversity for bacteria and fungi, and T-RFLP to assess the functional gene *pmoA*. Results indicated that community composition and diversity were important drivers of broad function resistantance, where relative abundance influenced specific functions. My work suggested that loss of diversity has direct consequences for function and resistance to disturbance of soil ecosystem.

After establishing that microbial BEF, in manipulative experiments, in the final chapter, I used observational study to support findings for the above studies. I used samples collected from global dryland ecosystems which represent an excellent model system for microbial biodiversity ecosystem function. In these ecosystems after water, N availability is the most limiting factor of microbial process and plant productivity. In this chapter, I investigated drivers of soil nitrifying microbial communities [ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB)] and consequences for processes involve in N cycling. Random forest and structural equation modelling were used to assess the most important drivers (including human impacts, climate, soil properties, nutrient availability, and plant influence) of the richness and composition of nitrifying microbial communities (determined by T-RFLP and q-PCR). Results indicated that samples collected under plant canopies consistently had lower richness of AOA bacterial taxa, but a greater richness of AOB bacterial taxa than samples collected in open areas between plant canopies. In both cases (AOA and AOB), plant microsites promoted the abundance of the most abundant taxa. Soil pH was identified as an important predictor for the total abundance of AOA and AOB bacterial taxa, to which T-RFLP and qPCR data were positively correlated. My analysis also indicated that nitrifying communities are linked to rate of potential nitrification. Further, my result suggested that any reduction in plant species and increased pH derived from increases in aridity (driven by climate change), will strongly influence the abundance, diversity and composition of nitrifying microbial communities with consequences for the rate of nitrification rates. We also found a link between diversity of bacterial nitrifiers and nitrification rates under vegetated microsites but not under open areas, suggesting that these organisms might be more active under certain micro-habitats.

In summary, my study provided direct evidence of relationship and the shape of relationships between microbial diversity and ecosystem functions and suggested that any loss of microbial diversity will have at least proportional decline in the process rates. Particularly microbial community richness and composition were found to be important, yet independent drivers of multiple ecosystem functions. These results highlight that, belowground microbial diversity is as important as above ground diversity in maintaining ecosystem services. This study is also the first to investigating the drivers of microbial nitrifiers at the global scale. Overall, results from this thesis demonstrate that microbial diversity should be explicitly considered in all biodiversity conservation debates and management decisions and indicated that inclusion of microbial data in predictive models are required to improve predictions to ensure that informed environmental policy decisions are made to sustain ecosystem function under predicted global climate change scenarios.

Chapter 1

General introduction

1.1. Importance of soil and its ecosystem services

Soils are the foundation of all terrestrial ecosystems and maintain multiple ecosystem functions and services which are vital for the existence of life on Earth and for human well-being (Victoria *et al.,* 2012; Figure 1). These ecosystem functions and services include, but are not limited, food and fibre production, nutrient and water cycling, climate regulation and habitats for biodiversity, among many others (Figure 1.1; Christensen *et al.,*1996; Costanza *et al.,* 1997; Victoria *et al.,* 2012). Ecosystem services provided by soil are often grouped under four categories (Lobo, 2001; de Groot *et al.,* 2002; MEA, 2005; Victoria *et al.,* 2012; Trivedi *et al.,* 2013**)** (Figure 1.1) including supporting services, regulatory services, provisioning services and cultural services:

1. **Provisioning services:**
   These services directly benefit humans by providing food, fibre, fuel, genetic resources (taxonomic and functional attributes), medium for construction and ornamental resources (animal skin, shells and plant products).
2. **Supporting services:**
   These services are obtained from natural environments and include nutrient cycling, water release/retention, plant productivity, habitat for biodiversity and degradation of complex materials.
3. **Regulatory services:**
   are defined as the benefits obtained from the regulation of ecosystem processes such as carbon (C) sequestration, greenhouse gas emissions, water purification and natural attenuation of pollutants.
4. **Cultural services:**
   These services provide the nonmaterial benefits such as spiritual enrichment, cognitive development, recreation, aesthetic experiences.

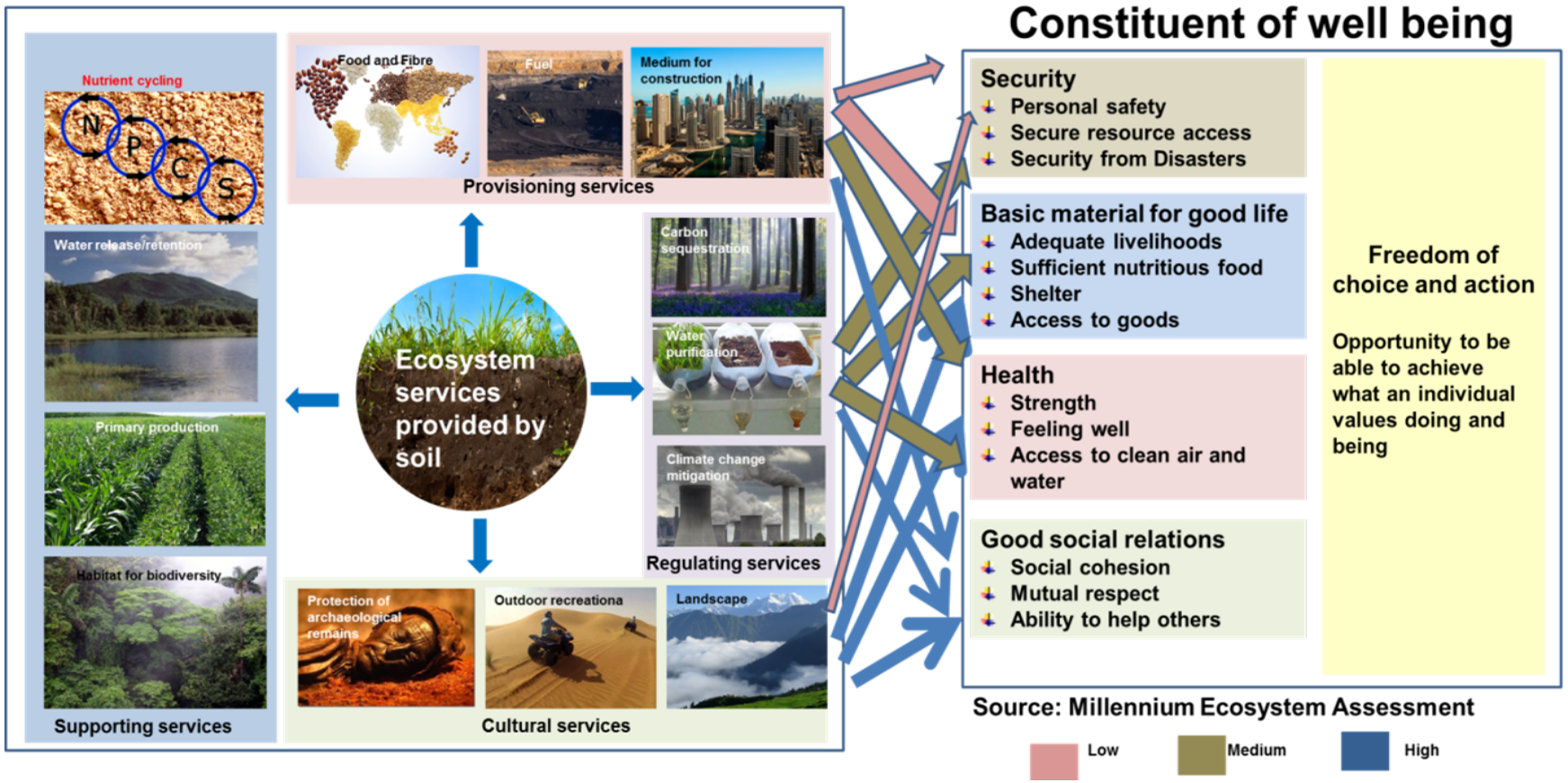


Figure 1.1. Links between ecosystem services provided by soil and human beings (modified from millennium assessment report, 2005).

A large number of studies have indicated that soil disturbances could largely alter the capacity of soil to maintain soil functions and plant productivity (Classen *et al.,* 2015, Delgado-Baquerizo *et al.,* 2017). Losses in soil health (Reynolds *et al.,* 2012), for example, are well-known to reduce the ability of soil to support key ecosystem services such as plant productivity, soil C storage, biodiversity habitat, pollutant degradation and climate regulation (via gasses emissions) (Tóth *et al.,* 2007). Soil health and soil functioning are largely maintained by biotic components within soils including soil fauna and microbial communities. For example, soil biodiversity is known to promote the rates of multiple single functions (Hooper *et al.,* 2005; Díaz *et al.,* 2011; Chapin, 2000; Wall *et al.,* 2012; Delgado-Baquerizo *et al.,* 2016) such as nutrient cycling and biogeochemical cycling. However, soil microbial communities are largely threatened by global environmental changes and anthropogenic disturbances (Gulledge and Schimel, 1998; Cardinale *et al.,* 2012) such as climate change, deforestation, use of excessive fertilizers and land use changes. For example, increases in aridity and changes in agricultural practices have been proved to alter microbial diversity and composition directly and indirectly via impacts on plant diversity and production (Vitousek *et al.,* 1997; Buckley and Schmidt, 2001; Gomez *et al.,* 2000). Such changes can create adverse conditions which may pose environmental hazards, diversity loss and microbial community change. Strikingly, the link between microbial diversity and multiple ecosystem functions simultaneously have never been assessed empirically in terrestrial ecosystems. Given the growing evidence linking the relationship between soil microbial diversity and ecosystem functionality, quantifying these links is of paramount importance to predict ecosystem functioning under global change scenarios.

1.2. Soil microbial community: diversity and patterns of distribution

1.2.1. Microbial diversity: Definition and current knowledge

Soil provides multiple niches for the growth and development of various life forms and represents the most biodiverse habitat on the planet (Bardgett and van der Putten, 2014) (Figure 1.2). It is estimated that one square meter of land may contain millions of different species of microorganisms (Pace, 1997; Torsvik and Øvreås, 2002; Bardgett and van der Putten, 2014; Leach *et al.,* 2017) **(**Figure 1.2). In fact, one gram of soil contains approximately 1 billion bacterial cells (Pace, 1997) representing thousands to as many as a million-bacterial species (Hong *et al.,* 2006; Torsvik and

Øvreås, 2002; Leach *et al.,* 2017); over 250 species of fungi (Bardgett and van der Putten, 2014) contributing to 200 m of fungal hyphae (Wagg *et al.,* 2014); and a wide variety of mites, earthworms, nematodes, arthropods, and protists (Wagg *et al.,* 2014). Most of the soil biodiversity remains unknown as over 95% of microbes are uncultivable (Singh *et al.,* 2010), however recent DNA sequencing technology advances are helping to fill knowledge gaps around soil biodiversity. It is worth noting here that we often rely on sequence similarities of taxa-specific DNA subunits to distinguish between distinct organisms rather than morphological characteristics. We refer to these as operational taxonomic units (OTUs) rather than species.

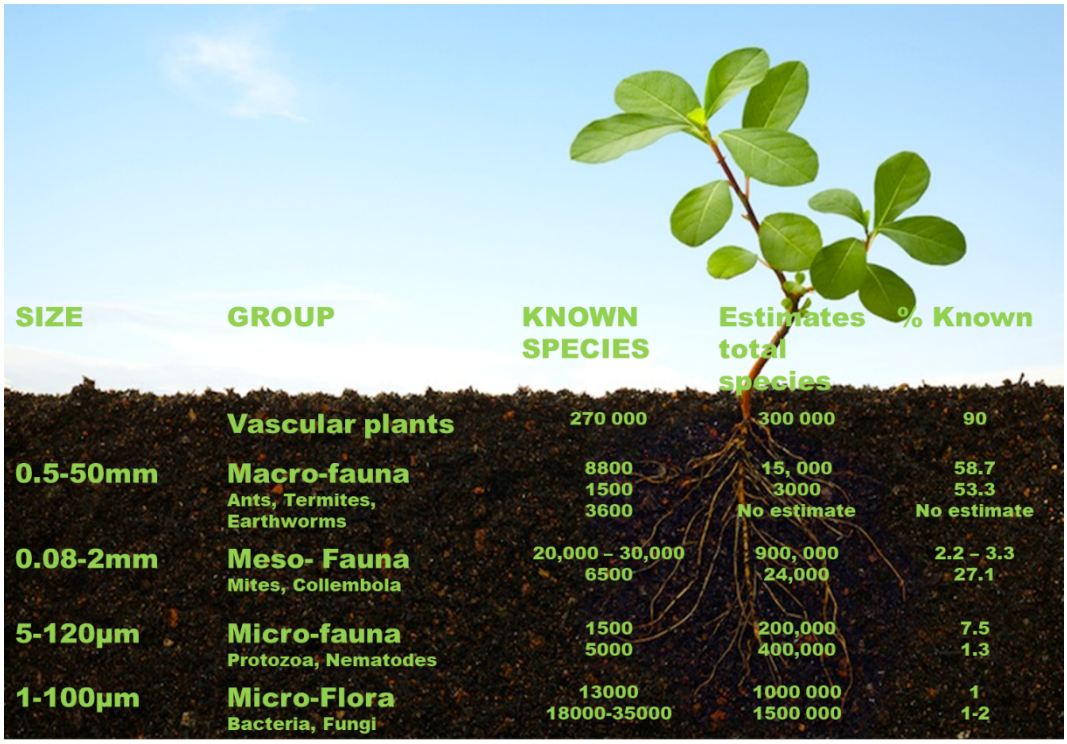


Figure 1.2. The biodiversity of soil categorised by size into macro-fauna, meso-fauna, microfauna and microflora. The number of known species falling into each category has been compared to the number of estimated species, providing a percentage of known organisms from each category. Image has been modified from Wall *et al*., (2012).

The development and application of high-throughput molecular tools –often called "omic" techniques – have provided greater insights into the diversity of soil microbiota at both continental and global scales (Fierer *et al.,* 2012; Delgado-Baquerizo *et al.,* 2016). These studies have challenged the traditional "everything is everywhere, but the environment selects" theory (Becking, 1934) and have provided concrete evidence for the restricted global distributions of soil microbiota due to

variations in climatic, soil and plant conditions (Bardgett and van der Putten, 2014). For instance, Fierer *et al.,* (2012) reported a significantly lower alpha diversity [diversity of each site (local species pool)] of soil microbes in deserts (both hot and cold) as compared to forest and grassland soils. Similarly, beta diversity (differences in species composition among sites) patterns were different among different biomes and the largest differences were between the desert and non-desert soils (Fierer *et al.,* 2012). Similarly, aridity and climatic conditions were reported as the main drivers of bacterial diversity at global scales (Maestre *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a). Recently, Tedersoo *et al.,* (2014); Maestre *et al.,* (2015), and Delgado-Baquerizo *et al.,* (2016a) reported global diversity and biogeography of fungi from soil samples collected from a wide variety of natural ecosystems across the globe. The authors reported strong effects of distance from the equator and precipitation on the taxonomic and functional group richness of fungi, suggesting the important role of global change in shaping soil microbial communities. These initial findings have advanced our understanding but the patterns of distribution from local to global scale remains a big challenge, particularly for critical functional [e.g. nitrifiers, nitrogen (N) fixing, methane (CH_4) oxidising] communities. More importantly, these findings suggest that microbial diversity largely shifts across different biomes and ecosystem types, with implications for the functioning of terrestrial ecosystems across the globe. There are different types of diversity attributes (richness, evenness, community structure, structural vs function) and these attributes can theoretically have different relationships with ecosystem functions but empirical evidence is scarce. However, such knowledge is key to develop management and conservation policies in order to harness microbial diversity for enhanced ecosystem services.

1.2.2. Soil microbial biodiversity and ecosystem functioning (Microbial BEF)

Since many microbial processes are essential for the sustainability of ecosystems (*e.g.* nutrient cycling, greenhouse gas modulation, plant production and soil health), understanding the factors that control these processes is crucial. Soil microbes regulate a wide range of soil functions such as nutrient cycling, degradation of organic matter, regulating the fluxes of trace greenhouse gases (e.g. N_2O, CH_4), sequestration of C and degradation of pollutants. They are essential components of biogeochemical cycles (Falkowski *et al.,* 2008) and represent approximately half of the total C contained in living organisms (Shively *et al.,* 2001). Microorganisms contribute

greatly to net C exchange through decomposition and heterotrophic respiration, through their interactions with plants and by modifying nutrient availability in the soil (Van der Heijden *et al.,* 2008; Trivedi *et al.,* 2013). Soil microbes are also important regulators of plant productivity through the mineralisation of nutrients, especially in nutrient poor ecosystems, where plant symbionts are responsible for the acquisition of limiting nutrients such as N and phosphorus (P, Chapin, 1980). For example, up to 80% of all N and 75% of P acquisition by plants come from the activity of N fixing bacteria and mycorrhizal fungi (Van der Heijden *et al.,* 2008). Microbes are also important regulators of plant production and diversity, determining plant abundance (Van Der Heijden *et al.,* 2008; Delgado-Baquerizo *et al.,* 2016; 2017) through its positive (symbiosis, mutualistic) and negative (pathogenicity) interactions. Despite their importance, *our knowledge about the quantitative role and control of microbial diversity in driving ecosystem functioning is still incomplete*.

A large body of the literature (Tilman *et al.,* 1996; Cardinale *et al.,* 2012; Reich *et al.,* 2012; Maestre *et al.,* 2012) has indicated that plant and animal biodiversity plays an important role for driving ecosystem functionality and stability in terrestrial ecosystems. However, much less is known about the role of microbial diversity in controlling ecosystem functioning, and thus the direct linkages between microbial diversity and ecosystem functions and services, such as productivity, nutrient cycling, and C storage are not well understood. Traditionally, microbial biomass was considered the major driver of soil functioning; as microbial diversity was expected to be so enormous, that microbial species were largely thought to be redundant. Due to the extreme diversity and complexity within soil microbiota, much of the research on biodiversity-functioning relationships in terrestrial environments has focused on aboveground diversity and, therefore, most of the principles overviewed here are derived from plant ecology. Belowground biodiversity effects on the functioning of terrestrial ecosystems are only starting to be identified (Delgado-Baquerizo *et al.,* 2016a; 2017). A complete understanding of the relationship between terrestrial biodiversity and ecosystem processes is of crucial importance in order to link above and belowground components in ecosystem modelling to better predict consequences of the loss of biodiversity.

Contemporary models, experiments and observations in ecology suggest a positive relationship between diversity of plant species and the magnitude and stability of ecosystem processes catalyzed by those species (Tilman *et al.,* 1997; Cardinale *et al.,* 2012; Reich *et al.,* 2012; Maestre *et al.,* 2012). However, we have a limited knowledge on the shape of the relationship between biodiversity and ecosystem functioning (BEF) (Figure 1.3). It has also been postulated that the shape of the relationship might vary in different ecosystems and communities, and will be variable over time (Schläpfer and Schmid, 1999; Loreau and Hector, 2001). In the developing field of BEF relationships, workers have already recognized multiple hypothetical trajectories (Schläpfer and Schmid, 1999; Loreau and Hector, 2001) which can roughly be classified into three categories: i) positive, non-redundant BEF relationship; ii) positive, functionally redundant BEF relationship; and iii) the BEF relationship being context dependent or idiosyncratic (Figure 1.3).

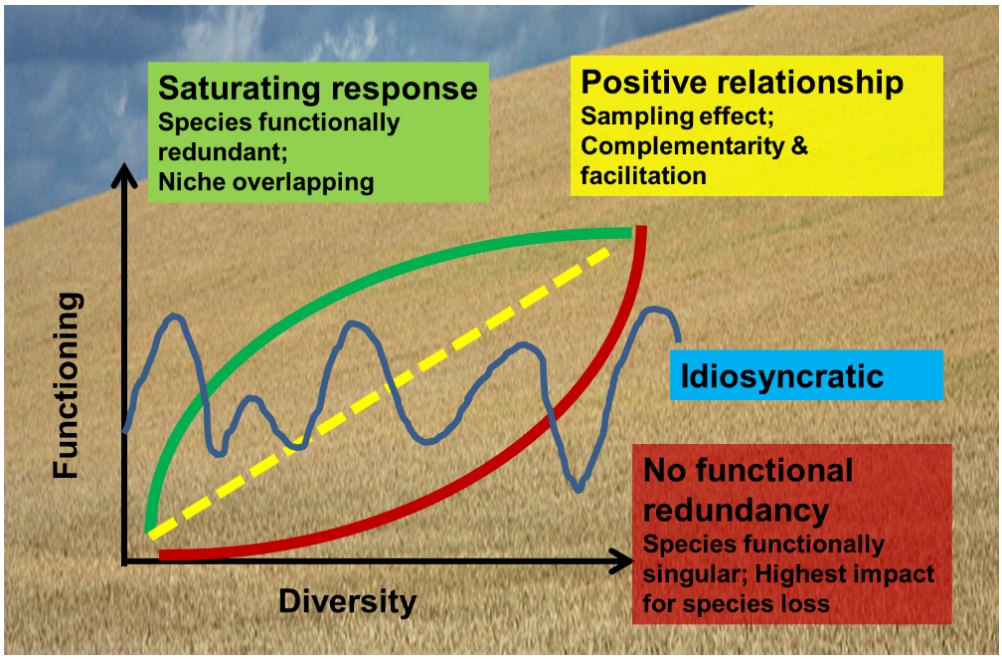


Figure 1.3. Patterns of the biodiversity-ecosystem functioning (BEF). Representation of the three generally accepted forms when a positive relationship is observed between species richness and the functioning of ecosystems. The linear relationship would occur if each species has unique characteristics that improve the process or function in question. The redundant or asymptotic form would occur if the species share common characteristics that improve the functioning of the system. Thus, the probability of adding a species with characteristics that are not present in the community becomes progressively small as the number of species increases, as it does their effect on soil functioning. The idiosyncratic relationship would occur if species with similar characteristics differ in their ability to improve function (Adapted from Nielsen et al., 2011).

Identifying the shape of microbial diversity and ecosystem functions is key to quantitatively estimate the consequences of diversity loss and use this information to support management decisions and policy advice. Interest in quantifying the BEF relationship has driven two decades of experimental and statistical modelling (Hector *et al*., 1999; Schmid *et al.,* 2002; Bell *et al.,* 2005; Kirwan *et al.,* 2007; Cardinale *et al.,* 2009; Isbell *et al.,* 2011; Delgado-Baquerizo *et al.,* 2016). In addition to describing BEF relationships, efforts have been made to link coefficients of BEF models to underlying mechanisms and process (Connolly *et al.,* 2013). Positive BEF relationships can arise via the complementarity effect, whereby diverse communities use more of the available resource space through niche differentiation or facilitation (Salles *et al*., 2009; Singh *et al*., 2015); or the selection effect (also termed the sampling effect), whereby diverse communities are more likely to contain species (termed as keystone species) which have a large impact on community functioning (Isbell *et al*., 2011; Reich *et al.,* 2012; Figure 1.3). Both complementarity and selection effects depend on the functional traits of constituent species and several studies have now shown functional diversity to be a better predictor of community function than phylogenetic diversity (Mokany *et al*., 2008; Salles *et al*., 2009; Krause *et al*., 2014).

1.2.3. Type of functions driven by soil microbial community

Microbial communities are largely assumed to be functionally redundant given their diversity; of the various ecosystem processes carried out by soil microbial communities, some are likely to be more sensitive to changes in the microbial community composition than others. For example, **narrow processes** are most likely to be affected by changes in the composition of the soil microbial community because manifestation of these processes requires specific physiological/metabolic pathways and/or are carried out by a phylogenetic clustered group of organisms (e.g. nitrification, CH_4 oxidation; Schimel and Schaeffer, 2012; Delgado-Baquerizo *et al.,* 2016). A process is considered physiologically/metabolically narrow (hereafter **'specialised functions')** when the pathway to carry out the specific processes is limited to an extremely narrow taxonomically related group (one or a few species) of microorganisms. These specialised functions include: N fixation (carried out by díazotrophs such as cyanobacteria, green sulphur bacteria, Azotobacteraceae, rhizobia and Frankia), CH_4 oxidation (Type 1 methanotrophs belonging to -γ-proteobacteria;

type II methanotrophs belonging to α-proteobacteria, and CH_4 production (performed by Archaea), aerobic ammonia oxidation is carried out by phylogenetically distinct groups of ammonia-oxidizing archaea (AOA) and bacteria (AOB) (Monteiro *et al.,* 2014). Because narrow processes are carried out by specialised microorganisms that carry the essential genes, loss of key species with that functionality will lead to a shift in abundances of the relevant functional genes and have a negative impact on the process rates (Pett-Ridge and Firestone, 2005; Philippot *et al.,* 2013; Powell *et al.,* 2015). When all species carrying the specialised functional genes are lost and the remaining species cannot maintain ecosystem functioning rates, that function is no longer available.

In contrast to the specialists performing narrow processes, most soil microorganisms are aerobic heterotrophs involved in **"broad"** or **"aggregate"** functions (Schimel and Mikan, 2005; Schimel and Schaeffer, 2012). Broad functions can be defined as functions carried out by a wide range of microbes or that can be measured as a single process but are actually the sum of multiple distinct processes (e g., soil respiration, litter decomposition). It is argued that some gain and loss of species will not influence the overall rate or stability of the broad processes as there is sufficient redundancy in metabolic process. However, the rate of the broad process will be influenced by changes in the microbial community, especially when functional groups have strong control over broad functions (Mooney and Drake, 1986; Hooper *et al.,* 2005). For example, soil microbes can be classified as "oligotroph" (slow growers requiring recalcitrant forms of C) and "copiotroph" (fast growers requiring labile forms of C) (Fierer *et al.,* 2007; Trivedi *et al.,* 2013) and shifts in the community composition have shown to impact decomposition and community respiration (Trivedi *et al.,* 2015). Moreover, organic matter decomposition requires the cooperation of multiple microbial species to perform individual functions simultaneously to efficiently conduct this process. Consequently, losses in microbial diversity might lead to reductions in essential soil processes. For example, differences in the community composition of litter samples showed functional dissimilarities in decomposition (Strickland *et al.,* 2009). Another recent study suggested that the relationship between multiple ecosystem functions and overall soil biodiversity resulted in a more linear and less saturating response curve for specific functions (Wagg *et al.,* 2014). These studies highlight our limited knowledge on the extent of

functional redundancies. Further studies with explicit consideration of broad and specialised ecosystem functions in relation to soil microbial diversity are needed to further test these hypotheses.

1.3. Biodiversity and ecosystem function (BEF) research in soil microbial communities and observation studies.

Considering the importance of the relationship between biodiversity and ecosystem functioning, understanding their linkages is a major challenge with societal implications (Cardinale *et al.,* 2012). However, the studies on BEF relationships either utilizing experimental manipulations in laboratory settings and/or comparative studies across treatments or natural gradients have provided contradicting and controversial results (Hooper *et al.,* 2005; Krause *et al.,* 2014). Due to the inherent limitations of comparative studies, BEF-research is moving towards direct manipulation approaches whereby community functions are measured after the diversity of the native community has been manipulated (Delgado-Baquerizo *et al.,* 2016; Philippot *et al.,* 2013). In spite of the importance in mediating many ecosystem functions, the BEF relationship for microbial communities remain largely unknown (Philippot *et al.,* 2013; Krause *et al.,* 2014). A previous meta-analysis has clearly shown that the studies performed to demonstrate the relationship between biodiversity and ecosystem functioning have primarily used higher organisms such as plants as models (Cardinale *et al.,* 2012). Although in recent years few studies address the BEF relationship in soil microbial communities (Philippot *et al.,* 2013; Wagg *et al.,* 2014).

Krause *et al.,* (2014) led a review effort to synthesize information on all published papers that refers to BEF relationships. We further extended the results of this meta-analysis to 2016 (was to 2012 in the study by Krause *et al.,* 2014). This meta-analysis showed that the number of publications in this field of research was surprising similar for plant and microbe related studies (Figure 1.4, left panel). However, as shown on the right in Figure 1.4, there are significantly fewer studies on BEF relationships that utilize a direct manipulation approach as compared to comparative designs where biodiversity is not directly manipulated. In 2012, only one study out of 610 used a direct manipulation approach to study the microbial role in BEF relationships.

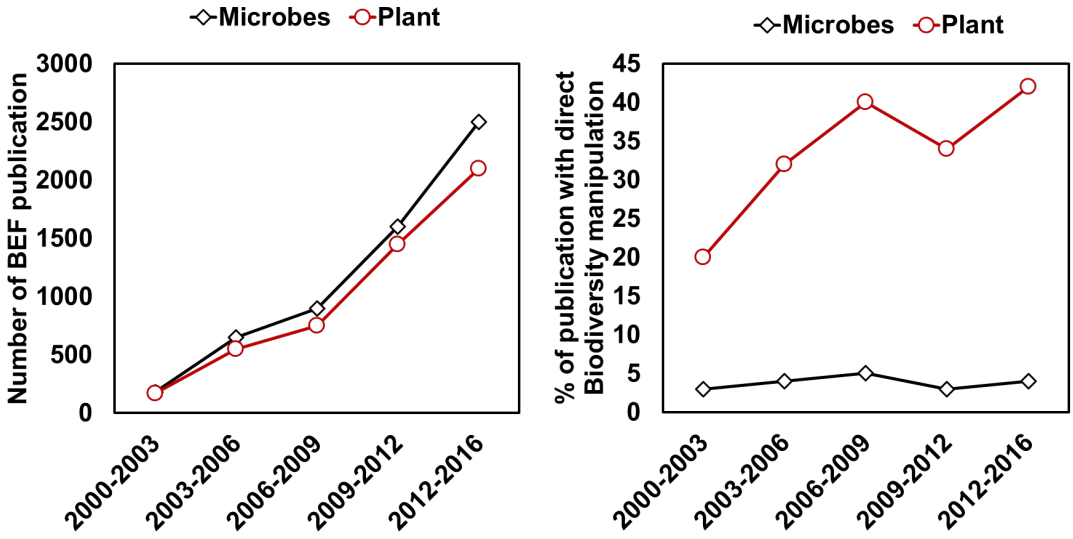


Figure 1.4. Temporal variations (left) in the number of publications of Biodiversity-Ecosystem Functioning, BEF, relationship in a broad sense for microorganisms as compared to plants, and the percentage of publications on microbial BEF or plant BEF where biodiversity was directly manipulated (right).

There are many examples of observational studies where the changes in the diversity of soil organisms and function of soil microbial community have been studied along environment gradients (Rouske *et al.,* 2010; Webster *et al.,* 2015; Yao *et al.,* 2015). Fierer *et al.,* (2012b) used metagenomic sequencing to compare the composition and functional attributes of soil microbial communities and showed that functional beta diversity of plant free cold desert soils was strongly correlated with phylogenetic beta diversity. Delgado-Baquerizo *et al*., (2016a) used two independent, large-scale databases with contrasting geographic coverage (from 78 global drylands and from 179 locations across Scotland, respectively), and showed that soil microbial diversity positively relates to multifunctionality in terrestrial ecosystems. The direct positive effects of microbial diversity were maintained even when accounting simultaneously for multiple multifunctionality drivers (climate, soil abiotic factors and spatial predictors). A recent study suggested that the active diversity (determined by metaproteomics) was related to the ecosystem multifunctionality index wherein dissolved organic carbon (DOC) determines the compartmentalization of functional niches among bacterial and fungal populations under arid and semi-arid environments.

Although it is widely postulated that biodiversity will have significant effect on ecosystem functioning, the nature and strength of BEF relationships in these observational studies are still widely debated (Wood *et al.,* 2015). These limitations arise because: (a) In these approaches diversity is an observed, dependent variable rather than an applied treatment and; (b) The relationships are not direct and can co-vary with diversity (Krause *et al.,* 2014). However, we lack studies using experimental evidence for shape of the relationship between the diversity of multiple microbial taxonomic and functional microbes in driving specialised and multiple ecosystem functions simultaneously in terrestrial ecosystems. Assessing the real importance of soil microbial diversity in driving soil processes is critical for developing a unified ecologically relevant theory about below-ground ecosystem functioning and to improve simulation model prediction and management policies for sustainable environments (Reed and Martiny, 2007; Delgado-Baquerizo *et al.,* 2016a).

1.3.1. Direct manipulation of diversity to study soil microbial BEF Relationships

There are limited studies that have manipulated microbial diversity to explore BEF relationships in soil; where they have occurred, different approaches have been used to manipulate diversity. First, one could isolate different microbial species in pure culture and assemble different numbers and combinations to study BEF relationship (Bell *et al.,* 2005). This approach is tedious and is biased towards the members of microbial community readily grown on nutrient rich media. Moreover, artificial assembly is unlikely to reflect the distribution of species in the regional species pool (Díaz *et al.,* 2007) and thus do not account for the evenness in community structure which is a trademark of natural communities. An alternative approach is based on the removal of species using a dilution-to-extinction technique where a natural community is stepwise diluted to create a diversity gradient of microbes (Wertz *et al.,* 2007; Baumann *et al.,* 2013; Crawford *et al.,* 2011; Philippot *et al.,* 2013). This approach allows investigation of a large range of diversity while maintaining the evenness between microbial communities. This approach has been used in fresh-water environments (Peter *et al.,* 2011; Delgado-Baquerizo *et al.,* 2016), but have been rarely performed in soils (Philippot *et al.,* 2013). Recent studies have used other methods to study BEF relationships including the sieve out technique that uses sieving to select soil microbes based on the size of soil aggregates (Wagg *et al.,* 2014). This approach is excellent to create overall soil diversity gradients, but not to explore the

effects of microbial diversity *per se* on soil functionality. Obviously, all approaches have some limitations, therefore, a combination of experimental approaches needs to be done to assess the direction and shape of the microbial BEF (Crawford *et al.*, 2012; Singh *et al.,* 2014; Delgado-Baquerizo *et al.,* 2016).

According to our current understanding based on different ecosystem such as terrestrial drylands, a large species pool of microbial diversity is required to sustain assembly, functioning, and stability of ecosystems (Bell *et al.,* 2005; Peter *et al.,* 2011). Several diversity manipulation studies in soil have clearly indicated a negative impact of diversity loss on soil ecosystem functioning on single specialised functions at the local scale in terrestrial ecosystems and in several functions at fresh-water ecosystems of Australia (Crawford *et al.,* 2012; Philippot *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016). These studies showed that decrease in the diversity and changes in the relative abundance of keystone species resulted in significant reduction in critical ecosystem functions such as soil porosity, denitrification and community respiration and pollutant degradation. Moreover, several studies have reported that microbial diversity enhances rhizosphere microbiome functions with positive effects on pathogen suppression (Van Elsas *et al.,* 2012; Hu *et al.,* 2016). In contrast, other studies did not find any relationship between diversity and ecosystem functions (Wertz *et al.,* 2007; Griffiths *et al.,* 2001). These studies suggest that biodiversity neither influenced the variability nor the stability of functions when the soils were disturbed (Griffiths *et al.,* 2001a). These divergent observations can be explained by the differences in the composition of microbial communities in the soil samples used in different studies. These results are in accordance with idiosyncratic response hypothesis and suggest that the composition of soil microbial community will determine the pattern of ecosystem response. This further leads to the assumption that as soil functional response will vary according to their resident microbial community, the relationship between diversity loss on ecosystem function will be context dependent.

Most of the studies in BEF relationship have measured only a single ecosystem process and in single taxonomic or functional groups (e.g. bacteria or denitrifiers). However, species perform multiple functions and therefore several workers have highlighted the importance of studying multi-functionality while describing BEF

Figure 1.5. A theoretical work-plan to study Biodiversity-Function Relationship of soil microorganisms across different scales and the benefits of such studies. Comparative studies on natural gradients at local to global scale will help in determining the patterns of interaction between soil microbial community and multiple ecosystem functions. Such studies will help in formulating theory and hypothesis of BEF relationships that can be tested in control lab based studies using simple microbial model microcosms. In these studies, the diversity can be easily manipulated and different microbial functions and their interactions (including those affected by climate change drivers) can be studied. The theoretical and practical knowledge from field and lab based studies can then be scaled up to small scale semi-natural ecosystems within which different manipulative studies can be performed. All of these steps will lead to the development of a framework for how soil microbes impact ecosystem functions. This will lead us to improved predictions of ecosystem functions particularly in relation to global change.

relationships (Hector and Bagchi, 2007; Maestre *et al.,* 2012; Wagg *et al.,* 2014), and multiple taxonomic and functional microbial groups co-exist in soils. Accordingly, analysis of only one response variable tends to overestimate the amount of functional redundancy. Recently Wagg *et al.,* (2014) measured multiple ecosystem functions including aboveground diversity, ecosystem productivity, C sequestration, litter decomposition, N turnover, and P leaching in grassland microcosm where a biodiversity gradient was achieved via sieving out soil communities based on size. The authors reported that reductions in the abundance and presence of soil organisms resulted in the decline of multiple ecosystem function. Using field studies,

soil plots receiving different levels of metals or management (water and N) treatments were used as model systems to study the impact of microbial diversity loss on various broad and specialised functions. The result indicated that even a moderate (5- 20%) loss of biodiversity could have significant negative impacts on broad (e.g. community respiration) and specialised (pesticide degradation) functions (Singh *et al.,* 2014; Colombo *et al.,* 2016). This suggests that there remain inconsistencies in reported findings on relationship and shape of the relationship in soil BEF and future studies are needed to develop a robust understanding with explicit consideration of experimental approaches (manipulative vs observational studies), function (broad vs specialised), diversity (taxonomical vs functional, richness vs community structure) and ecosystem (high vs low diverse) types (Figure 1.5).

1.3.2. Effect of aridity on specialised BEF relationships of soil microbial communities: a global scale perspective

The observational evidence for microbial BEF is important to provide the theoretical and practical knowledge that have advanced our current understanding of BEF relationships within soil microbial communities. Manipulative studies are criticised because as they are artificial, wherein the shape of BEF relationship can be identified, but neglects other factors that may affect the relationship and masks the true diversity (Ducklow, 2008), if carried out without validation by field studies. Only a few studies have analysed the relationship between soil microbial diversity and functions on a large scale (Fierer *et al.,* 2012; 2013; Delgado-Baquerizo *et al.,* 2016a, 2017a) and have mainly focussed on the taxonomic diversity of microbial communities. In some cases, functional (N fixing, nitrifiers, CH_4 oxidising) communities can provide a better relationship and understanding of key functions given these communities carry specific genes required for those functions (Trivedi *et al.,* 2016). I propose that the development of an experimental framework will require integration of the knowledge gained both by laboratory-based microcosm studies and global scale observation studies to establish microbial BEF (Figure 1.6).

Global dryland ecosystems provide an excellent model system for microbial BEF as a loss of microbial diversity is associated with increasing aridity (Maestre *et al.,* 2015) and is directly linked to reduced multifunctionality (Delgado-Baquerizo *et al.,* 2016a). Drylands cover 45% of the Earth's land surface (Reynolds *et al.,* 2007)

and support over one third of the total global population (Maestre *et al.,* 2012; Delgado-Baquerizo *et al.,* 2013a). Drylands provide habitat to many endemic plants and animal species (Millennium Ecosystem Assessment, 2005) and include 20% of the major centres of global plant diversity (White *et al.,* 2003). These ecosystems are particularly vulnerable to ongoing global environmental change causing desertification, increased aridity and shrub encroachment (Valencia *et al.,* 2015). It has been suggested that global change drivers will change the functional structure of biological communities leading to alterations in the multi-functionality of dryland communities.

A body of work on the biodiversity-multifunctionality relationships in drylands has accumulated in recent years. Pioneering studies have investigated the role of biodiversity in maintaining multi-functionality at a large number of sites that represent a wide range of spatial variability in resource availability, abiotic factors, and species richness and composition (Maestre *et al.,* 2012; Valencia *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016b). These studies have provided empirical evidence of the importance of biodiversity in maintaining and improving ecosystem multi-functionality in drylands such as C storage, productivity, litter decomposition, water infiltration and the build-up of nutrient pools (Maestre *et al.,* 2012; Valencia *et al.,* 2015; Delgado-Baquerizo *et al.,* 2013a). Maestre *et al.,* (2012) reported positive effects of plant species richness on multifunctionality, which are mediated by increases in net primary production (NPP) leading to cascading effects on multiple organisms and ecosystem processes. Valencia *et al.,* (2015) evaluated the effect of changes in the plant functional structure of Mediterranean drylands on the variations in multifunctionality. The authors reported that maintaining and enhancing functional diversity in plant communities might help to buffer negative effects of global changes on dryland multifunctionality. However, much less is known on the microbial BEF relationship (but see Delgado-Baquerizo *et al.,* 2016a) in these ecosystems and no experimental or observational study has explored this relationship in drylands worldwide for functional microbial communities.

In drylands, after water, N is the most important factor limiting net primary productivity and organic matter decomposition (Robertson and Groffman, 2007). As many reactions in N cycle (such as nitrification, denitrification) are mediated by

narrow groups of microbes, community shifts within these specialised groups will significantly affect multifunctionality of dryland ecosystems. Wang *et al.,* (2014) suggested that the patterns of N cycling along the aridity gradient in arid and semi-arid ecosystem are non-linear. This observation further suggests that denitrifier/nitrifiers should respond to aridity and/or rainfall in extremely dry areas differentially from that of more mesic areas. Uncoupling of the N cycle (Delgado-Baquerizo *et al.,* 2013a) through the change in plant and microbial community responses (Wang *et al.,* 2014) due to increased aridity makes dryland ecosystems highly vulnerable to future climate change. Hu *et al.,* (2014) provided evidence that water addition had more important roles than N fertilizer application in influencing the autotrophic nitrification in dryland ecosystems, and AOA are increasingly involved in ammonia oxidation when dry soils become moist. These studies warrant further investigations on how the changes in the community composition of the guilds of specialised microbial functional groups (for example, nitrifiers/denitrifiers) modulates ecosystem functionality and whether the relationship between species richness and functions shows a general pattern that reflects cause-and-effect linkages.

1.4. Biodiversity-stability hypotheses

Ecosystem function stability refers to the ecosystem's ability to minimize dynamic fluctuations and the ability to defy changes after disturbances (McCann, 2000). The study of BEF relationships is intrinsically linked to the conservation and management of natural resources. In fact, biodiversity is often the center of environment protection and sustainability debates. Classically, **ecosystem sustainability** is defined as the limits set by the carrying capacity of the natural environment, so that human-use does not irreversibly alter the proper functioning of its processes and components (Lubchenco *et al.,* 1991). Many authors dealing with sustainability relate it to the concept of ecosystem stability, which is defined by two components, **resistance** and **resilience** (Figure 1.6). In this thesis, I will use resistance –the ability of a function to remain unchanged in response to a particular disturbance – as our metric of stability (Orwin and Wardle, 2004).

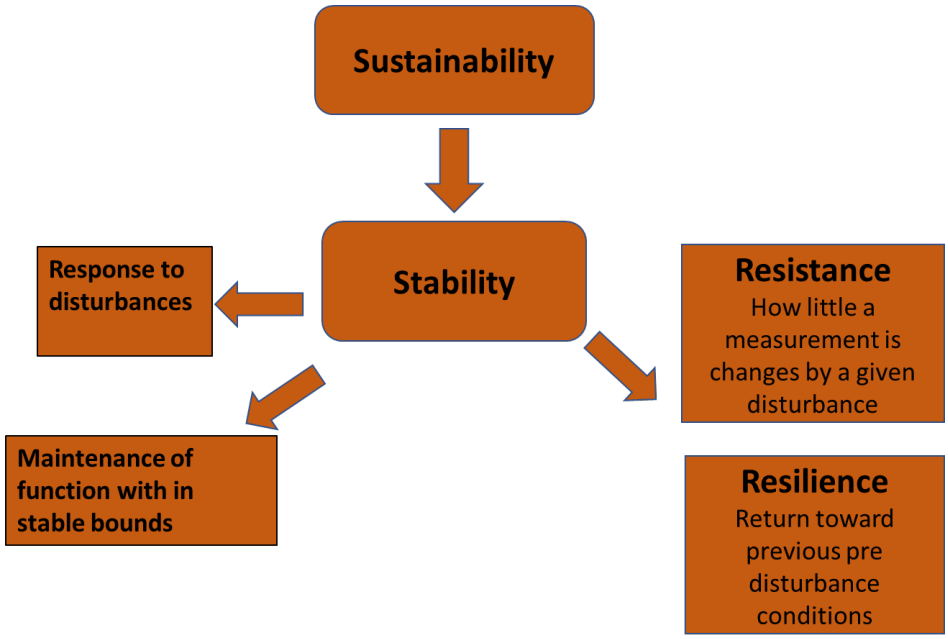


Figure 1.6. Conceptual framework of the relationship between sustainability and different components of stability.

Stability determines the ability of a system to continue functioning under changing conditions, which can occur through natural processes or perturbation related to human activities (Orwin and Wardle, 2004). Stability is therefore an important parameter in the ecological functioning of the soil (Seybold *et al.,* 1999) and directly refers to the concept of "ecological insurances" proposed by Yachi and Loreau in 1999. This concept predicts that in fluctuating environments, the presence of a large number of species assures the maintenance of the functioning of an ecosystem. This is based on the idea that ecosystem functions (e.g., nutrient cycling and C sequestration) are each provided by a functional group of species with the same capabilities in the system. The size of each pool determines the level of functional redundancy of the group. Thus, in undisturbed environments, the presence of a minimum number of functional groups is essential to the functioning of the ecosystem. On the other hand, in disturbed environments, the presence of a great diversity of species increases the probability that at least some will survive and even thrive, under disturbance and thus guarantee the stability of ecosystem functions. For this reason, the level of diversity can be regarded as an insurance of ecosystem stability, which is

particularly important in the case of agro-ecosystems that are regularly subjected to disturbances related to agricultural practices.

The composition of most microbial communities is sensitive and not immediately resilient to disturbance, as microbial communities can fail to recover even after several years of facing disturbances (Allison and Martiny, 2008). Many studies have shown that the composition and functions of soil microbial communities are linked (Reeve *et al.,* 2010; Fierer *et al.,* 2012; 2013; Zhou *et al.,* 2015), therefore, a change in microbial composition will alter the process rates. A detailed meta-analysis by Shade *et al.,* (2013) has clearly demonstrated that soil microbial communities are equally sensitive to pulse (short-term discrete events such as extreme weather events) and press (long-term or continuous events such as increases in atmospheric CO_2 concentration and the associated rise in global temperature).

Soil microbial communities are major players in controlling the rate of ecosystem processes; they play important roles in global nutrient cycling, which determine the sustainability and productivity of terrestrial ecosystems (Singh *et al.,* 2010; Trivedi *et al.,* 2013; Leff *et al.,* 2015). An intensive area of research is to understand the effects of climate change on the structure and function of soil microbial communities and their feedback to global processes (Bardgett *et al.,* 2008; Singh *et al.,* 2010; Trivedi *et al.,* 2013). A practical assumption behind such research is the option to mitigate the effects of climate change by managing soil microbial communities and their associated functions (Singh *et al.,* 2010). Although some general trends have emerged on the direct and indirect effect of global climate change on assembly, composition, function and feedback of soil microbial communities, many uncertainties remain about the controls on soil microbial community stability (de Vries and Shade, 2013). Moreover, only a few studies have directly tested how shifts in community structure and function in response to climate change may affect the stability of the BEF relationship (Knelman and Nemergut, 2014). Recently, Hawkes and Keitt (2015) proposed different scenarios on the microbial-community level functional responses to environmental change based on resilience and historical contingencies. The authors suggested that functional resilience in soil microbial communities can result from: (a) individual level physiological plasticity; (b) shifts in community composition; or (c) rapid evolution which results in better adaptation.

Microbes can adapt to the change in environmental conditions through multiple mechanisms and there is therefore, a lot of uncertainty on the shape of their response to environmental changes. Soil microbial communities generally show functional plasticity wherein individuals in the community adjust themselves to a certain degree to a natural change in the environment. A community that consists of diverse members with versatile physiology will be more resistant to a change in the environment (Evans and Hofmann, 2012). On the other hand, low physiological and functional plasticity or trait breadth within local species assemblages may preclude community acclimatization via shifts in relative abundance of native taxa (Hawkes and Keitt, 2015). For example, ecological trade-offs such as those imposed by drying-rewetting cycles alters community composition, which in turn influences ecosystem processes measured in terms of rates of C mineralization independently of moisture effects (Hawkes *et al.,* 2011; Yuste *et al.,* 2011). Alternatively, compositional shifts may have no impact on ecosystem processes if microbial taxa are functionally redundant (Allison and Martiny, 2008). A number of factors are known to influence the resistance of microbial diversity and communities to disturbances, which make it difficult to predict microbial responses to specific stressors (Bissett *et al.,* 2013). A recent global dryland study from Delgado-Baquerizo *et al.,* 2017a suggest that functional resistance was reported to be linked to microbial community compositions but not diversity or abundance. The study further suggested that the effect of particular microbial taxa on multifunctionality resistance could be controlled by altering soil pH. Contrastingly, other studies reported that microbial diversity promotes functional resistance after environmental perturbations (Girvan *et al.,* 2005; Awasthi *et al.,* 2014).

Theoretical models have suggested multiple relationships between diversity and stability (Cleland *et al.,* 2012) with several hypotheses about the importance of biodiversity for the stability of ecosystems highlighted (Figure 1.7):

a) **'rivet popper' hypothesis** (Ehrlich and Ehrlich, 1981): This states that within an ecosystem elimination of a certain number of species may not impact the stability of the system. However, the next species that disappears, depending on which species that is, will cause the system to become unstable. In this hypothesis, the diversity is compared to the rivets on an airplane: each species plays a small but significant role

in the working of the whole, and the loss of any rivet weakens the plane by a small amount. If too many rivets are popped the plane will crash, or in the case of diversity loss a vital function will collapse (Figure 1.7).

b) **functional redundancy hypothesis** (Walker, 1992): This hypothesis is based on the division of the ecosystem's species into functional groups, *i.e.* a set of species having similar effects on a specific ecosystem process or similar responses to environmental conditions (Hooper *et al.,* 2005). In every group, there is one species that is optimally adapted to the prevailing environmental conditions and that reaches the highest abundance. Other species in that group seem to be redundant, since they have the same function. However, if the environmental conditions change, one of the redundant species could turn to be the one that is better adapted to the new conditions, and would take over as the most important species.

c) **idiosyncratic hypothesis** (Lawton, 1992): This suggests that stability changes when diversity changes, but the magnitude and direction of change is unpredictable, because the roles of individual species are complex (Lawton, 1997; Figure 1.1).

d) **keystone species** (Paine, 1966): According to this hypothesis, only one species has been reported to be crucial for the stability of the system (Figure 1.7). The impacts of a keystone species on its community or ecosystem are large and disproportionate to its abundance (Paine, 1966).

e) **insurance hypothesis** (Yachi and Loreau, 1999): The **Insurance hypothesis** put forth by Yachi and Loreau (1999) states that diversity ensures ecosystem functioning in face of environmental fluctuations because multiple species could buffer the system against the loss of other species. In this context species, functional traits assume more importance than species richness.

Despite of the strong theoretical background we lack empirical evidence to link soil microbial diversity and the resistance of multiple ecosystem functions to global change.

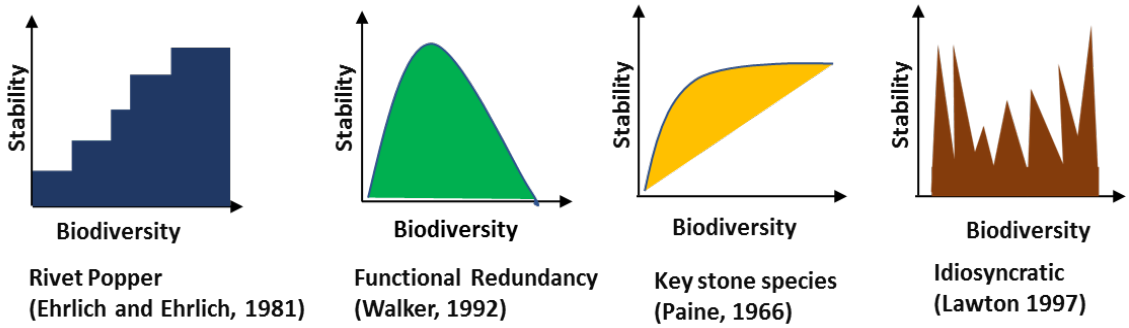


Figure 1.7. Schematic summary of biodiversity-stability hypotheses.

1.5. Knowledge gaps in the link between microbial diversity and the resistance of soil functions to global change

The microbial community is essential for ecosystem functioning but is considered a black box in predictive ecosystem and climate models. The reason is partly due to the lack of knowledge and also because microbes are considered functionally redundant, omnipresent and it is assumed (in the models) that changes in the microbial community will not affect soil functionality (Singh *et al.,* 2010; Bodelier, 2011; Trivedi *et al.,* 2013). These assumptions are only valid if the microbial community is resistant, or functionally redundant (loss of microbial taxa is replaced by others capable of carrying out the same function). The problem is that this may not be true in most cases (Singh *et al.,* 2010; 2014). Tests of these issues are scarce, and barring a few studies, no consistent relationship between microbial diversity and functional resistance and resilience has been found (Peter *et al.,* 2011; Singh *et al.,* 2014; Phillippot *et al.,* 2013). There are very few studies that have directly manipulated soil microbial diversity. This is partly because the soil system is extremely diverse (Torsvik *et al.,* 1994).

Most of the developed theories are based on the study of above-ground ecosystems, so it is important to understand the drivers of specialised microbes in natural ecosystems. Previously, several studies have claimed that plant biomass, productivity and/or nutrient retention increases with plant species diversity (Tilman *et al.,* 1997a; Hooper and Vitousek, 1998; Symstad *et al.,* 1998), although there are exceptions (Hooper and Vitousek, 1997; Wardle *et al.,* 2003). Some studies have also found positive effects of diversity on some aspects of the stability of plant (Frank and

McNaughton, 1991; Tilman and Downing, 1994; Tilman, 1996), aquatic (McGrady-Steed *et al.,* 1997), and microbial (Naeem and Li, 1997) communities. Because of the difficulty of manipulating soil microbial diversity directly, most studies have investigated how the diversity of other organisms or factors, which may indirectly alter soil microbial diversity, and affect soil microbes and their function. It is argued that species diversity in nutrient-poor ecosystems is often high, containing different plant species, able to produce a wide range of secondary metabolites and defence compounds (Lambers and Poorter, 1992). I argue that at least a minimum number of species is essential for ecosystem functioning under steady conditions and that a large number of species are essential for maintaining stable processes in the changing environment. Therefore, it is important to further investigate the drivers of ecosystem resistance with explicit consideration of broad and specialised functions, taxonomic and functional communities under changing environments. Such system-level understanding on the major predictors of community richness, composition, abundance and functioning of ammonia oxidizers [ammonia oxidizing archaea (*amoA*) and bacteria (*amoB*)] in terrestrial ecosystems at the global scale is required to improve nutrient cycling modelling and predicting the responses of nitrification to global change.

1.6. Aims and objectives of this research

The overall aim of this thesis was to improve our understanding of (1) the empirical links between taxonomic and functional microbial diversity and multiple ecosystem functions and (2) the empirical and observational links of microbial diversity in driving the response of multiple ecosystem functions to global changes including N deposition, wetting-drying cycles and temperature changes. To accomplish this, both observational studies (environmental gradient) and laboratory controlled experiments were performed, followed by a similar methodological approach where microbial diversity and functions both broad and specific were measured, soil physicochemical properties were determined, diversity and community composition was assessed by using Miseq (Bacteria and Fungi) and TRFLP (*amoA, pmoA, nosZ*) and qPCR was used to measure the abundance of microbial groups. These objectives were reached in four distinct studies. Chapter 1 includes a general introduction to the topic. Chapter 2-4 evaluate the link between microbial diversity and ecosystem functions at multiple spatial scales using both observational and microcosm approaches. Chapter 4-

evaluates the link between microbial diversity and niche differentiation in driving the response of multiple ecosystem functions to global changes in a microcosm experiment Chapter 5 evaluates the major drivers of the diversity of nitrifiers in global drylands. (Figure 1.8). Chapter 5 will synthesise my research findings in a summary that brings together both observational studies and laboratory-controlled experiments. Experimental chapters are elaborated on further below:

Chapter 2: Losses in taxonomic and functional microbial diversity reduce the rate of key soil processes and multi-functionality

Aim: To test the empirical link of the direction and shape of the microbial BEF considering broad, specialised and multiple functions and taxonomic and functional diversity.

Hypothesis: Rates of multiple broad and specialised functions will be positively linked to microbial diversity in a non-redundant fashion.

Chapter 3: Microbial richness independently drives soil multifunctionality

Aim To identify the role and relative importance of microbial richness and composition in predicting multifunctionality.

Hypothesis Microbial richness is important and operates independently from community composition in driving terrestrial multifunctionality.

Chapter 4: Examination of the role of microbial diversity in driving the responses of soil functions to global change drivers including: a) elevated temperature (ambient vs +3°C increase); b) N addition and; c) wetting drying cycle treatments. This study took place in controlled-environment facilities.

Aim: To examine- the resistance of broad and specialised soil functions across soils with different levels of microbial diversity following severe perturbations for both broad and specific microbial functions.

Hypothesis: Higher microbial diversities will be more resistant against perturbations.

Chapter 5: Niche differentiation mediated by vegetation is the major regulator of nitrifying microbial communities across the globe.

Aim: To identify the main environmental drivers of the diversity, abundance and community composition of bacterial and archaeal nitrifiers in global dryland ecosystem.

Hypotheses:

1. Plant canopies strongly influence the distribution of AOA and AOB in drylands in global drylands.
2. AOA organisms might benefit from increases in aridity, while AOB might benefit from increases in ammonium derived from human activities.

Chapter 6: General conclusion and future directions.

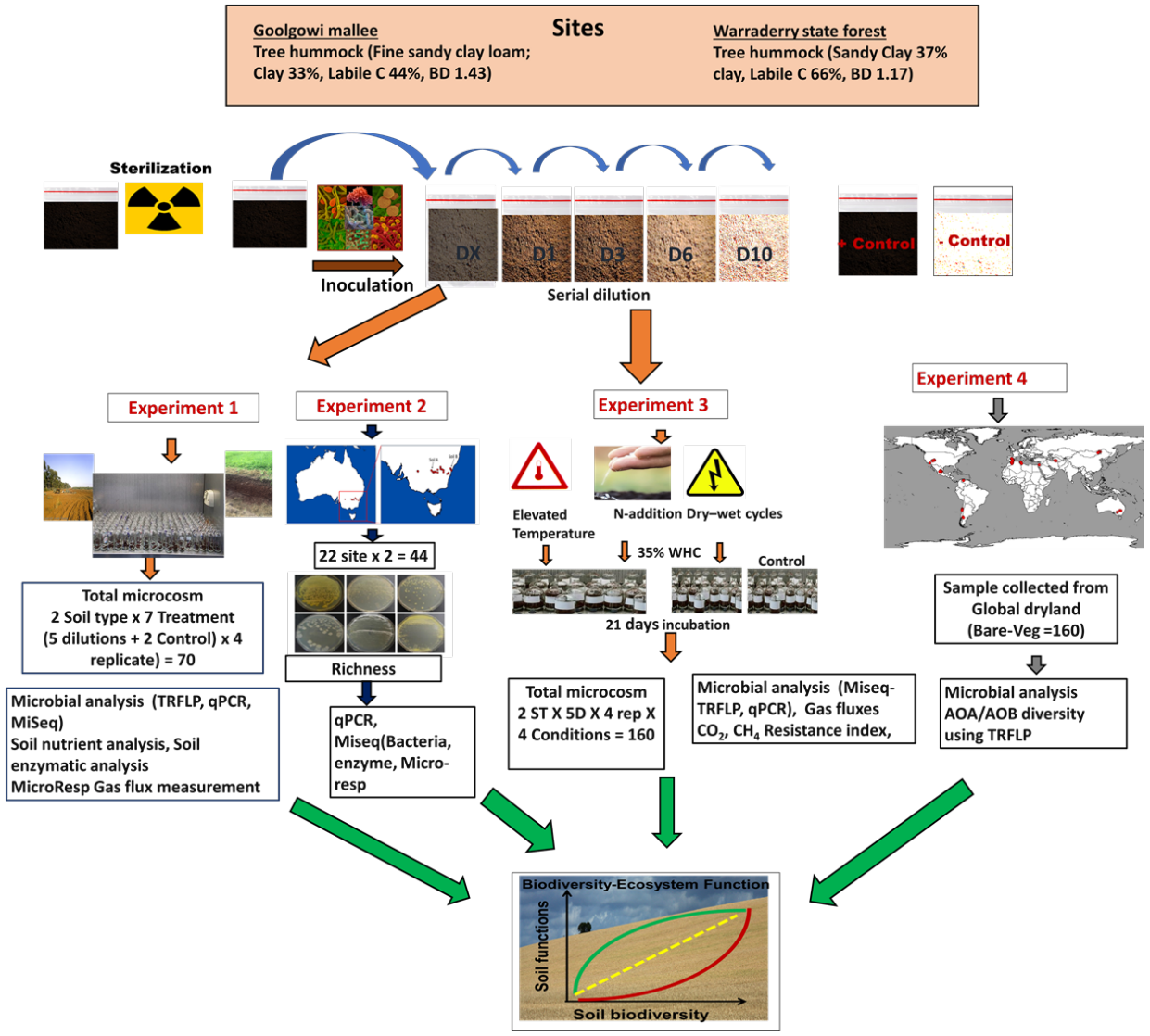


Figure 1.8. Flowchart of experimental plan for Chapters 2-5.

Chapter 2*

Losses in taxonomic and functional microbial diversity reduce the rate of key soil processes and multifunctionality

*This work has been peer reviewed in **Functional Ecology** for publication and a revision is requested.

2.1. Abstract

Microbial communities are the basis of life on earth, catalysing biogeochemical reactions that drive global nutrient cycles and support food and fibre production. However, unlike plants and animals, microbial diversity is not on the biodiversity–conservation or land management agenda due to the assumption of functional redundancy. There is a lack of experimental evidence reporting a link between soil microbial biodiversity and ecosystem functioning (microbial BEF); which limits our capacity to predict changes in ecosystem functioning in response to land use intensification and climate change. Here, we conducted a microcosm experiment to empirically test for a link between taxonomic and functional microbial diversity and ecosystem functioning using the dilution-to-extinction approach. Our results show reductions in functional and taxonomic microbial diversity led to proportional declines in the rates of key ecosystem processes (e.g. CH_4 consumption), and multiple ecosystem functions related to carbon, nitrogen and phosphorus cycles simultaneously (i.e. multifunctionality). Statistical modelling provided evidence for a lack of redundancy in the soil microbial BEF relationship. These results provide strong experimental evidence to support that microbial food web diversity is critical for maintaining the rates of key processes and multiple ecosystems functions in terrestrial ecosystems, similarly to plant and animal diversity. Moreover, our findings suggest that any loss in microbial diversity resulting from climate change and land use intensification will likely reduce key soil processes and multifunctionality, negatively impacting the provision of ecosystem services such as climate regulation, soil fertility and food and fibre production by terrestrial ecosystems from which future human generations depend.

Key words: Microbial diversity; Ecosystem functions; Multi-functionality; Nutrient cycling; Ecology theory; Functional redundancy.

2.2. Introduction

Experimental and observational approaches over the last 20 years have led to the conclusion that plant biodiversity is positively linked to ecosystem functioning (Tilman *et al.,* 1997a; Loreau *et al.,* 2001a; Hooper, 2005; Duffy *et al.,* 2015). Predictions of a decline of terrestrial biodiversity have raised substantial concerns over the consequences that losses in microbial biodiversity may have on key

ecosystem processes and functions, which regulate the provision of ecosystem goods and services such as nutrient cycling, food production, climate regulation and ultimately affect human well-being (Hooper *et al.,* 2005; Cardinale *et al.,* 2011; 2012; Díaz *et al.,* 2011; Wall and Nielsen, 2012). Soil microbes are the most abundant and diverse organisms on the planet (Fierer and Jackson, 2006; Singh *et al.,* 2009), they drive almost every ecosystem function in terrestrial ecosystems including, but not limited to, nutrient cycling, primary production, litter decomposition and climate regulation (van der Heijden *et al.,* 2008; Bodelier 2011; Wagg *et al.,* 2014; Bardgett and van der Putten 2014). Despite their importance, the relationship between soil microbial diversity and ecosystem functions (i.e., soil microbial BEF) remains poorly understood (Graham *et al.,* 2016). Some evidence of a strong relationship between soil microbial diversity and ecosystem functions based on observational correlational studies have been reported from local environments to a global scale (He *et al.,* 2009; Colombo *et al.,* 2015; Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a; d). However, observational links have been questioned because of their inability to conclusively establish a cause-and-effect relationship between diversity and process outcomes. Nonetheless, there is evidence supporting the microbial BEF relationship from freshwater ecosystems (e.g. Peter *et al.,* 2011; Delgado-Baquerizo *et al.,* 2016) where microbial diversity is presumably lower than in more complex terrestrial environments (Zeglin, 2015).

Lack of strong experimental support for the soil microbial BEF relationship is not solely due to a small number of studies, but also from apparently inconsistent results from those studies. Several studies reported that soil microbial diversity promoted single ecosystem functions (van Elsas *et al.,* 2012; Philippot *et al.,* 2013; Vivant *et al.,* 2013), but others have reported no or weak relations (Griffiths *et al.,* 2000; 2001; Wertz *et al.,* 2006). These contradictory results may be the consequence of three major gaps in previous studies that attempted to assess the microbial BEF relationship. Firstly, previous experimental approaches did not statistically account for the role of microbial biomass and composition in the interpretation of the microbial BEF results (Peter *et al.,* 2011; Vivant *et al.,* 2013). Secondly, we lack studies that simultaneously and experimentally evaluate the role of both taxonomic and functional diversity of soil bacteria and fungi in driving ecosystem functioning. Finally, most studies focused on one or several soil functions only, which ultimately may have led

to the reported contradictory results; multiple ecosystem functions need to be considered simultaneously to achieve an integrative understanding on the role of biodiversity for soil functioning (Byrnes *et al.,* 2014). Thus, any attempt to assess the link between microbial diversity and ecosystem functions needs to consider multiple taxonomic and functional gene markers and ecosystems functions simultaneously. The link between soil biodiversity (i.e. soil fauna and microbes together) and multifunctionality has been previously reported using a manipulative experimental approach (Wagg *et al.,* 2014); however, we lack empirical evidence for the soil microbial diversity and multifunctionality relationship. Clarifying the role of microbial diversity for ecosystem functioning is of paramount importance if we intend to develop an appropriate conceptual framework for the impact of microbial diversity loss on ecosystem function and further identify the consequences for humanity.

In addition to the three major gaps raised above, we also lack a comprehensive understanding on the shape of the microbial BEF relationship. In fact, the significance of microbial diversity losses for ecosystem functioning is currently challenged by the concept of functional redundancy (Loreau, 2004). Coupled with the large microbial diversity found in soils (Bradgett and van der Putten 2014; Orgiazzi *et al.,* 2016), functional redundancy is believed to occur when a loss in soil microbial species does not necessarily alter the rate of ecosystem functioning because the same functions can be performed by multiple species (Loreau, 2004). However, recent evidence from fresh water ecosystems suggest a lack of redundancy for the microbial BEF relationship of both broad (conducted by many microbial species) and specialised (conducted by few microbial species) functions (Delgado-Baquerizo *et al.,* 2016). Little is known on whether the knowledge from aquatic ecosystems may apply to terrestrial environments as the shape of the soil microbial BEF remains largely unexplored and poorly understood.

In terrestrial ecosystems, it has been hypothesized that certain ecosystem processes are likely to be more sensitive to changes in microbial diversity than others (Schimel *et al.,* 2005; chapter 1 Figure 1.1). Specialised functions (Schimel and Schaeffer, 2012; Wood *et al.,* 2016) are most likely to be affected by changes in diversity because they require a specific physiological pathway and/or are carried out by a small group of species possessing specialised functional genes (Schimel *et al.,*

2005; Philippot *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016). In addition, the theoretical importance of microbial diversity has been highlighted in the instance of aggregated functions – those functions that require the cooperation of multiple microbial communities from different taxonomic groups (Schimel *et al.,* 2005). This concept also applies to the prevalent role of microbial communities in maintaining multiple ecosystems and services simultaneously (combination of multiple specialised and aggregate functions; 'multifunctionality' hereafter) (Wagg *et al.,* 2014; Delgado-Baquerizo *et al.,* 2016a). Drawing on this theoretical knowledge, a proportional rather than redundant microbial BEF relationship would be expected for terrestrial ecosystems. However, we lack studies using experimental evidence for shape of the relationship between the diversity of multiple microbial taxonomic and functional microbes in driving specialised and multiple ecosystem functions simultaneously. Assessing the real importance of soil microbial diversity in driving soil processes is critical for developing a unified ecologically relevant theory about below-ground ecosystem functioning and to improve simulation model prediction and management policies for sustainable environments (Reed and Martiny 2007; Delgado-Baquerizo *et al.,* 2016a).

Here, we used the dilution-to-extinction (e.g. Franklin *et al*., 2001; Peter *et al.,* 2011; Philippot *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016) experimental approach on two independent soils from Australia to explore the relationship between microbial diversity and ecosystem functioning. In this study, we explicitly consider the links between: (1) taxonomic diversity (derived from the number of operational taxonomic units) with multifunctionality (calculated as the standardized sum of 17 functions including utilization of C sources; production of extracellular enzymes involved in the C, N, P cycling, CO_2 production and basal respiration, and specialised functions according to Maestre *et al.,* 2012); and (2) microbial functional diversity and the rates of specialised functions (e.g. CH_4 consumption, NO_3 production, and N_2O consumption. All these specialised functions require specific genes that encode enzymes capable of performing these functions which are limited to relatively few microbial species). By addressing the link between microbial taxonomic and functional diversity and multiple and specialised functions we aim to provide, to the best of our knowledge, the most comprehensive evidence on links between microbial diversity and ecosystem functioning in terrestrial ecosystems. Based on the most

accepted soil ecological theories (Schimel *et al.,* 2005), we hypothesized that: (a) experimental losses in functional and taxonomic microbial diversity will lead to reductions in key ecosystem soil processes and multifunctionality, respectively; and (b) given the expected importance of soil functional and taxonomic microbial diversity for key soil processes and multifunctionality, the microbial BEF relationship should follow a non-redundant shape.

2.3. Material and Methods

2.3.1. Site description

To provide replication for the study, we collected soil samples from two sites in Australia with different soil properties. Soil sampling was carried out in March 2014. Soil samples were collected from the top 10 cm from Goolgowi mallee (site A; NSW 33.9667° S, 145.7000° E) and Warraderry State Forest (site B; NSW, 33.7035° S, 148.2612° E), New South Wales, Australia; both of them dominated by *Eucalyptus Spp.* Site characteristics and soil properties for both the soils are presented in Table 2.1.

2.3.2. Microcosm preparation

A complete workflow of our experimental approach is presented in Figure. 2.1. Soil samples from each site were sieved to < 2mm and divided in two portions: (1) soil for sterilization, and (2) soil for microbial inoculum and experimental controls (non-sterilized original soils). The first portion was sterilised using a double dose of gamma radiation (50kGy each) at ANSTO Life Sciences facilities, Sydney. Gamma radiation was used as it is known to cause minimal change to the physical and chemical properties of soils compared with other methods such as autoclaving (Wolf *et al.,* 1989; Lotrario *et al.,* 1995). The dilution-to-extinction approach was used to prepare soil microcosms (Peter *et al.,* 2011a; Philippot *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016). A parent inoculum suspension was prepared by mixing 25 g soil in 180 ml of sterilized Phosphate buffer saline (PBS). The mixture was vortexed on high speed for 5 min to mix the contents. The sediment was then allowed to settle for 1 min and serial dilutions were prepared from the suspension. For each soil (soils A and B), 5 dilutions were used as the microbial inoculum to create a diversity gradient; these dilutions were undiluted (10x; DX); 1/10 dilution (D1); $1/10^3$ dilution (D3); $1/10^6$ dilution (D6); and $1/10^{10}$ dilution (D10). Microcosms with non-sterilized soil served as references

but not included in our statistical analyses. A total of 48 microcosms (500g each) (5 dilutions level + 2 control x 4 replicates x 2 soil types) were prepared. Moisture content in these microcosms was adjusted to 50% water holding capacity and maintained (adding sterile water if needed) during the incubation period. These microcosms were established under sterile conditions; aseptic techniques were used throughout the experiment to avoid contamination.

Table 2.1. Environmental characteristics, location, and soil properties of sampling sites.

Characteristics (Soil properties)	**Sampling sites**	
	Site A (**Goolgowei mallee**)	Site B (**Warraderry state forest**)
Rainfall (mm year^{-1})	400	657
Latitude	-34.998025	-33.729918
Longitude	145.726367	148.203347
Soil texture	Clay loam	Sandy clay
Clay %	32	37
Bulk density	157	117
pH (H_20)	6.01	5.68
Total carbon (%)[a]	1.73	1.84
Total nitrogen (%)[a]	0.13	0.15
NH_4^+-N (mg kg^{-1}) [c]	14.44	27.25
Olsen_P (mg kg^{-1}) [d]	5.23	4.9
MB_P (mg kg^{-1}) [e]	9.58	6.93

[a] Measured with a CN analyzer (Leco CHN628 Series, LECO Corporation, St Joseph, MI, USA) following the Dumas combustion method.

[b] Measured as described in Anderson and Ingram (1993).

[c] Analysed calorimetrically (Sims *et al.,*1995) from K_2SO_4 0.5 M soil extracts using a 1:5 soil: extract ratio as described in Jones and Willett (2006).

[d] Measured by $NaHCO_3$ extracts of the Olsen method (Watanabe & Olsen, 1965).

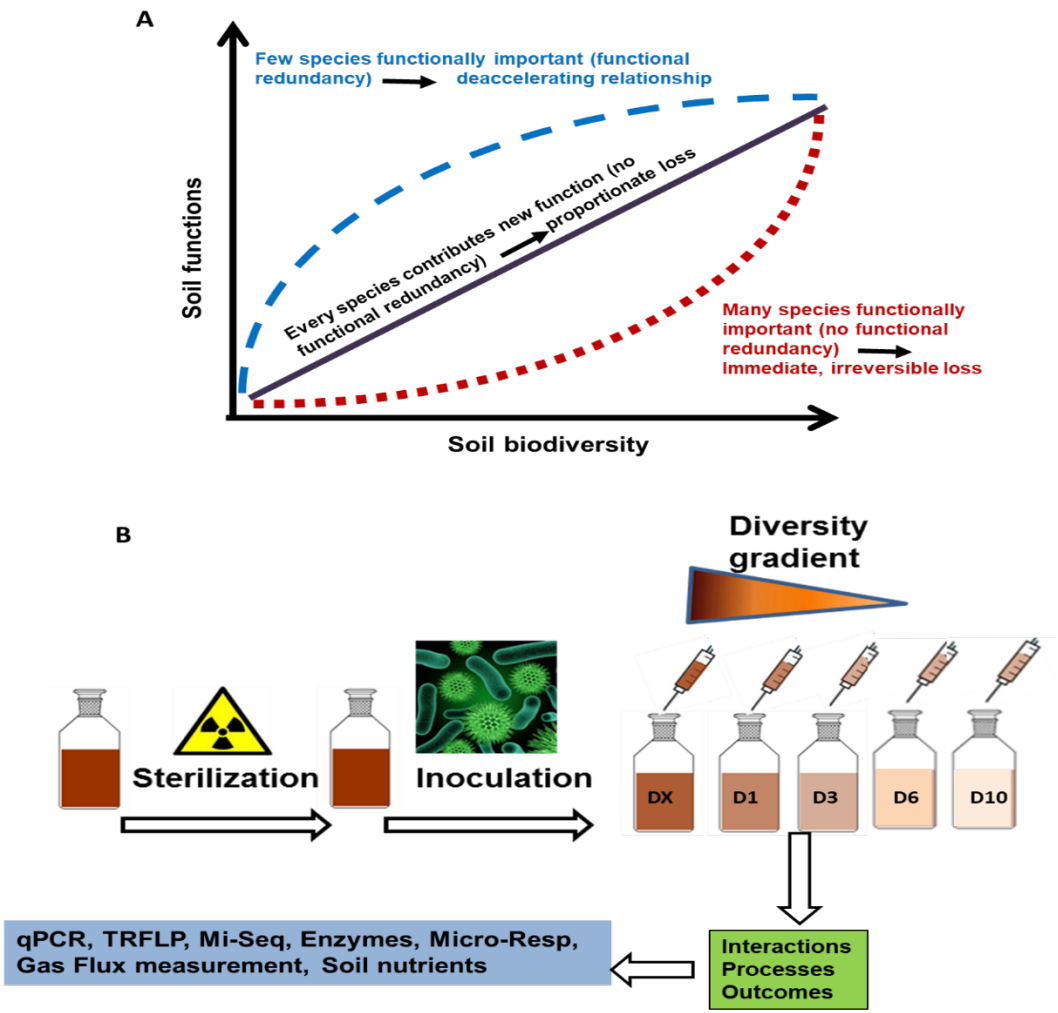


Figure 2.1. Theoretical relationship between microbial diversity and ecosystem functions (a) and workflow of the experimental set-up followed in the study (b).

Soil microcosms were incubated at 20°C for 6 weeks for microbial colonization and biomass recovery as described in Delgado-Baquerizo *et al.,* (2016). This is critical for the dilution-to extinction method (Delgado-Baquerizo *et al.,* 2016); microcosms with the highest dilution are expected to have the lowest microbial biomass initially, which may affect any interpretation regarding the relationship between microbial diversity and ecosystem functioning. Biomass recovery is needed to properly address the link between microbial diversity and ecosystem functioning controlling for biomass interferences. Thus, we started measuring microbial diversity and functions only after the microbial biomass had recovered across all dilutions of the microcosm. We also examined the major differences in microbial composition across the different dilution levels and statistically controlled our analyses for these issues as explained below.

2.3.3. Microbial community analysis and quantification

2.3.3.1. DNA extraction

Total genomic DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) as per the manufacturer's instructions, with a slight modification in that a FastPrep bead beating system (Bio-101, Vista, CA, USA) at a speed of 5.5 m s^{-1} for 60 s was used at the initial cell-lysis step. The quantity and quality of extracted DNA were checked photometrically using a NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.3.3.2. Abundance of total bacteria and fungi and functional genes

The abundances of total bacteria (using the 16s rRNA gene), fungi (using the ITS gene), ammonia-oxidizing archaea (using *amoA* gene), N_2O reducing bacteria (using *nosZ* gene), and methanotrophs (using *pmoA* gene) were quantified on a CFX-96 thermocycler (Biorad, USA) using primers and conditions described in Supplementary Table 2.1. Standard curves were generated using ten-fold serial dilutions of plasmids containing the correct insert of each respective gene. The 10 µl reaction mixture contained 5 µl SensiMix SYBR No-ROX reagent (Bioline, Sydney, Australia), 0.3 µl of each primer (20 mM), 0.4 µl BSA (20 mM), and 1 µl of diluted template DNA (1-10 ng). Melt curve analyses were conducted following each assay to verify the specificity of the amplification products, and the PCR efficiency for

different assays ranged between 86% and 99%. Amplified products were run on 2% agarose gel to confirm product size and specificity.

2.3.3.3. Diversity and community compositions of total bacteria and fungi

The bacterial and fungal communities were examined using 16S rRNA [primer set 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC); Herlemann *et al.,* (2011)] and internal transcribed spacer region (ITS1) of fungal rRNA gene [primer set ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC); McGuire *et al.,* (2013)], respectively using the Illumina MiSeq® system platform at the Next-Generation Sequencing Facility at Western Sydney University (Richmond, Australia).

2.3.3.4. Diversity of functional genes

T-RFLP for *pmoA*, *amoA* (only for ammonia oxidising archaea), and *nosZ* were performed using florescent labelled primer pairs A189F/Mb661R (Dunfield *et al.,* 2003, Stralis- Pavese *et al.,* 2004), CrenamoA23F/CrenamoA616R (Nicol *et al.,* 2008), and nosZ121f/nosZ1622 (Enwall *et al.,* 2005), respectively. The PCR reactions in a 50 µl mixture contained 2.5 µl of BioTaq DNA polymerase (Bioline, Sydney, Australia), 0.5 µl of each primer (20 mM), 1 µl dNTP mix (20 mM), 5 µl 10×NH_4 reaction buffer, 2 µl BSA (20 mM), 2 µl $MgCl_2$ solution (50 mM), 2 µl of five-fold diluted template DNA (1-10 ng). Thermal-cycling conditions for each gene are provided in supplementary Table 2.1. The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, San Louis, CA, USA). The concentrations of PCR products were fluorometrically quantified using the NanoDrop® ND-2000c UV-Vis spectrophotometer. PCR products obtained from individual reactions were digested separately with *Hhal* (for *amoA*), *MspI* (for *nosZ*), *Rsal* (for *pmoA*) restriction enzymes in 10 µl volume containing approximately 200 ng purified PCR products, 20 µl of the restriction enzymes (BioLabs, Sydney, Australia), 0.1 µl BSA and 1 µl of 10 × NE Buffer. Digests were incubated at 37°C for 3 h, followed by 95°C for 10 min to deactivate the restriction enzyme. Terminal restriction fragments (TRFs) were resolved on an ABI PRISM 3500 Genetic analyzer (Applied Biosystems, CA, USA).

2.3.4. Assessment of multiple soil functions

A total of 17 functions related to C, N and P cycles were measured including three specialised (NO_3 production, CH_4 consumption, and N_2O consumption) and broader functions including CO_2 production, basal respiration. We also measured processes or proxy of processes which contribute to broad functions including seven enzyme activities [β-Glucosidase (BG; starch degradation), β-D-celluliosidase (CB; cellulose degradation), β-Xylosidase (XYL; hemicellulose degradation), α-Glucosidase (AG; starch degradation), Phosphatase (PHOS; P mineralization), N-acetyl- β-Glucosaminidase (NAG; chitin degradation) and Leucine amino-peptidase (LAP; protein degradation)], and induced respiration from carbon substrates (including a range of compounds from labile to recalcitrant: glucose, alanine, cellulose, protocatechuic acid and lignin; Schimel *et al.,* 2005; Singh *et al.,* 2014).

2.3.4.1. Gas flux measurement

Soil gas flux for nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) were monitored by placing 20 g of soil from each microcosm in a glass jar (12 cm depth, 75 cm diameter, Ball, USA), and then sealed with a gas-tight lid, which had a rubber stopper in the middle. Gas samples were collected in 25 ml gas-tight syringes at 0, 30 and 60 min after sealing. Gases were measured with an electron capture detector in an Agilent-7890a gas chromatograph equipped with a flame ionization detector (FID) and an electron capture detector (ECD) (Agilent Technologies, Wilmington, DE, USA). A linear model was then applied to estimate the gas flux rate inside the jar headspace (Matthias *et al.,* 1980) and expressed as micrograms of N_2O-N/ CH_4-C per square meter per hour (mg N_2O-N/CH_4-C m^{-2} h^{-1}) or milligrams of CO_2-C per square meter per hour (mg CO_2-C m^{-2} h^{-1}).

2.3.4.2. MicroResp analysis

MicroResp™, as described by Campbell *et al.,* (2003), was used to measure community respiration and substrate-induced respiration. Soil samples were placed in a 96- deep well plate delivering approximately 0.4 g of soil to each well, and incubated for two days at RT in the dark before the assay was carried out. A total of seven different carbon substrates (cellulose, glucose, lignin, alanine, oxalic acid, protocatechuic acid, tween80) and water (30 mg g^{-1} soil water) were used and the rate of respiration determined over a 6 h incubation period at 25°C as previously described

by Campbell *et al.,* (2003). After incubation, the CO_2 production rate (CO_2-C g^{-1} h^{-1}) was calculated based on the change in absorbance (A_{570}) of the indicator. All C sources were obtained from Sigma Aldrich (Australia) and dissolved in deionised water and filter sterilised. Substrates were added to soils in quadruplicate at concentrations of either 30, 15 or 1 mg ml^{-1}, depending on the solubility of the compound in water. Sterile deionised water was delivered to four replicate wells to measure basal respiration. The absorbance of CO_2 detection plates was initially determined prior to soil incubation and then deep-well plates were sealed with the pre-read CO_2 detection plates and incubated at 25 °C for 6 h in the dark, as recommended by the manufacturer (Macaulay Scientific Consulting, UK). The change in optical density after incubation was then measured on a spectrophotometer microplate reader (EnSpire® 2300 Multilabel Reader, Perkin Elmer, USA) at a wavelength of 570 nm. The rate of CO_2 respiration expressed per gram of soil per well was calculated using the formula provided in the MicroResp™ manual (Macaulay Scientific Consulting, UK). The total substrate respiration responses were calculated by subtracting the water response from each individual substrate response and then summing the single responses for each sample. After incubation, the CO_2 production rate (CO_2-C g^{-1} h^{-1}) was calculated based on the change in absorbance (A_{570}) of the indicator plate measured on a spectrophotometer microplate reader (EnSpire® 2300 Multilabel Reader, Perkin Elmer, USA).

2.3.4.3. Extracellular enzyme analysis

We determined the activities of six enzymes involved in C, N, and P as additional proxies of ecosystem functions (Bowker *et al.,* 2011; Bailey *et al.,* 2013). Through extracellular enzyme activities, soil microbial communities regulate soil organic matter turnover and nutrient cycling in terrestrial ecosystems (Bell *et al.,* 2013; Trivedi *et al.,* 2016). Moreover, extracellular enzymatic activities are also considered a good indicator of nutrient demand by plant and microorganisms in soil (Bell *et al.,* 2013). Activities of AG, BG, XYL, CB, PHOS and NAG were measured using 4-methylumbelliferyl (MUB) substrate while LAP activity was measured using 7-amino-4-methylcoumarin (MUC) substrate. Both substrates yield highly fluorescent cleavage products upon hydrolysis (Wallenstein *et al.,* 2008). All enzyme assays were set up in 96-well microplates as described by Bell *et al.,* (2013). Twelve replicate wells were prepared for each sample and each standard concentration. The assay plate

was incubated in the dark at 25 °C for 3 h to mimic the average soil temperature. Enzyme activities were corrected using a quench control (Wallenstein *et al.,* 2008). Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 460 nm emission on a spectrophotometer microplate reader (EnSpire® 2300 Multilabel Reader, Perkin Elmer, USA). Fluorescence was converted to potential enzyme activity in nmol g^{-1} soil dry weight h^{-1} based on the MUB standard curve as reported by Bell *et al.,* (2013).

2.3.4.4. Potential nitrification rate (PNR)

Potential nitrification rate (PNR) was assessed using the chlorate inhibition soil-slurry method as previously described (Hu *et al.,* 2015, details in supplementary information). In brief, 5 g of fresh soil was placed in a 50-ml tube containing 20 ml of 1 mM $(NH_4)_2SO_4$. Potassium chlorate with a final concentration of 50 mg L^{-1} was added to inhibit nitrite oxidation. The suspension was incubated in the dark for 24 h at 25°C, NO_2^--N in the soil was extracted using 5 ml of 2 M KCl, then the NO_2^--N concentration was determined spectrophotometrically at 540 nm with N-(1-naphthyl) ethylene diamine dihydrochloride as an indicator. Potential nitrification rate was calculated as the linear increase in nitrite concentrations during incubation.

2.3.5. Data analysis

2.3.5.1. Diversity of total bacteria and fungi

Miseq data analysis was performed using the 'Quantitative Insights into Microbial Ecology' (QIIME v 180) software package (Caporaso *et al.,* 2010). Paired ends were joined using the 'SeqPrep' method (https://githubcom/jstjohn/SeqPrep). Barcode, linker primer and reverse primer sequences were removed from the raw sequence reads while setting minimum quality score of Q20. Sequences were then screened for the presence of chimeric sequences using USEARCH (Edger, 2010 and Edger *et al.,* 2010) against the Green Genes database V13_8 (McDonald *et al.,* 2012 and Werner *et al.,* 2012). Similar sequences were binned into operational taxonomic units (OTUs) using 'UCLUST' method (minimum pairwise identity of 97%, Edger, 2010). Taxonomic designations of OTUs were assigned using Greengenes, as described by McDonald *et al.,* (2012) and Werner *et al.,* (2012) using UCLUST. The number of sequences per sample was rarefied to ensure even sampling depth across all samples. The diversity indices [Shannon diversity, Chao 1, abundance-based coverage

estimators (ACE)] were then calculated on relative abundance OTU tables. We used the Shannon diversity matrix to further explore relationships between diversity and functions; it is reported to be a better predictor for microbial diversity as it considers both species richness and evenness within the samples (Delgado-Baquerizo *et al.,* 2016). Furthermore, Shannon diversity has been widely used in animal (Saleem *et al.,* 2012; Lemieux *et al.,* 2014 and references within) and plant (Zhang *et al.,* 2012; Zhu *et al.,* 2016 and references within) ecology to explore the relationship between diversity and ecosystem functions. Taxonomic richness, *i.e.* the number of taxa present, was expressed as the number of OTUs. NMDS and PERMANOVA analysis were performed by PRIMER v 6113 and PERMANOVA^{+} (PRIMER-E, Plymouth, UK) software (Clarke and Gorley 2006).

2.3.5.2. Diversity of functional genes

Raw T-RFLP data were analysed using the GeneMapper v5 software (Applied Biosystems) with the advanced peak detection algorithm. A GeneScan 600-LIZ internal size standard was applied to each sample. The T-RFLP profiles were analyzed using a local southern size calling method (peaks between 50 and 650 bp in size) and a peak amplitude threshold setting of 50, using Genemapper version 40 (Applied Biosystems). TRF peaks that differed by less than 1 bp were binned into the same fragment. The relative fluorescence abundances of all TRFs were exported for microbial community analysis. A binary table of peak presence/absence was generated and exported for further statistical analysis for determining the Shannon diversity index (Singh *et al.,* 2006). All multivariate statistical analysis of T-RFLP data were conducted using PRIMER v 6113 and PERMANOVA^{+} (PRIMER-E, Plymouth, UK) (Clarke and Gorley, 2006), data underwent standardisation and log +1 transformation, taxonomic distinctness was assessed using the Bray-Curtis dissimilarity measure (Bray and Curtis, 1957).

2.3.5.3. Multifunctionality index

To assess the performance of the overall microbial diversity, we averaged the standardized scores (z scores) of all ecosystem functions to obtain a single index of ecosystem multifunctionality (Maestre *et al.,* 2012; Wagg *et al.,* 2014). To obtain a quantitative multifunctionality index for each site, we first normalized (log- transform when needed) and standardized all the functions measured using the Z-score

transformation. These standardized ecosystem functions were then averaged to obtain a multifunctionality index. This averaging multifunctionality index is widely used in the multifunctionality literature and provides a straightforward and easy-to-interpret measure of the ability of different communities to sustain multiple functions simultaneously (Maestre *et al.,* 2012; Wagg *et al.,* 2014; Bradford *et al.,* 2014; Delgado-Baquerizo *et al.,* 2016a). In addition, averaging multifunctionality have been demonstrated to provide similar results to other popular multifunctionality indexes such as multi-threshold approach (see Delgado-Baquerizo *et al.,* 2016, 2016a for comparison) and PCA-based multifunctionality (Maestre *et al.,* 2012).

2.3.6. Statistical Analysis

Differences in diversity and ecosystem function were analysed statistically using analysis of variance (ANOVA). A $P < 0.05$ was considered significant in our ANOVA analysis. Linear regressions were used to evaluate the relationship between diversity and aggregate, specialised and multiple functions. We used Spearman's correlation analysis to evaluate the relationship between diversity of different microbial groups and individual functions. We conducted partial correlation analysis to evaluate whether microbial biomass (qPCR data) and community composition (1st axis of NMDS plots) also influenced the relationship between microbial diversity and ecosystem function in this study (see Delgado-Baquerizo *et al.,* 2016 for a similar approach). All analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) and the resulting figures were generated by using Sigmaplot 12.0.

2.3.6.1. Modelling the shape of the microbial BEF

We assessed the direction (i.e. positive, negative or invalid) and shape of the relationship between: (a) microbial diversity (total bacteria, fungi, and multi-diversity determined using Miseq) and multifunctionality; and (b) diversity of functional groups [ammonia-oxidizing archaea (using *amoA* gene), N_2O reducing bacteria (using *nosZ* gene), and methanotrophs (using *pmoA* gene) determined by TRFLP analysis)] and their associated functions at the two sites. The best shape describing the relationship between bacterial diversity and functioning was identified by fitting three different functions that involve different biological interpretations (logarithmic, linear, and exponential; Cardinale *et al.,* 2011; Reich *et al.,* 2012). These functions provide information about the two groups of ecological shapes used to describe the

relationship between microbial diversity and ecosystem functioning (Delgado-Baquerizo *et al.,* 2016). The logarithmic shape describes functional redundancy whereas the linear or exponential shapes describe no functional redundancy depending on each particular case. Best model fits were selected by Akaike information criteria (AICc; Burnham and Anderson 2002) where a lower AICc value represents a model with a better fit. AICc is a corrected version of AIC that is highly recommended when dealing with small sample sizes, as in our case (Burnham and Anderson 2002). We further used a difference in AICc values of 2 (ΔAICc > 2) to determine substantial differences between models (Burnham and Anderson 2002; Burnham *et al.,* 2011). In some cases, we were able to identify a particular model (i.e, linear, logarithmic and exponential) characterizing the shape of the microbial BEF. In these cases, a particular model was substantially different (ΔAICc > 2) to the other two possibilities. In other cases, we were not able to identify a unique model characterizing the shape of the microbial BEF, but we were still able to differentiate between functional groups: functional redundancy vs. no functional redundancy. This case implies that logarithmic model is different to the linear and exponential ones (ΔAICc > 2), but we are not able to differentiate between linear and exponential (ΔAICc > 2) as best models shaping the microbial BEF. The analysis was performed using R package (https://www.r-project.org/).

2.4. Results

2.4.1. Recovery of microbial biomass and community structure

After a six-week incubation, we measured the microbial abundance - as a proxy for biomass - by qPCR of taxonomic (bacteria: 16S rRNA; fungi: ITS sequence) and functional genes (N_2O reducing bacteria using *nosZ* gene; methanotrophs using *pmoA* gene and ammonia oxidising archaea and bacteria using (*amoA*) in our microcosms from two different soils (Soil A and B; see Methods). Our results showed globally similar abundances in all dilutions for bacteria, fungi and all measured functional groups in both soils (Supplementary Figure 2.1). It is essential that all dilution microcosms should have a comparable abundance of microbes as ecosystem functions are highly correlated with microbial abundance (Reeve *et al.,* 2010). These analyses showed that microbial abundance had successfully recovered in all diversity dilution microcosms.

Our nonmetric multidimensional scaling [NMDS; Supplementary Figure 2.2a-2.2b)] and PERMANOVA (Supplementary Table 2.2) showed some general differences in microbial composition of total bacteria, total fungi and specialised groups across dilution levels in soils from both sites (Site A and B). However, further post-Hoc analyses comparing microbial composition at a finer scale provided evidence for a lack of significant differences in microbial composition across different dilution levels Supplementary Table 2.3). These observations ensured that biomass and the community composition between dilutions were not influencing our results on microbial BEF relationships. Even so, the relationship between microbial diversity and ecosystem function was statistically controlled by these important factors to further verify these results.

2.4.2. Microbial community diversity

2.4.2.1. MiSeq analysis for total bacteria and fungi

Samples were rarefied to 22,342 and 16,521 sequences for bacteria and fungi, respectively. The decrease in microbial diversity between dilution treatments was supported by decreases in Shannon diversity and species richness (Figure 2.2). Similar results were obtained with the use of other diversity metrics such as Chao1 and ACE (supplementary Table 2.4). As expected, the undiluted soil harboured the higher average species richness (measured in terms of number of OTUs) in samples from both sites; bacterial richness was reduced by 60% and 80% in the D10 dilution as compared to undiluted microcosms for Site 1 and Site 2, respectively. Similar trends were observed for fungal richness which was reduced by 44% and 55% in the D10 dilution as compared to undiluted microcosms for Site 1 and Site 2, respectively (Figure 2.2).

2.4.2.2. TRFLP analysis of functional microbial groups

Similar to the microbial abundance results described above, TRFLP analysis showed significant differences in the richness and diversity of N_2O reducing bacteria (using *nosZ* gene); methanotrophs (using *pmoA* gene); and ammonia oxidising archaea (using *amoA*) for both sites (Figure 2.3). The diversity of *amoA, nosZ,* and *pmoA* genes reduced by 45% and 57%; 80% and 77%; and 60% from undiluted to D10 dilutions for Site 1 and Site 2, respectively. Similarly, the richness of all the functional groups was reduced by more than 75% from undiluted to D10 dilutions for both sites.

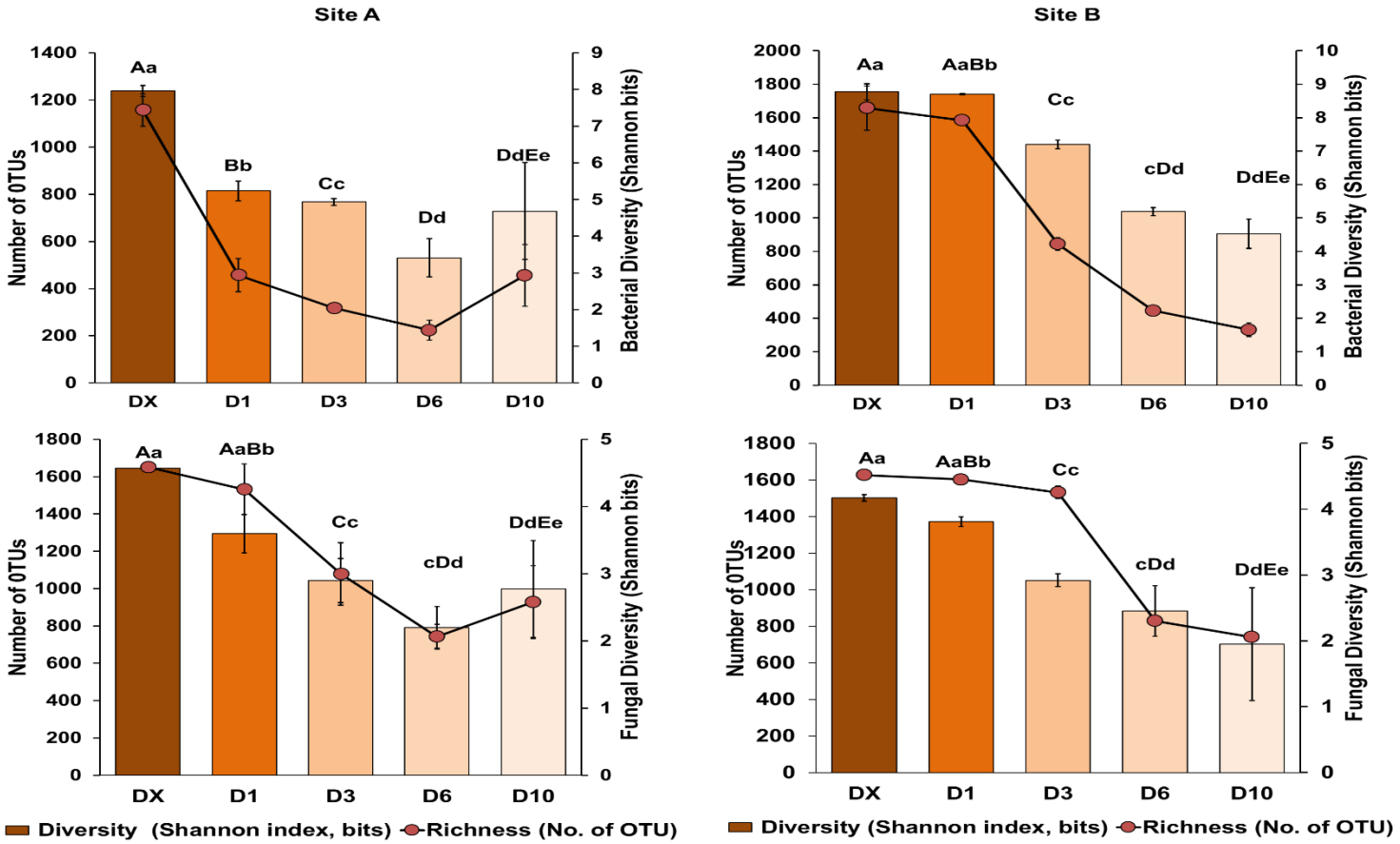


Figure 2.2. Bacterial and fungal Shannon diversity and number of operational taxonomic units (OTUs) in diversity gradients microcosms as determined by MiSeq analysis for two sampling sites. Each bar represents the different diversity of soil at each dilution and error bars represent the standad error. The solid line indicates the number of OTUs and error bars within the line indicate the standard error. Different capital and small letters indicate statistically significant differences ($P < 0.05$) between Shannon diversity and the number of OTUs, respectively. Dx represents the positive control. The negative control is not included in this figure. The left side of the figure represents site A and the right side represents site B.

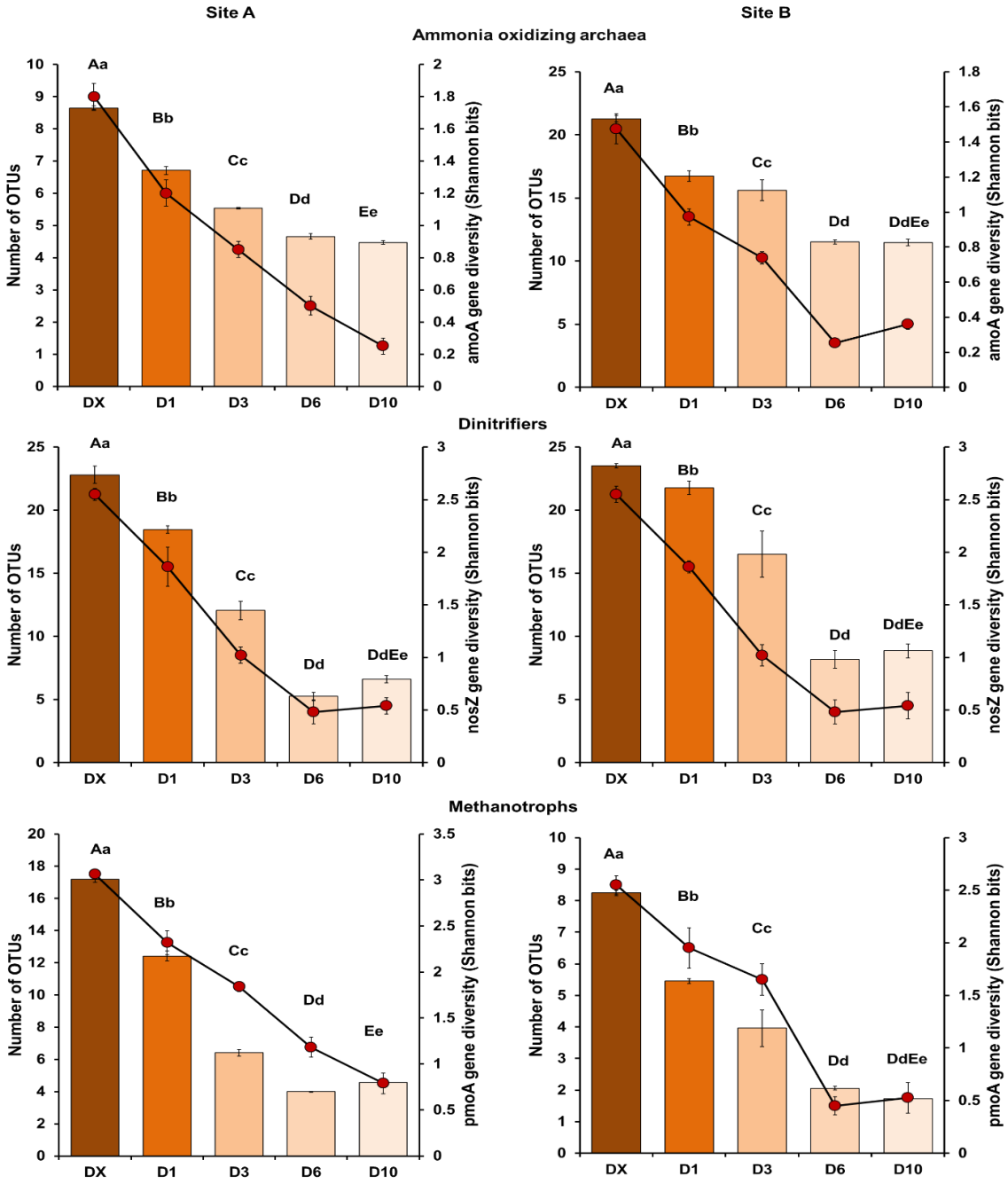


Figure 2.3. Diversity and number of operational taxonomic units (OTUs) in diversity gradients microcosms of ammonia oxidising archea (using the *amoA* gene); methanotrophs (using the *pmoA* gene), and dinitrifiers (using the *nosZ* gene) as determined by TRFLP analysis for two sampling sites. Each bar represents the different diversity of soil at each dilution and error bars represent the standard error. The solid line indicates the number of OTUs and error bars within the line indicates the standard error. Different capital and small letters indicate statistically significant differences ($P < 0.05$) between Shannon diversity and the number of OTUs, respectively. Dx represents the positive control. The negative control is not included in this figure. The left side of the figure represents site A and the right side represents site B.

Note that the AOB (ammonia-oxidizing bacteria) community was not included in our study because of low AOB abundance according to our results from qPCR. Thus, the PCR products did not satisfy the requirements for T-RFLP. The same problem has been reported in a previous study including samples from a region near our sampling locations (Liu *et al.,* 2016).

Despite low resolution, TRFLP has been used to determine the diversity-function relationships in several studies (Korhonen *et al.,* 2011; Giaramida *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016). This technique is especially important for the determining the diversity and composition of specialised microbial groups using functional genes wherein the diversity is low and the groups represent only a minor fraction of the overall microbial community (Stralis *et al.,* 2004; Singh and Tate 2007; Hu *et al.,* 2015a). Recent studies have provided evidence that TRFLP and next generation sequencing (including 454 pyrosequencing and MiSeq) provide similar results in terms of diversity estimation (Van Dorst *et al.,* 2014; Delgado-Baquerizo *et al.,* 2016). Overall, we were able to create strong diversity gradients in our microcosms and these provided us with an appropriate system to explore the functional responses of changes in microbial diversity and the consequences of these changes for the functioning of the soil ecosystem.

2.4.3. Relationship between microbial diversity and functions

2.4.3.1. Links between taxonomic diversity of fungi and bacteria with multifunctionality

Multifunctionality in both soils was positively related to the taxonomic diversity of bacteria and fungi ($P<0.001$), as demonstrated by different models (Table 2.2). Although all the models significantly explained the variance; the best fit was observed for linear models (Figure 2.4 Table 2.2). The relationships between bacterial and fungal diversity with multi-functionality were similar across soil types, (r^2 for linear models = 0.79 and 0.80 for bacteria, fungi, respectively at Site B), (r^2 for linear models = 0.72, 0.717 for bacteria and fungi, respectively at site A). The relationship between microbial diversity and multifunctionality showed a significant positive relationship even when we combined results from both soil types ($r^2 = 0.79$, $P<0.001$; Figure 2.6). Further statistical modelling suggested a lack of redundancy in the relationship between microbial taxonomic diversity and multifunctionality (Table 2.2). In all

cases, models that describe non-redundancy in BEF relationships (both exponential and linear models) were better (ΔAICc >2) than logarithmic models that describe redundancy. Our analysis also revealed the linear models were better than the exponential models (ΔAICc >2 in all cases) suggesting a proportionate reduction in multifunctionality with decreasing microbial diversity.

2.4.3.2. Links between taxonomic diversity of fungi and bacteria with individual functions

Correlation analysis evaluating the link between taxonomic diversity and single functions for both soils were variable and are available in Table 2.3. The values of the measured functions from different dilutions are presented in Supplementary Figure (2.3-2.5). Production of AG, NAG, PHOS was significantly correlated (P<0.001) with microbial diversity for both soils. Similarly, Finally, utilization of cellulose and protocatechuic acid was correlated with microbial diversity (P<0.001) for both soil types. The production of BG was significantly correlated (P<0.001) with microbial diversity at Site B, however its production was correlated only with fungal diversity at Site A. The production of BG, LAP, XYL and utilisation of glucose was significantly correlated (P<0.001) with microbial diversity only at Site B. In contrast lignin utilisation was linked to microbial diversity only at site A (P<0.001). The production of CO_2, basal respiration and alanine utilisation were not significantly correlated with microbial diversity at both sites (Table 2.3).

2.4.3.3. Links between functional diversity and specialised functions

Relationships between the diversity of functional groups of soil microbes [as determined by TRFLP analysis of functional genes *pmoA, amoA and nosZ*)] and their specialised functions were determined using different models (Table 2.2; Figure 2.5). We observed a significant correlation between the diversity of functional groups and their specialised functions for both soil types using all three models tested (Table 2.2). However, we observed differences in best fitting models that explained the relationship between diversity and specialised functions. For example, linear models best explained the positive relationship between the diversity of the *pmoA* gene and CH_4 consumption (R^2 = 0.68 and 0.93 for Site A and B, respectively). A positive relationship between *nosZ* and N_2O production for Site A and B were best explained

Table 2.2. Model fit statistics and AICc index describing the relationship between microbial diversity and ecosystem functions. Green coloured boxes show the relationship between the diversity of total bacteria (Miseq), total fungi (MiSeq), and multi-diversity (bacteria fungi standardize z score) with multifunctionality. Grey coloured boxes show the relationship between the diversity of specialised microbial groups [determined by TRFLP (a*moA, nosZ* and *pmoA*)] and their associated functions. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely it is that this model is correct. Two models with an ΔAICc value > 2 are substantially different. GG- Goolgowei mallee WSF-Warraderry State Forest.

	Function	Site	Model	R^2	P	AICc	DeltaAICc	Selected Model(s)	Model group
Bacteria	**Multifunctionality**	**GG**	Logarithmic	0.671	<0.001	9.357	3.183		
			Linear	0.72	<0.001	6.174	0	✓	No redundancy
			Exponential	0.606	<0.001	19.24	13.066		
		WSF	Logarithmic	0.755	<0.001	8.134	3.557		
			Linear	0.795	<0.001	4.577	0	✓	No redundancy
			Exponential	0.715	<0.001	25.07	20.493		
Fungi	**Multifunctionality**	**GG**	Logarithmic	0.655	<0.001	10.328	3.71		
			Linear	0.713	<0.001	6.6184	0	✓	No redundancy
			Exponential	0.686	<0.001	17.221	10.603		
		WSF	Logarithmic	0.694	<0.001	12.554	8.225		
			Linear	0.797	<0.001	4.32866	0	✓	No redundancy
			Exponential	0.768	<0.001	22.346	18.017		
Multidiversity	**Multifunctionality**	**GG**	Logarithmic	0.713	<0.001	6.659	3.804		
			Linear	0.763	<0.001	2.8554	0	✓	No redundancy
			Exponential	0.653	<0.001	17.9313	15.076		
		WSF	Logarithmic	0.611	<0.001	17.344	4.086		
			Linear	0.683	<0.001	13.258	0	✓	No redundancy
			Exponential	0.614	<0.001	23.418	10.16		

	Function	Site	Model	R^2	P	AICc	DeltaAICc	Selected Model(s)	Model group
amoA	**Nitrate production**	**GG**	Logarithmic	0.819	<0.001	-92.56	0	✓	Redundancy
			Linear	0.76	<0.001	-86.867	5.693		
			Exponential	0.661	<0.001	-79.8	12.76		
		WSF	Logarithmic	0.811	<0.001	-97.124	7.096		
			Linear	0.844	<0.001	-100.945	3.275		
			Exponential	0.868	<0.001	-104.22	0	✓	No redundancy
pmoA	**Methane consumption**	**GG**	Logarithmic	0.667	<0.001	-320.14	0.92		
			Linear	0.684	<0.001	-321.06	0	✓	No redundancy
			Exponential	0.626	<0.001	-321.06	0	✓	No redundancy
		WSF	Logarithmic	0.915	<0.001	-352.182	4.798		
			Linear	0.934	<0.001	-356.98	0	✓	No redundancy
			Exponential	0.896	<0.001	-353.56	3.42		
nosZ	**Nitrous oxide consumption**	**GG**	Logarithmic	0.497	<0.001	-196.79	8.61		
			Linear	0.405	<0.001	-193.417	11.983		
			Exponential	0.699	<0.001	-205.4	0	✓	No redundancy
		WSF	Logarithmic	0.666	<0.001	-197.59	3.7		
			Linear	0.723	<0.001	-201.29	0	✓	No redundancy
			Exponential	0.641	<0.001	-195.52	5.77		

Logarithmic: $Y = a + b \cdot \log(X)$; Linear: $Y = a + b \cdot X$; Exponential: $Y = a \cdot e^{bX}$

by exponential ($R^2 = 0.70$) and linear ($R^2 = 0.72$) models, respectively. On the other hand, a negative relationship between the diversity of *amoA* gene and NO_3 production at Site A and B were best explained by logarithmic ($R^2 = 0.82$) and exponential ($R^2 = 0.87$) models, respectively. Our correlation analysis also showed a significant relationship between the diversity of functional genes and their associated functions for both sites (Table 2.4).

Moreover, statistical modelling suggested a general lack of redundancy in the relationship between functional microbial diversity and specialised functions (Table 2.2). I was able to successfully identify a best group of models (functional vs. no functional redundancy) shaping the microbial BEF relationship in all cases (Table 2.2). In five of six cases (except for *amoA* diversity and nitrate production in site A), the best models supported a lack of functional redundancy in the microbial BEF relationship (linear and/or exponential shapes), indicating a major loss of function with the initial loss of diversity (Table 2.2).

2.4.3.4. Statistical analyses to control for the role of microbial biomass and composition in the interpretation of the BEF relationship

It can be argued that microbial abundance and composition can influence the relationship between biodiversity and functions in our results. To account for this important factor, I conducted partial correlations using microbial diversity as a predictor of multi-functionality and accounting for abundances (qPCR data) and composition (first axis of NDMS plots) (Supplementary Table 2.5) The results were similar to those observed in Figure (2.4-2.5) (where diversity was directly related to functions) and thus provided evidence that the significant relationship between microbial diversity and ecosystem functionality is maintained after controlling for biomass and composition.

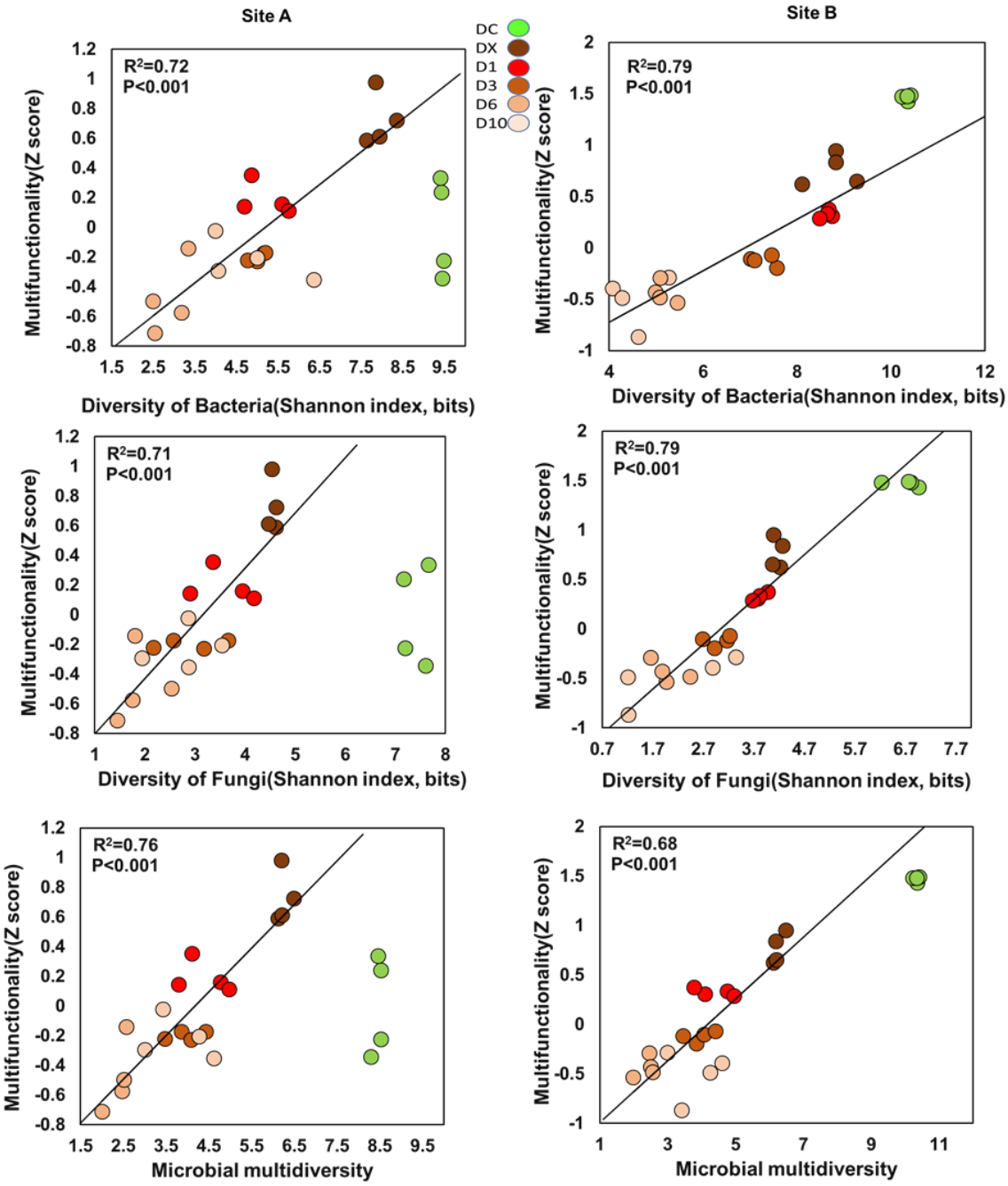


Figure 2.4. Relationship between bacterial, fungal, and combined (combined Z score of bacterial and fungal diversity) and multifunctionality at different sites. The solid line represents fitted regressions. Different colours represent different dilutions darker to light (DX-D10). Green represents the positive control. R square and P values were obtained without including the positive control.

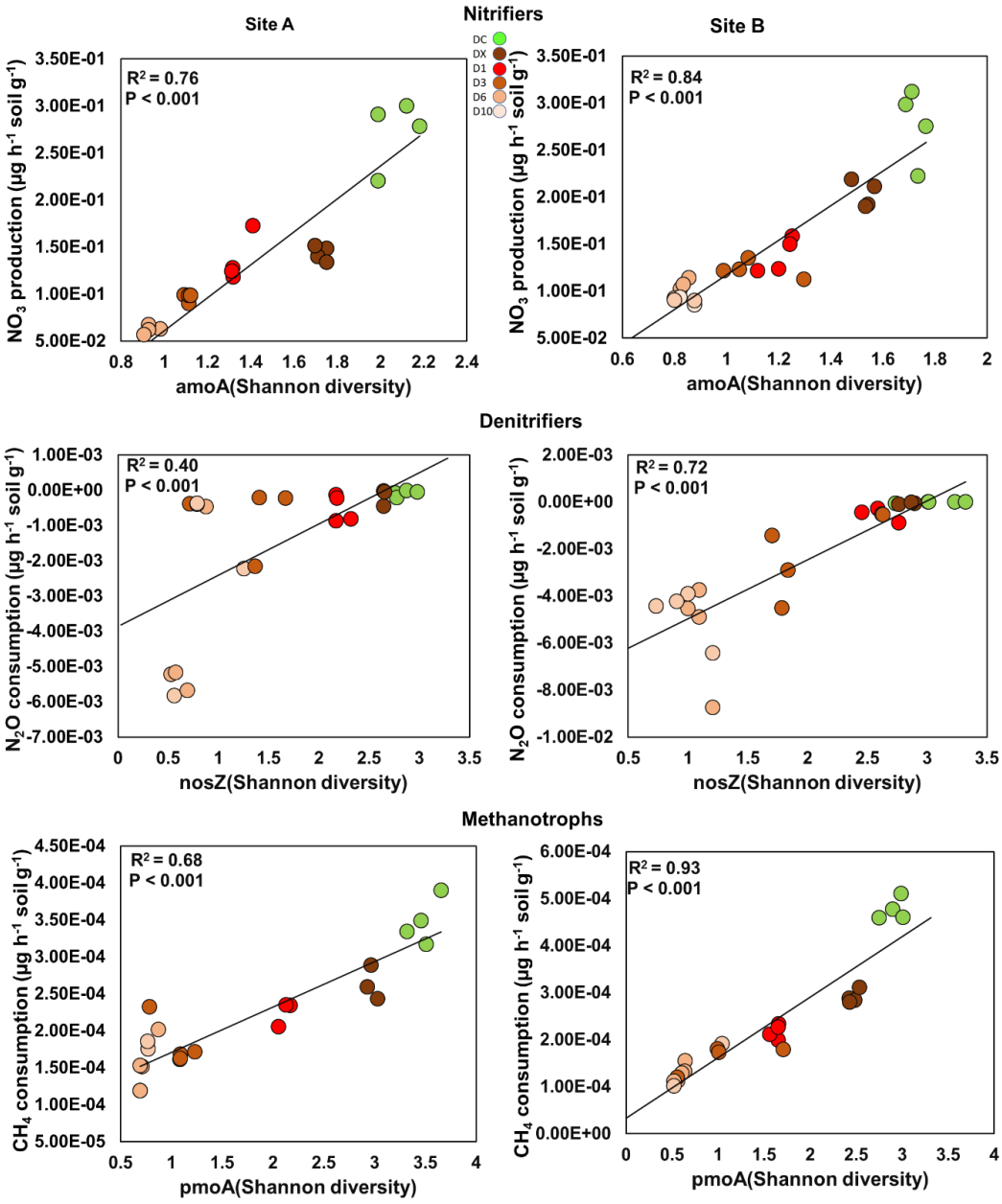


Figure 2.5. Relationship between diversity of functional groups of soil microbes [as determined by TRFLP analysis of functional genes *pmoA, amoA and nosZ*)] and their specialised functions. The solid line represents fitted regressions. Different colours represent different dilutions darker to light (DX-D10). Green represents the positive control. R square and P values were obtained without including the positive control.

Table 2.3. Spearman correlation between categorized functions and microbial diversity [bacterial, fungal, and multi-diversity (combined z scores for bacterial and fungal diversity)] in two soil types. Bold letters represent significant correlations. * and ** represent significance levels at $P < 0.01$ and $P < 0.001$, respectively

Functional categorization	**Function measured**	**Diversity**					
		Site A			**Site B**		
		Bacteria	**Fungi**	**Multi-diversity**	**Bacteria**	**Fungi**	**Multi-diversity**
Specialised functions	Methane consumption	**0.58***	**0.56***	**0.51***	**0.91****	**0.67****	**0.68****
	N_2O consumption	**0.86****	**0.84****	**0.86****	**0.87****	**0.77****	**0.82****
	Nitrate production	**0.63***	**0.68****	**0.67****	**0.88****	**0.81****	**0.86****
Soil Enzyme activity	β-Glucosidase (BG)	**0.81****	**0.82****	**0.85****	**0.95****	**0.78****	**0.91****
	α-Glucosidase (AG)	0.57	**0.65****	0.63	**0.73****	**0.77****	**0.79****
	β-D-celluliosidase (CB)	0.52	**0.54***	0.54	**0.94****	**0.80****	**0.91****
	N-acetyl- β-Glucosaminidase (NAG)	**0.75****	**0.79****	**0.79****	**0.91****	**0.82****	**0.91****
	Phosphatase (PHOS)	**0.63***	**0.70****	**0.69****	**0.83****	**0.86****	**0.88****
	β-Xylosidase (XYL)	0.46	0.39	0.44	**0.95****	**0.88****	**0.96****
	Leucine amino-peptidase (LAP)	0.46	0.397	0.449	**0.755****	**0.79****	**0.80****
Substrate Utilization	Glucose utilization	0.23	0.32	0.29	**0.69****	0.50	**0.63***
	Cellulose utilization	**0.70****	**0.76****	**0.76****	**0.84****	**0.73****	**0.83****
	Proto utilization	**0.74****	**0.75****	**0.77****	**0.75****	**0.76****	**0.79****
	Lignin utilization	**0.62***	**0.56***	**0.61***	0.29	0.31	0.32
	Alanine utilization	0.34	0.31	0.34	0.31	0.14	0.23
	Basal respiration	0.24	0.15	0.21	0.02	0.01	0.03
Soil respiration	CO_2 production	0.05	0.01	0.03	0.37	0.20	0.40
Multifunctionality		**0.69****	**0.82****	**0.78****	**0.95****	**0.93****	**0.91****

Table 2.4. Spearman correlation between specialised functions and microbial groups responsible for performing associated functions in two soil types. Bold letters represent significant correlations. * and ** represent significance levels at $P<0.01$ and $P<0.001$, respectively.

Functional gene Diversity	**Function**		
		Site A	Site B
amoA	NO_3 production	**0.94****	**0.85****
pmoA	CH_4 consumption	**0.59***	**0.66****
nosZ	N_2O consumption	**0.81****	**0.81****

2.5. Discussion

My findings provide strong empirical evidence that the microbial diversity of multiple taxonomic and functional groups positively relates to multifunctionality and specialised ecosystem functions in terrestrial ecosystems. These results provide experimental support to previous observational studies linking microbial diversity with ecosystem functions across environmental gradients from local to global scales (e.g. He *et al.,* 2009; Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016; 2016a). Moreover, my results suggest that reductions in the functional and taxonomic microbial diversity mostly lead to proportional or exponential (i.e. lack of redundancy) declines in the rates of key ecosystem processes and multifunctionality. Altogether, our results challenge the classical conceptual idea of large functional redundancy in soil microbial communities and support the inclusion of microbial diversity as an integral component of the land-management policy and biodiversity-conservation agenda. Thus, our results provide a strong framework to assess microbial diversity and ecosystem functions by explicitly considering taxonomic and functional gene diversity, a range of specialised functions and multifunctionality if the actual consequences of microbial diversity are to be evaluated. This knowledge is essential for developing a predictive understanding of microbial community responses to environmental perturbations (Girvan *et al.,* 2005; Singh *et al.,* 2014; Blaser *et al.,* 2016).

2.5.1. Link between fungal and bacterial diversity and ecosystem multifunctionality

Building on previous experimental and observational studies with plants (Hector *et al.,* 2007; Eisenhauer *et al.,* 2016; Jing *et al*., 2015), soil animals (Wagg *et al.,* 2014; Soliveres *et al.,* 2016) and several observational studies investigating soil microbial communities (Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a), my results show that broad scale changes in soil microbial food web diversity are likely tightly linked to ecosystem multifunctionality. Up until now, this has remained an important knowledge gap on the applicability of biodiversity-ecosystem function theories to soil microbial communities. In accordance with my initial hypothesis based on a manipulated experimental approach, these results clearly demonstrate a positive relationship between microbial diversity and ecosystem multifunctionality. Partial correlation analysis has further revealed that the relationship between the diversity of soil bacteria, fungi and functions is maintained even after accounting for biomass and community composition, supporting the robustness of our results. My results provide strong evidence that unlike the positive but decelerating BEF relationship observed most frequently in plant and animals, multifunctionality in soil ecosystems is related to microbial diversity in a non-redundant fashion. This observation suggests that losses of microbial diversity resulting from climate change or soil degradation (Maestre *et al.,* 2015) will likely reduce the ability of terrestrial ecosystems to provide multiple ecosystem functions on which human life depends (Wagg *et al.,* 2014; Bradford *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a).

2.5.2. Link between functional diversity and specialised functions

The relationship between functional microbial diversity and three ecosystem level processes that are catalysed by specialised microbial groups (Levine *et al.,* 2011; Hu *et al.,* 2015; Phillipot *et al.,* 2013) was investigated: nitrification, denitrification and methane consumption. In accordance with my hypothesis, the results demonstrate that specialised functions are most likely to be affected by changes in diversity because they require a specific physiological pathway and/or are carried out by a phylogenetically clustered group of organisms (Schimel and Schaeffer, 2012; Wood *et al.,* 2015); and the organisms involved are less frequent and abundant when diversity is low. As postulated, there was a positive relationship between CH_4 consumption, NO_3 production, and N_2O

consumption and the diversity of *pmoA* genes (for methanotrophs), *amoA* genes (for ammonia oxidisers) and *nosZ* genes (for denitrifiers), respectively. Our results were supported by previous observational studies (Levine *et al.,* 2011; Singh *et al.,* 2014; Ho *et al.,* 2014; Powell *et al.,* 2015; Trivedi *et al.,* 2016) and experimental work (Peter *et al.,* 2011; Phillipot *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016) that have reported positive relationships between diversity of specialised microbes with narrowly distributed traits. In general (but for one case see Table 2.2), I found that the relationship between microbial diversity and specialised functions tends to be exponential or linear suggesting that specialised functions possess a lower degree of functional redundancy, if any. I therefore postulate that these functions will be much more sensitive to losses of biodiversity in natural settings.

My analysis also showed significant correlations between total taxonomic diversity of bacteria and functions, multi-diversity and specialised functions (Table 2.3) suggesting that the functions driven by specialised microbial guilds are either reflective of total microbial diversity or dependent on the richness of other microbial groups (Ho *et al.,* 2014). This suggests that even if some members of specific functional guilds of microbes are more resilient than others (Ho *et al.,* 2011; Levine *et al.,* 2011), diversity loss/or shifts in the composition of the overall soil microbial community may have important ecological implications amongst specialised microbial communities that control greenhouse gas emissions, particularly for future climate scenarios. For example, reductions in the diversity of *amoA* genes will largely reduce the availability of nitrate – one of the most limiting factors for plant and microbial growth in terrestrial ecosystems (Robertson and Groffman, 2007). Moreover, reductions in the diversity of methanotrophs (*pmoA* genes) and denitrifiers (*nosZ* genes) will have negative consequences for climate regulation on Earth. Both methanotrophs (*pmoA* gene) and denitrifiers (*nosZ* genes) are essential microbial communities in terrestrial ecosystems as they constitute the ultimate barrier that prevents the release of CH_4 and N_2O gasses both responsible for global warming - from deeper soil layers to the atmosphere (Smith *et al.,* 2003; Heimann and Reichstein, 2008).

2.5.3. Link between bacterial and fungal taxonomic diversity and individual's functions

The current paradigm supported by only a few studies states that a limited loss in diversity will not influence the overall rate or stability of processes with sufficient redundancy in metabolic processes (particularly broad functions, Levine *et al.,* 2011; Miki *et al.,* 2014; Wood *et al.,* 2015). In accordance with these studies, I did not observe a significant relationship between such functions (basal respiration and CO_2 production) and the diversity of soil bacteria and fungi. The CO_2 generating metabolic pathways are ubiquitously distributed in extremely diverse microbial communities and therefore there was no discernible relationship between microbial diversity and CO_2 production (Levine *et al.,* 2011). My results are interesting as I observed significant positive relationships between microbial diversity and the activity of most of the enzymes involved in C degradation and substrate utilization of various C sources (Table 2.3) and therefore, expected that CO_2 production and basal respiration would be related to microbial diversity.

My study suggests that broad functions such as respiration that incorporate several metabolic pathways merely reflect the average response of the microbial community to environmental conditions (in particular nutrient status). Supporting this idea, Delgado-Baquerizo *et al.,* (2015; 2016d) found that soil respiration is mostly controlled by soil properties such as pH and soil carbon and microbial abundance, with a minor effect from microbial composition. However, a mechanistic understanding of the link between activity and ecosystem process rates might require analysis of each individual component that comprise the broad functions (Frossard *et al.,* 2012; Trivedi *et al.,* 2016). In natural ecosystems, substrate inputs will vary in response to management practices and can stimulate decomposition of soil organic carbon (priming effect, Kuzyakov *et al.,* 2010) with considerable influence on soil C storage (Wieder *et al.,* 2013; Trivedi *et al.,* 2013). This is especially true for low soil C ecosystems such as those included in this study, in which microbial activity may be strongly limited by the availability of C in soil. The fact that taxonomic microbial diversity was strongly but differentially related to supplemented substrate use further support this idea and also indicated that a relationship between some

broad functions (e.g. labile carbon utilisation) and biodiversity can be ecosystem dependent. Based on our observations, I propose that linking diversity with the individual pathways for extracellular enzyme production or utilization of a particular substrate will provide a more appropriate framework to generate ecosystem models that predict the rate and fate of C in natural ecosystems rather than through the measurement of broad functions (Mau *et al.,* 2015; Trivedi *et al.,* 2016). Nonetheless, given limitations of the methods used, these observations should be considered with caution and confirmed with more experimental studies.

2.6. Conclusions

Soil microbes are well known to contribute and regulate most of the terrestrial ecosystem functions, however the linkage between diversity and the rate of soil functions remain debatable due to a lack of empirical studies. Distinguishing the microbial regulation of soil functions at multiple-levels is critical to understanding the overall effect of perturbation on ecosystem functions. My study provides strong evidence that microbial diversity positively relates to ecosystem functioning in terrestrial ecosystems; even after controlling for microbial biomass and composition. Thus, my results suggest that any reduction in microbial diversity resulting from climate change or soil degradation, will significantly reduce the provision of multiple ecosystem functions related to C, N and P cycles; these functions support important ecosystem services such as climate regulation, food production and specialised processes such as gaseous emissions that support climate regulation worldwide. By making these links, I propose that multiple aspects of microbial diversity, ecosystem functions and ecosystem variables need to be considered when studying microbial BEF, formulating sustainable management and conservation policies, and when predicting the effects of global change on ecosystem functions. These findings significantly advance our understanding on the relationship between biodiversity and ecosystem functionality in terrestrial ecosystems and emphasise the need for the development of approaches to conserve microbial diversity for their positive effects on ecosystem functions.

Chapter 3*

Microbial richness independently drives soil multifunctionality in terrestrial ecosystems

*This work has been published in **Functional Ecology** and available on as an Early view (http://onlinelibrary.wiley.com/doi/10.1111/1365-2435.12924/full) as part of bigger dataset.

The thesis chapter emphasizes the role of microbial richness in driving multifunctionality using observational and experimental study and was a part of the bigger dataset which was published that explores the role of both richness and composition. The initial idea was conceived by lead author Dr Manuel Delgado-Baquerizo and the experiment was designed in consultation with Drs Brajesh K Singh, Peter Reich, and Pankaj Trivedi. I led most ofexperimental works including carrying out experiments and laboratory analysis [e.g. microcosm preparation and maintenance (including growing bacterial cultures, density adjustments, plate counts); measuring soil functions; and molecular analysis (including qPCR analysis and preparing samples for MiSeq analysis)]. I also isolated and characterized (by 16 S rRNA sequencing) bacterial isolates used in the observational study. During the course of this experiment, I learned statistical modeling approaches under the guidance of lead author and my supervisor Dr Manuel Delgado-Baquerizo who conducted the statistical analysis for the published paper. Through this training on the use of advanced statistical techniques to analyze complex ecological datasets, I was able to analyze my dataset for other chapters. Finally, I also contributed in writing the first draft of this manuscript.

3.1. Abstract

Soil microbes provide multiple ecosystem functions including primary production, nutrient cycling, decomposition and climate regulation. However, we have limited knowledge on the quantitative and relative importance of microbial diversity community composition in regulating ecosystem multi-functionality. This is critical knowledge gaps which limit our capacity to predict influence of biotic attributes on ecosystem services. Two independent approaches (i.e. experimental and observational), combined with applied statistical modelling were used to identify the role and relative importance of microbial richness along with other main component of biodiversity viz community composition in regulating multifunctionality. In the observational study, soil microbial communities and functions were measured in soils from 22 locations across a 1200 km transect in south-eastern Australia. In the experimental study, soils from two of those locations were used to develop gradients of microbial diversity and composition through inoculation of sterilized soils. The results demonstrated that microbial richness was positively related to multifunctionality in both the observational and experimental approaches. Moreover, from two different approaches, this study provides evidence that microbial richness is important and an independent driver of multifunctionality. Overall, these findings advance our understanding of the mechanisms underpinning relationships between microbial diversity and ecosystem functionality in terrestrial ecosystems, and further suggest that information on microbial richness needs to be considered when formulating sustainable management and conservation policies, and when predicting the effects of global change on ecosystem functions.

Key words: Microbial richness; Community composition; Ecosystem functions; Multi-functionality; Statistical modelling; Random forest analysis.

3.2. Introduction

Biological diversity is the major drivers of multiple ecosystem functions including primary production, nutrient cycling, climate change regulation (Hopper *et al.,* 2005; Cardinale *et al.,* 2012); and these functions have been valued at trillions of US dollars per year (Costanza *et al.,* 1997). In recent decades, biodiversity loss has become a global

concern and various studies have demonstrated a negative effect of these losses on ecosystem services on which the society depends (Hopper *et al.,* 2005; Cardinale *et al.,* 2012). Although the importance of biodiversity for ecosystem functioning has been reported (Cardinale *et al.,* 2011; Tilman *et al.,* 2014), biodiversity is extremely complex, and involves different components including but not limited to, species richness (number of taxa); composition (i.e. the identity of the different organisms comprising a community expressed in terms of their relative abundance, Díaz *et al.,* 2001); traits of individual members; and the emerging properties of communities based on the interactions of individuals. These components (either individually or in combinations) are likely to change markedly under future climate change scenarios or more intense land uses (Díaz *et al.,* 2001; Hooper *et al.,* 2005). Therefore, it is critical that to quantify the relative importance of these biodiversity components for multifunctionality so that appropriate management and conservation policies can be formulated to predict the likely changes in ecosystem functioning under changing environments.

Our current understanding of the relationships between microbial diversity, and ecosystem functioning is limited, particularly in terrestrial environment (Bardgett and van der Putten, 2014), unlike plants and animals (Hooper *et al.,* 2005; Lefcheck *et al.,* 2015). Microbes are considered by far the most abundant and diverse life forms on Planet Earth (Singh *et al.,* 2009; Leach *et al.,* 2017), and play key vital in maintaining multiple ecosystem functions including litter decomposition, primary production, soil fertility and gaseous emissions (He *et al.,* 2009; Peter *et al.,* 2011a; Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a). In recent years several studies have shown that global environmental drivers such as land use change, nitrogen enrichment and climate change reduce overall microbial diversity (Wall *et al.,* 2010; Maestre *et al.,* 2015; Leff *et al.,* 2015; Trivedi *et al.,* 2016a). This has triggered increasing concern that reduced microbial biodiversity in soils may impair numerous ecosystem functions mediated by soil microbes (Wagg *et al.,* 2014). In order to evaluate the global consequences of shifting microbial diversity on multifunctionality, it is critical that direct evidence of the effect of species richness intendent of other diversity variables (i.e composition) on multiple ecosystem functions is provided (Downing and Leibold, 2002; Hooper *et al.,* 2005).

In recent years, experimental and observational studies provided evidence that microbial diversity promotes ecosystem multifunctionality in terrestrial and aquatic ecosystems from local to global scales (He *et al.,* 2009; Peter *et al.,* 2011a; Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a). However, despite these findings we lack empirical evidence from either observational and/or manipulative studies highlighting the importance of the biodiversity of soil microbial communities towards the overall performance of an ecosystem. It is still unclear whether ecosystem multifunctionality is influenced by soil microbial richness independent of microbial composition (but for see Wagg *et al.,* 2014; Delgado-Baquerizo *et al.,* 2016a). Assessing the relative importance of different variables of microbial community diversity in driving multifunctionality is critical to include microbial communities and processes in ecosystem and earth system simulation models, and to consider their status when making policy or management decisions. This study combined a regional field survey and a microcosm experiment manipulating the diversity of bacterial in two soils to identify the role and relative importance of microbial richness in predicting multifunctionality. It was hypothesized that microbial richness was important and operate independently (along with other components of microbial biodiversity herein microbial composition), as the drivers of terrestrial multifunctionality.

3.3. Material and Methods

3.3.1. Study sites and soil sampling

Two independent but complementary approaches were used to evaluate the role and relative importance of microbial richness in supporting multifunctionality: an observational study that utilized a broad regional soil survey (Field survey), and an experimental microcosm approach (Microcosm study). Note it was not the intention to directly compare results between experimental approaches. Rather, the goal was to address the research question by using two very different, but complementary, approaches (experimental and observational studies) and thus provide further robust scientific support to findings. Moreover, using both observational and microcosm experimental studies gives us a unique opportunity to separate the differential effects of taxa richness

with other components of microbial biodiversity (e.g. microbial composition) on multiple ecosystem functions.

3.3.2. Observational study by the field survey (observational approach)

The observational study was carried out in 22 sites from eastern Australia across a gradient of about 1200 km (Figure 3.1; Supplementary Table 3.1). Locations were intentionally chosen to represent a wide range of climatic and soil property conditions. Details of site characteristics and selected soil properties are presented in Supplementary Table 3.1. Soil sampling was carried out in March 2014. At each site, three soil cores (0-5 cm depth) were collected from two microsites: under trees (*Eucalyptus* spp.) and in open (bare soil) -dominated sites. Soil cores were then mixed to obtain a composite sample for each microsite at each site. A total of 44 soil samples (22 sites x 2 microsites) were analysed in this study. Following field sampling, the soil was sieved (<2 mm mesh). A portion of the soil was immediately frozen at -20ºC for characterizing bacterial abundance, composition and diversity. The other fraction was air-dried and stored before functional analyses. This storage approach is well established and commonly used when analysing soil variables such as those evaluated here in large-scale surveys (Maestre *et al.,* 2012; Tedersoo *et al.,* 2014).

Soil DNA was extracted from 0.25 g of defrosted soil samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). Total abundance of bacteria was quantified in all soil samples (Field and Microcosm studies) with primers Eub 338-Eub 518 using 96-well plates on a CFX96 Touch™ Real-Time PCR Detection System (Foster city, California, USA, see Supplementary Table 3.2 for details). The bacterial diversity and composition in the soil surface (top 5 cm) along the observational gradient were characterized by using the Illumina Miseq profiling of 16S rRNA genes (Illumina Inc.) using the 341F/805R (Herlemann *et al.,* 2011) primer set (see details on analysis in chapter 2 Section 2.3.3.3).

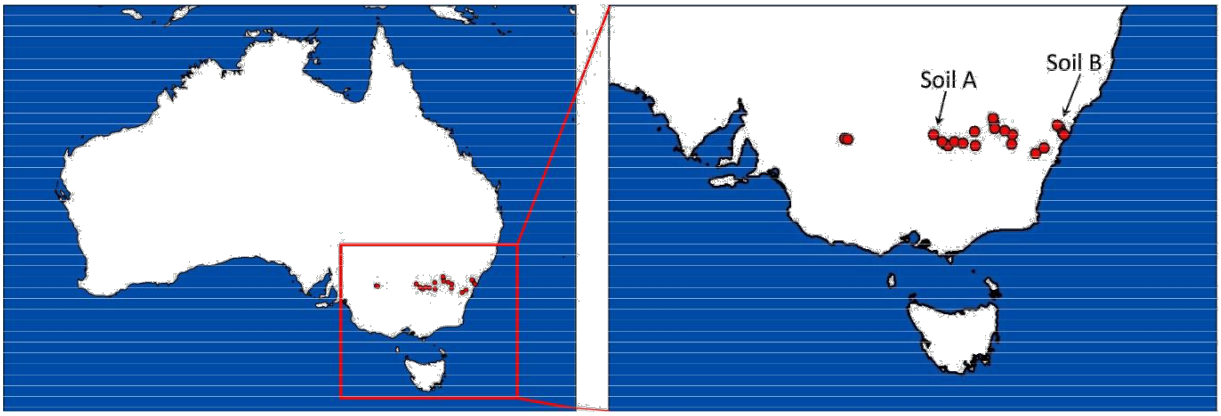


Figure 3.1. Locations of the 22 study sites in this study including Field and Microcosm (soils A and B) studies.

3.3.3. Experimental study using the microcosm study (Experimental approach)

In addition to above, a large quantity of soil (~5kg) from two sites of contrasting aridity and total soil carbon (Soils A and B; Figure 3.1; JM072-TREE and Site 1-TREE in Supplementary Table 3.1) were collected. Soil A had a lower soil carbon than Soil B (3.03% vs. 8.45%). In addition, Soil A had a higher pH than Soil B (6.36 vs. 5.63; Supplementary Table 3.1). In both cases, soil samples were collected from under tree canopies. Following field sampling, the soil was sieved (<2 mm mesh), one part stored immediately at 4ºC (non-sterile soil used for the microbial inoculums), and the other sterilized using gamma radiation (50kGy) as described in detail in chapter 2 section 2.3.2.

The richness treatment consisted of one, two, four and six bacterial taxa per microcosm. For each of these richness levels, all the possible equally distributed taxa combinations were prepared. A total of 37 (6+15+15+1 combinations corresponding to richness levels one, two, four and six) treatments were prepared per soil. The level "six" of diversity to improve the balance of this treatment was duplicated to ensure the success of this important level of diversity (6+15+15+2). In addition, and to reduce the correlation between diversity and composition in this experiment, additional microcosms was prepared with diversity 'two' but with 75/25% and 25/75% of bacterial composition to reduce correlation between taxa richness and composition. This is a critical point, as most previous biodiversity research has not adequately separated composition effects from

richness effects due to experimental design constraints (Huston, 1997; Allison, 1999; Hooper *et al.,* 2005). This provided 30 new treatments per soil (Supplementary Table 3.3). A total of 67+1 combinations were used in this study (a complete list of combinations is shown in Supplementary Table 3.3). To ensure the success of inocula, three microcosms were established for each combination (68 x 3), resulting in a total of 204 microcosms per soil (Soils A and B).

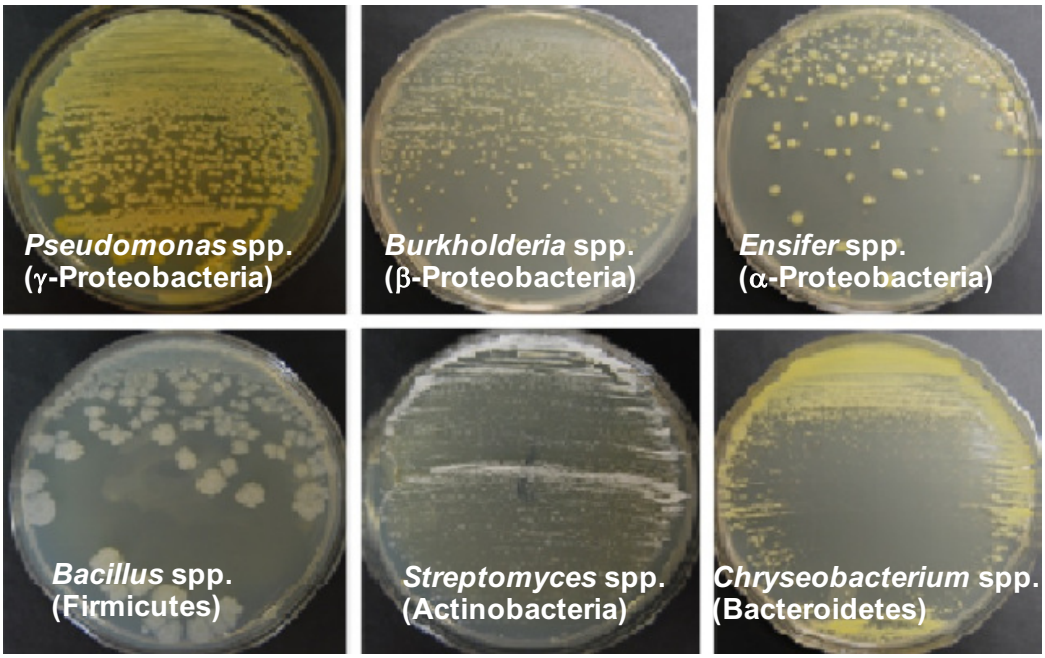


Figure 3.2. Bacterial isolates used in the study.

Bacterial strains from six terrestrial dominant phylogenetic taxa belonged to phylum Actinobacteria (*Streptomyces* spp.), Firmicutes (*Bacillus* spp.), Bacteroidetes (*Chryseobacterium* spp.), and Proteobacteria classes α-Proteobacteria (*Ensifer* spp.), β-Proteobacteria (*Burkholderia* spp.), and γ-Proteobacteria (*Pseudomonas* spp.) (Figure 3.2), were isolated across both "soils A and B". The identification of the bacterial isolates was performed using full-length 16S rDNA sequencing. Bacterial cultures were kept in glycerol stocks at -80 °C and grown in nutrient medium (from DIFCO laboratories, USA). A single colony of each bacterial culture was picked, grown overnight in nutrient broth, washed in phosphate buffered saline and adjusted to an OD_{600} of 0.001 (equivalent to 10^8 cells of bacteria). Bacterial cultures were left for 6 h at room temperature before assembling the communities. To ensure this number of cells, their abundance was

monitored using quantitative PCR (qPCR) approaches (see qPCR details Supplementary Table 3.2), before soil inoculation.

3.3.4. Construction of Microcosms

Sterile soil samples (10 g) were placed in hermetic containers. Soil samples were inoculated to achieve a total amount of 10^8 cells per microcosm. Thus, the final cell densities in all microcosms were the same, that is, the six-taxa assemblage had the same number of cells (1/6 of each strain) as those in the single taxon assemblage. These microcosms were positioned in a laminar flow cabinet to avoid contamination. Microcosms were incubated in the darkness at 50% soil water content (SWC) and 25ºC for 8 weeks under sterile conditions. Soils were opened to the air every 5 days in a laminar flow cabinet to prevent the samples becoming anaerobic. After incubation, a portion of the soil was immediately frozen at -20 ºC, and the abundance of different bacterial taxa determined using quantitative PCR (qPCR). The other fraction was used to assess multiple ecosystems functions as described below. Soil DNA extraction and bacterial 16S rRNA gene quantification were done as explained above.

To check whether the original composition assigned to the different microcosms was maintained by the end of the experiment and take into account changes in bacterial abundance in microcosms, the abundance of each of Actinobacteria, Bacteroidetes and α, β-and γ-Proteobacteria and Firmicutes was quantified using qPCR (details provided in Supplementary Table 3.2). Both original assigned (when microcosms were constructed) and corrected (after qPCR analyses) relative abundances of bacteria were highly related (Spearman $\rho > 0.935$; $P < 0.001$ in all cases) therefore, the corrected values are used in further analyses.

3.3.5. Functional measurements

In all soil samples, seven variables (hereafter functions) were measured: activity of β-glucosidase (starch degradation), cellobiosidase (cellulose degradation), N-Acetylglucosaminidase (chitin degradation), phosphatase (phosphorus mineralization), basal respiration and glucose and lignin induced respiration. Extracellular soil enzyme activities were measured from 1g of soil by fluorometry as described in Bell *et al.,* 2013 (details provided in chapter 2 Section 2.3.4.3). In addition, the Microresp® approach from

Campbell *et al.,* (2003) was used to measure basal respiration and glucose and lignin-induced respiration (details provided in chapter 2 Section 2.3.4.2). Altogether, the selected soil variables (hereafter functions) constitute a good proxy of nutrient cycling, organic matter decomposition, biological productivity, and build-up of nutrient pools (Campbell *et al.,* 2003; Schade and Hobbie, 2005; Perroni-Ventura *et al.,* 2009; Jax 2010; Maestre *et al.,* 2012; Bell *et al.,* 2014; Bradford *et al.,* 2014; Jing *et al.,* 2015). Extracellular enzymes are also considered a good indicator of nutrient demand by soil microorganisms (Bell *et al.,* 2013).

3.3.6. Assessing multifunctionality

Averaging multifunctionality approach was used to obtain to evaluate the role of microbial diversity and composition in driving multifunctionality. To obtain an averaging multifunctionality index for each sample, each of seven ecosystem functions using the Z-score transformation first normalized (log-transformed when needed) and standardized as described in Maestre *et al.,* (2012). Following this, the standardized ecosystem functions were averaged to obtain a multifunctionality index (Maestre *et al.,* 2012). Averaging multifunctionality is widely used in the multifunctionality literature and provides a straightforward and easy-to-interpret measure of the ability of different communities to sustain multiple functions simultaneously (Maestre *et al.,* 2012; Wagg *et al.,* 2014; Bradford *et al.,* 2014; Jing *et al.,* 2015).

3.3.7. Statistical analyses

3.3.7.1. Exploring the relationship between bacterial diversity/composition and multifunctionality

For the Field survey (non-replicated approach), the relationship between bacterial richness and composition (α-, β- and γ-Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria) was first explored with multifunctionality and each single function by fitting linear multiple regressions. In addition, partial correlations were conducted between bacterial richness and composition with multifunctionality accounting for latitude/longitude and total bacterial abundance (qPCR) to take into account any bias derived from these important factors. Bacterial diversity was x^2-transformed to improve normality before these analyses.

For the Microcosm study (replicated approach), the effects of diversity on multifunctionality was examined using a nested ANOVA, with diversity as a fixed factor and bacterial combination (Table 3.1) as a random factor nested within diversity (Quinn and Keough, 2002). These analyses were repeated using bacterial abundance as a covariate (ANCOVA) to account for any bias derived from a potential shift of bacterial yield in the microcosms. Then Spearman's correlations were employed to explore the relationship between the relative abundance of the main bacterial phyla/classes with single functions, averaging multifunctionality and with the number of functions at or above 25, 50 and 75% thresholds of the maximum observed function. Finally, the effects of each bacterial phyla/classes identity in supporting multifunctionality in both mono- and mixed cultures (i.e. presence or absence of each taxon across all microcosms) was evaluated by conducting ANOVA analyses.

3.3.7.2. Distance-based multimodel inference

To identify the relative importance of richness and composition of bacteria (α-, β-, γ-Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes) as drivers of multifunctionality, a multi-model inference approach was used based on information theory and non-parametric distance-based linear regressions (DISTLM; McArdle and Anderson 2001). These analyses were done using the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK). The Euclidean distance was used as the measure of multifunctionality dissimilarity between pairs of samples. Bacterial richness represents the number of inoculated phylotypes in the case of the Microcosm study, and the number of OTUs (species) of all bacteria in the case of the Field survey. In the Microcosm study, the composition of bacteria represents the relative abundance of the six inoculated taxa. In the case of the Field survey two approaches were used to represent the composition of bacteria including: (1) relative abundance of the six selected taxa (those in the experimental approach) accounting for 28-74% (average 53%) of the relative abundance of all bacteria. Thus, the aim was to directly compare results from the field and experimental approaches; and (2) a representation of the composition of the entire community of bacteria (100% of species) (using the axes from a NMDS). To obtain a metric of community composition at the lowest taxonomic rank, a non-metric

multidimensional ordination (NMDS) was used on the matrix of bacterial composition at the OTU level (i.e. species level). Given a low stress in these analyses (0.05), the axes of a NMDS are considered a good representation of the variation in the composition of entire bacterial communities across samples. The three-dimensional NMDS solution was used for further analyses. The NMDS ordinations was conducted with the package Vegan from R (Oksanen *et al.,* 2015) using the Bray-Curtis distance. Including a representation of the entire community composition of bacteria in the models is needed to clarify the relative importance of bacterial composition and diversity in driving multifunctionality in the Field survey (i.e., real world) where multiple bacterial species co-exist together.

In addition to these analyses, for the Field survey, the model was repeated including richness and composition of bacteria, spatial variables (latitude and longitude) and soil properties (soil carbon and pH). Finally, for the Field survey, analyses were also repeated including spatial influence, soil properties, bacterial richness, and composition at the OTU level (using the axes from a NMDS) instead of only including selected microbial taxa in this study (α-, β-, γ-Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes).

All the models were ranked that could be generated with independent variables according to the second-order Akaike information criterion (AICc). Here, a $\Delta AICc > 2$ threshold was considered to differentiate between two substantially different models and then select the best of those models (Burnham and Anderson 2002; Burnham *et al.,* 2011). Then, the AICc of the best model were compared including both taxa richness and composition to that of the corresponding model with only composition or richness. Differences < 2 in AICc between alternative models indicate that they are approximately equivalent in explanatory power (Burnham and Anderson, 2002). Finally, the relative importance of bacterial richness and composition (relative abundance of six selected taxa) was calculated as predictors of multifunctionality as the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which each predictor appears (Burnham and Anderson, 2002; Maestre *et al.,* 2012). In general, analyses were not influenced by high collinearity between richness and

composition, as only weak relationships were found between bacterial richness and composition for both Field and Microcosm studies (Supplementary Table 3.5).

3.3.7.3. Partial correlation and Random Forest

Partial correlation analyses were conducted to thoroughly check whether the relationship between bacterial richness or composition was still maintained after controlling for the rest of microbial attributed selected in the best model. To further clarify the relative importance of bacterial richness and composition in predicting multifunctionality, a classification Random Forest analysis (Bierman, 2001) was employed, as described in Delgado-Baquerizo *et al.,* (2015). Random Forest analysis for the field study includes as predictors: bacterial richness, composition and total abundance, as well as latitude, longitude, soil carbon and pH. Random Forest analyses for the experimental soils A and B include as predictors: bacterial richness, composition and total abundance. This technique is a novel machine-learning algorithm that extends standard classification and regression tree (CART) methods by creating a collection of classification trees with binary divisions. Unlike traditional CART analyses, the fit of each tree is assessed using randomly selected cases (1/3 of the data), which are withheld during its construction (out-of-bag or OOB cases). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy (i.e. increase in the mean square error between observations and OOB predictions) when the data for that predictor is randomly permuted. This decrease is averaged over all trees to produce the final measure of importance. These analyses were conducted using the rfPermute package (Archer *et al.,* 2016) of the R statistical software (http://cran.r-project.org/).

3.4. Results

3.4.1. Field survey

The distance-based multi-modeling approach indicated that bacterial richness along with microbial composition (relative abundance of β-Proteobacteria, γ-Proteobacteria, Bacteroidetes and Actinobacteria) provided independent and complementary information to predict multifunctionality (Table 3.1). The best-fitting model accounted for over 60% of the variation in multifunctionality; and always included both bacterial richness and composition as predictor variables (Table 3.1). Model fit declined substantially when

either bacterial richness or composition was removed as a predictor variable (Table 3.1; ΔAICc >2 threshold), suggesting that both microbial components are important predictors of ecosystem multifunctionality. Models including only bacterial richness had a markedly higher ΔAICc (+23.11) than the best-fitting model (Table 3.1). The analysis based on the calculating the relative importance of all microbial attributes in predicting multifunctionality using weighted information from all models showed that bacterial richness was the fourth most important predictor of multifunctionality after the relative abundance important microbial groups (Figure 3.3).

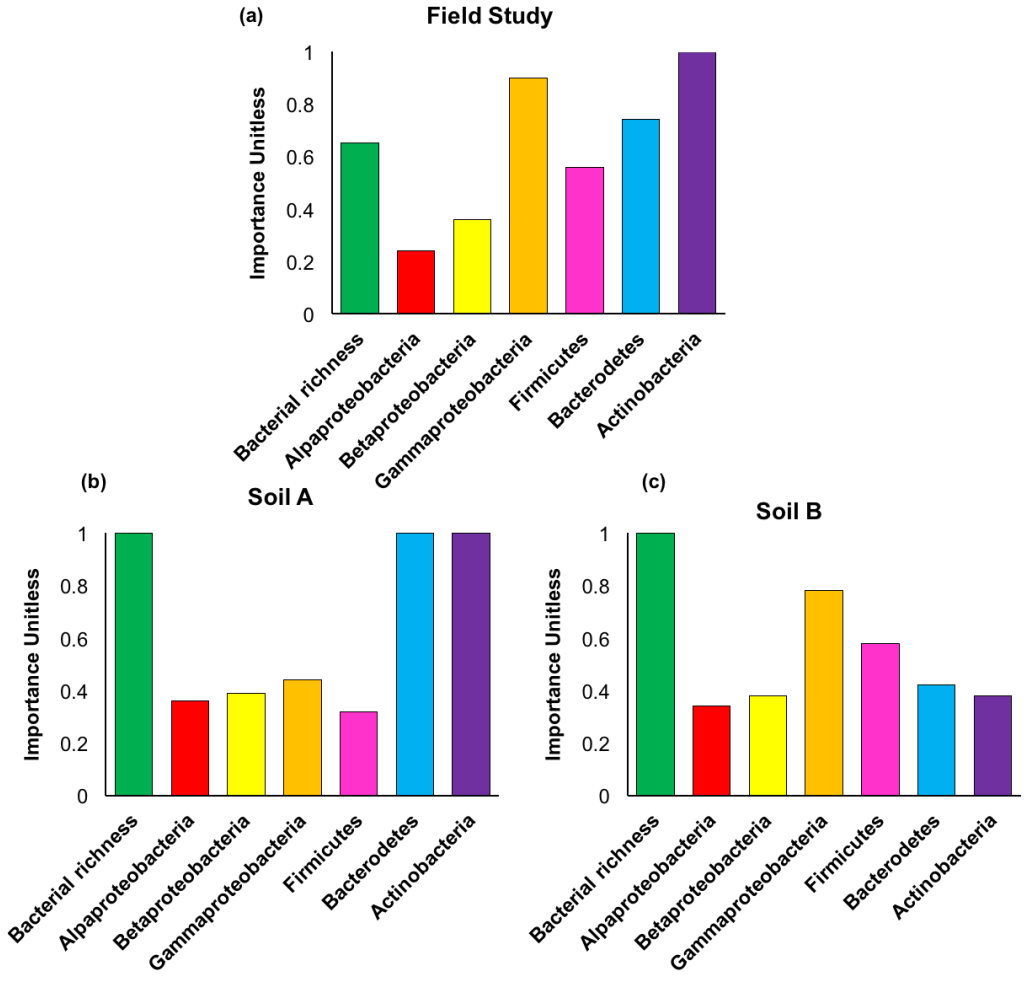


Figure 3.3. Relative importance of bacterial richness and composition in models of multifunctionality for the field (a) and experimental studies (b-c). The height of each bar is the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which each predictor appears.

Table 3.1. Best-fitting model (including microbial richness and composition) and the same model with either bacterial richness or composition (but not both) included as predictors of multifunctionality for the field and microcosm ("soils A and B") studies. Shaded cells indicate that the variable has been included in the model. Models are ranked by AICc. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely the model to be correct. AICc is the difference between the AICc of each model and that of the best model. AICc indicates substantially different models. A = α-Proteobacteria; B = β-Proteobacteria; C = γ-Proteobacteria; D = Firmicutes; E = Bacteroidetes; F = Actinobacteria.

Approach	Diversity	Composition	R^2	AICc	ΔAICc
I (Field study)	**Richness**	γ-Proteobacteria + Firmicutes + Bacteroidetes+ Actinobacteria	0.599	-53.75	0
	Excluded	γ-Proteobacteria + Firmicutes + Bacteroidetes+ Actinobacteria	0.511	-51.52	2.23
	Richness	Excluded	0.134	-30.64	23.11
II (Soil A)	**Richness**	Bacteroidetes+ Actinobacteria	0.43	-445.74	0
	Excluded	Bacteroidetes+ Actinobacteria	0.344	-419.05	26.69
	Richness	Excluded	0.19	-378.39	67.35
II (Soil B)	**Richness**	γ-Proteobacteria	0.084	-276.88	0
	Excluded	γ-Proteobacteria	0.014	-264.04	12.84
	Richness	Excluded	0.06	-273.78	3.1

Table 3.2. Summary of the effects of microbial composition on the multiple ecosystems functions in this study for the field and microcosm (soil A and B) studies. Microbial composition effects are based on Spearman correlations available in supplementary table 3.4. Symbols + and - indicate positive and negative interactions.

Study	Functions	Richness	α-Proteobacteria	β-Proteobacteria	γ-Proteobacteria	Firmicutes	Bacteroidetes	Actinobacteria
Field	β-Glucosidase	+		+	+		+	+
	Cellobiosidase			+	+		+	
	N-Acetylglucosaminidase	+		+	+		+	
	Phosphatase		+	+	+			
	Basal Respiration		+	+	+		+	+
	SIR Glucose	+		+			+	+
	SIR Lignin			+	+		+	
Microcosm (Soil A)	β-Glucosidase	+		-	-		+	+
	Cellobiosidase	+					+	+
	N-Acetylglucosaminidase	+		-	-		+	+
	Phosphatase	+						+
	Basal Respiration	-		-	+			
	SIR Glucose			+				
	SIR Lignin		-		-	+		+
Microcosm (Soil B)	β-Glucosidase							
	Cellobiosidase							
	N-Acetylglucosaminidase	+			+	+	+	
	Phosphatase	+						+
	Basal Respiration			-	+	-		
	SIR Glucose				+	-		
	SIR Lignin				+	-		

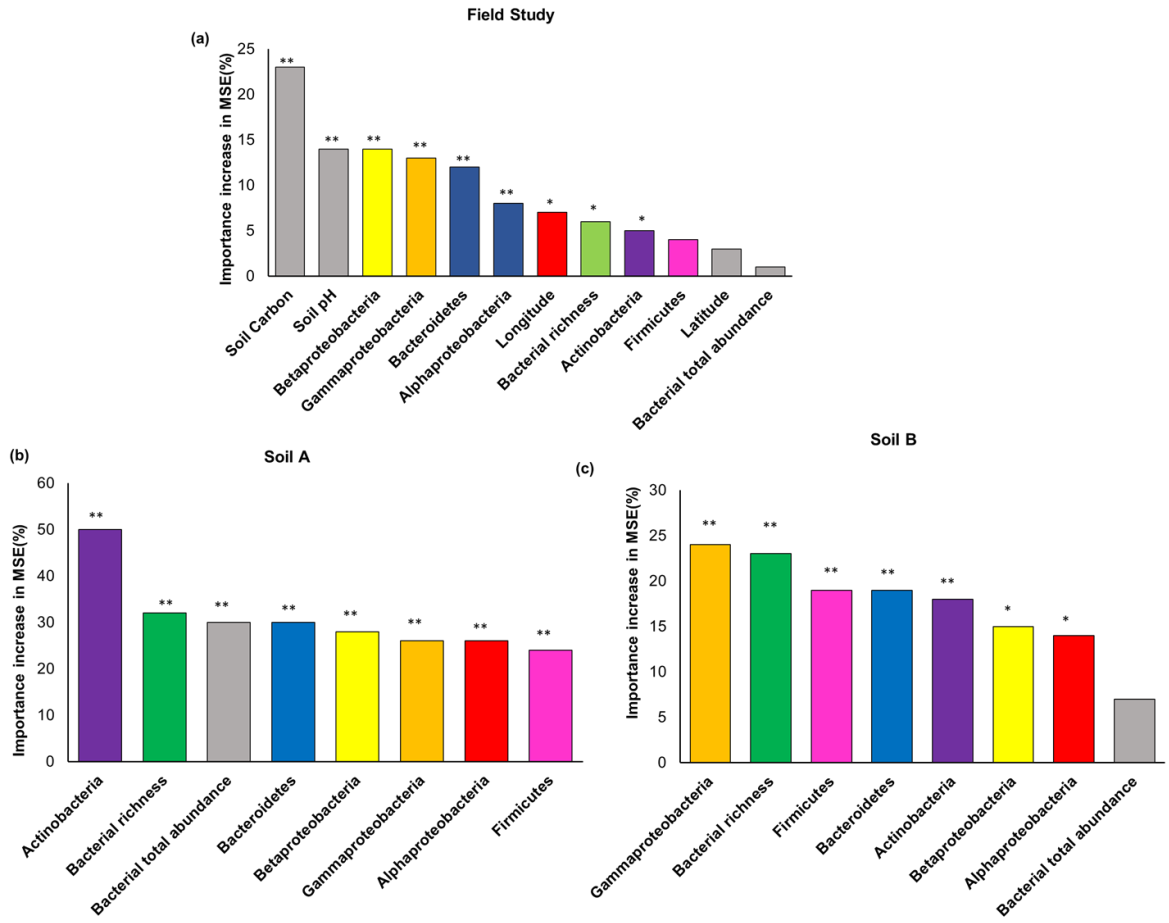


Figure 3.4. Random forest mean predictor importance (percentage of increase in mean square error) of bacterial richness, composition, total abundance and location and soil properties (i.e., for the observational study) on multifunctionality. $^{**}P < 0.01$; $^{*}P < 0.05$.

Random Forest analyses provided further evidence that bacterial richness is a significant predictor of multifunctionality after accounting for multiple multifunctionality drivers (Figure 3.4).

These results showed that Bacterial richness was positively related to multifunctionality (Figure 3.5). Moreover, positive effects of bacterial richness were found on some individual functions (enzyme activities and carbon degradation assays; Table 3.3). For example, I found positive correlations (Spearman) between bacterial richness and β-glucosidase (P=0.01), N-Acetylglucosaminidase (P=0.08) and SIR Glucose (P<0.01) (Table 3.3). Similar results were obtained when the linear relationships

among bacterial richness and single functions was used, with cellobiosidase, but not N-Acetylglucosaminidase, being positively related to bacterial richness in these analyses (Supplementary Figure 3.1).

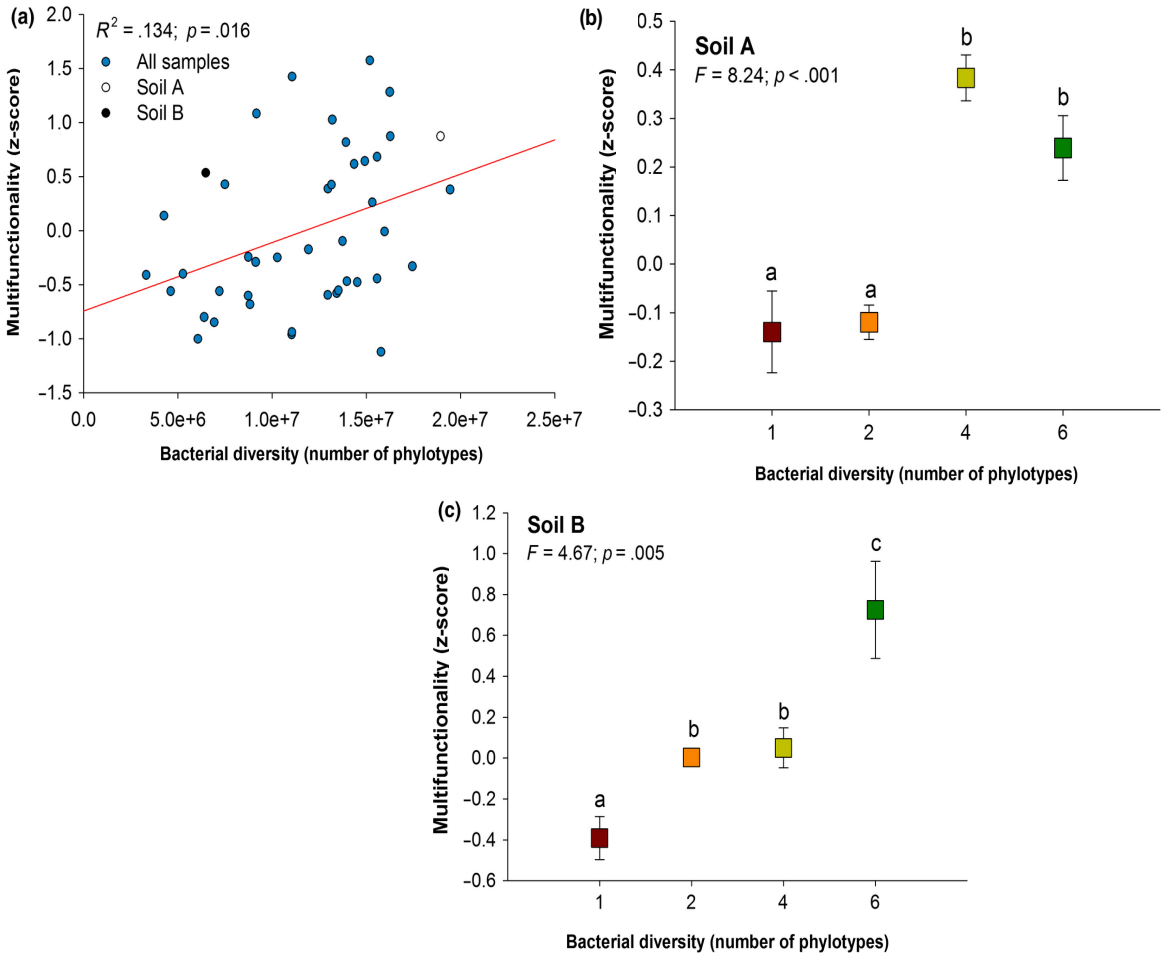


Figure 3.5. Mean (±SE) values for multifunctionality across different bacterial taxa for mono- (a and c) and mixed cultures (b and d) of bacteria in the experimental approach. Different letters in panels a) and c) indicate significant differences in multifunctionality among bacterial taxa ($P<$ 0.05) as tested using post-hoc tests after one-way ANOVA. Panels b) and d) represent averaging multifunctionality index in mixed cultures including (presence) or excluding (absence) each bacterial phylum/class. In these panels significance levels are as follows: ** $P<0.01$, *** $P <$ 0.001.

3.4.2. Microcosm study (Experimental approach)

Supporting the results from the Field survey, the distance-based multi-modeling approach indicated that bacterial richness and composition (relative abundance of Bacteroidetes and Actinobacteria for Soil A and γ-Proteobacteria for Soil B provided independent and complementary information to predict multifunctionality (Table 3.1). The best-fitting models accounted for significant but modest (8% for soil A) and substantial (43% for Soil B) percentages of the variation in multifunctionality for the two soils; and always included both bacteria richness and composition as predictor variables (Table 3.1). Also, similar to the results found for the Field survey, model fit declined substantially when either bacterial richness or composition was removed as a predictor variable (Table 3.1; ΔAICc > 2 threshold). Models including only bacterial richness had a higher ΔAICc for Soil A (+67.35) and Soil B (+3.10).

Although models including both bacterial richness and composition always improved multifunctionality predictions (vs. those models lacking one of these components; Table 3.1), the results for the Microcosm study also suggested that the relative importance of bacterial richness compared with composition is soil-dependent. Thus, richness was more important than composition in Soil B, while the opposite pattern was observed for Soil A (Table 3.1). Similar results are found when the relative importance was calculated of bacterial richness and composition using weighted information from all models (Figure 3.3). Alternatively, the Random Forest model indicated that bacterial richness was the most important predictor of multifunctionality, but only after the relative abundance of Actinobacteria for soil A and Gammaproteobacteria for soil B (Figure 3.4).

Actinobacteria, Alpha-Proteobacteria and Acidobacteria were most dominant class in both soil (Figure 3.6a). The highest multifunctionality was found in the soil microcosms with the highest bacterial richness in both Soils A and B (Figures. 3.5 and c; $P<0.01$). These results remained consistent after statistically controlling for total bacterial abundance (Supplementary Figure 3.2). For single functions, bacterial richness was positively related to N-Acetylglucosaminidase and phosphatase activities in both soils from the Microcosm study and to β-glucosidase and cellobiosidase activities in Soil

A (*P<0.05*; Tables 3.2 and Supplementary Figures 3.3 and 3.4). There is no significant difference in abundance of bacteria in these soils after incubation period suggesting that abundance have similar value against all diversity level (Figure 3.6b).

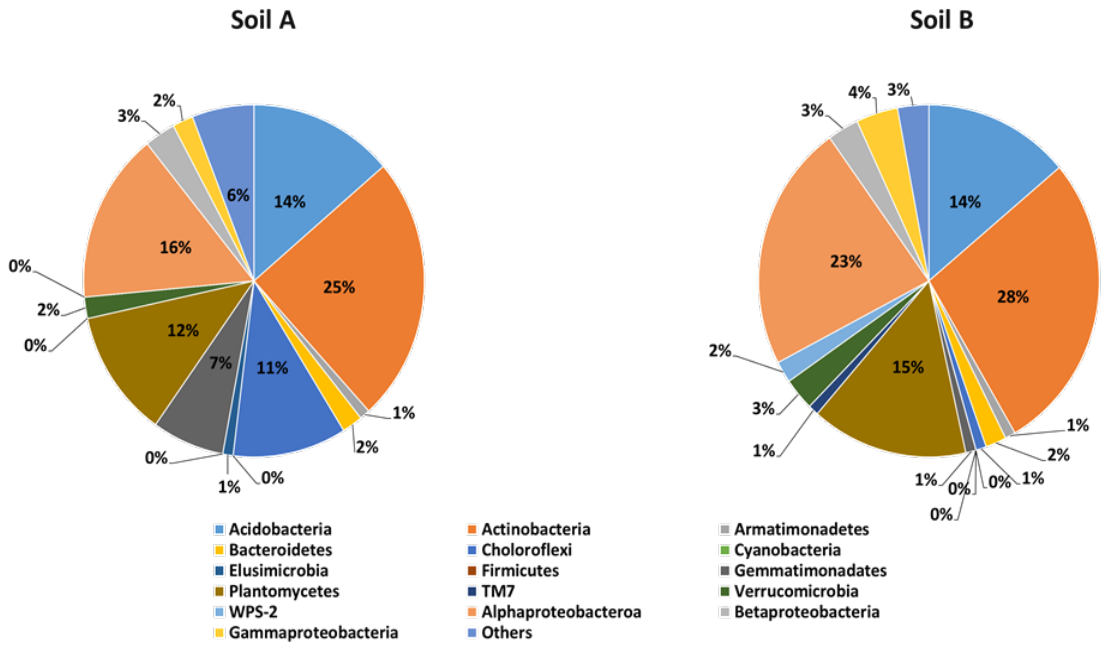


Figure 3.6a. Relative abundance of main bacterial phyla in soil A and B used for the microcosm study.

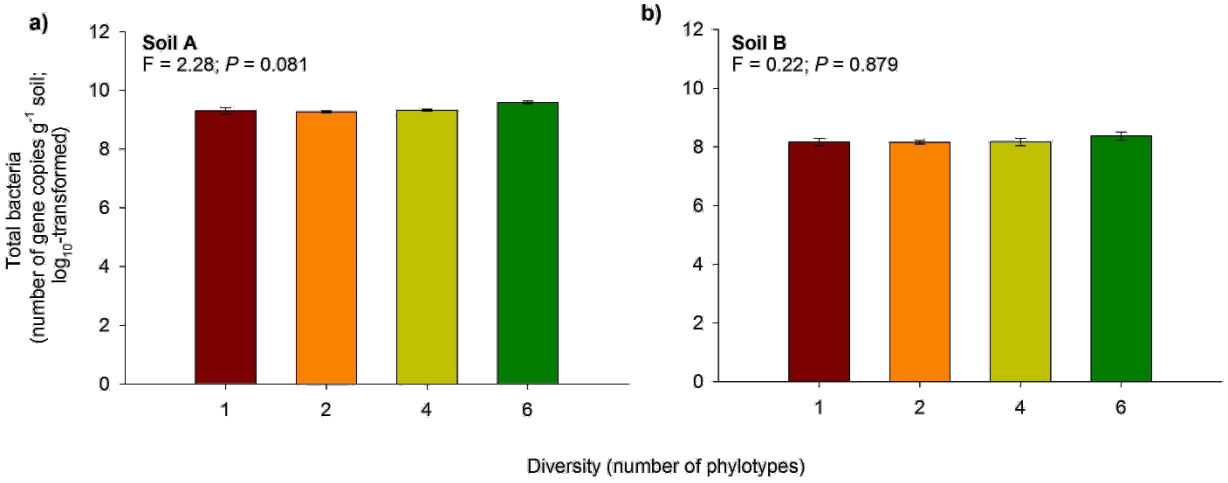


Figure 3.6b. Total abundance of bacteria (16s rRNA gene) for the different bacterial richness levels in the Microcosm study at the end of the incubation period (soils A and B).

3.5. Discussion

Despite the growing body of literature providing evidence that microbial diversity influences the way in which ecosystems function (Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a), the form of the relationship between microbial diversity and functioning remains poorly understood. Most studies have tended to focus on a particular component of diversity (richness or composition), and no previous study, to the best of my knowledge, has empirically and statistically examined the relative importance of bacterial richness as compared with other major component of biodiversity, composition in supporting multiple functions in terrestrial ecosystems. Using observational and experimental data, this chapter provides evidence that both bacterial richness and composition are key drivers of multiple ecosystems functions in terrestrial ecosystems. Most importantly, the multi-model approach indicates that these two microbial diversity components provide independent and complementary information on the role of bacteria in ecosystem processes. These results provide strong support for the hypothesis that the effects of bacterial biodiversity on ecosystem functioning are due to the combined effects of bacterial richness and identity of key taxa within a community.

Both the field survey and microcosm study provide evidence that bacterial richness is strongly and positively related to multifunctionality. These results were maintained after controlling for microbial abundance using partial correlations and ANCOVA analyses, and provided experimental support to previous observational studies showing positive relationships between soil microbial diversity and multiple soil functions, such as those used here (He *et al.,* 2009; Jing *et al.,* 2015; Delgado-Baquerizo *et al.,* 2016a). The mechanisms behind the positive effects of bacterial richness on multifunctionality could include an increase in the interactions among microbial taxa (complementarity effects; Loreau and Hector, 2001) and the so-called "sampling effect" (i.e. increasing taxa richness increases the likelihood that key taxa would be present; Hooper *et al.,* 2005). Species interactions are especially important for microbial communities that rely heavily on aggregated processes (Schimel *et al.,* 2005) such as break down of complex and recalcitrant polymers into simpler, more labile monomers, which are rapidly consumed and largely respired (Schimel *et al.,* 2005). Thus, losses in

bacterial richness may inactivate critical functions (e.g. chitin degradation), but also can reduce the rates in which multiple ecosystems functions are being produced, as supported by the observational and experimental data. Bacterial richness also showed similar positive trends with each of the single functions studied. Of interest was the fact that bacterial richness showed a strong and positive effect on N-Acetylglucosaminidase (chitinase) in all the experimental approaches used here. In agreement with Chapter of the thesis, this result further supports the notion that complex processes such as organic matter decomposition are favored by the existence of a diverse collection of microbes all contributing to the overall process to promote the highest degradation rates (Schimel *et al.,* 2005). The Microcosm study also showed that a "sampling effect" may be, at least in part, responsible for driving multifunctionality, as microcosms including certain key taxa tended to have the greatest multifunctionality. Consistent with the results reported by Hooper *et al.,* (2005) for plant communities, it is suggested that microbial taxa interaction and sampling effects are not mutually exclusive.

This study further showed that the relative importance of richness compared with composition in controlling multifunctionality is also soil-dependent, as supported by the Microcosm study (Soil A vs. B). In particular, that richness was more important than composition in Soil B, with the higher soil organic matter, while the opposite pattern occurred in Soil A. Although it is difficult to extrapolate from only two soils, if these results were generally true, they would suggest that bacterial richness might play a predominant role in organic soils, where the interaction among multiple microbial communities is needed to break down complex and recalcitrant polymers into simpler and more labile monomers (organic matter degradation; Schimel *et al.,* 2005). Conversely, species identity (Bacteroidetes and Actinobacteria in Soil A) may play a major role in mineral soils. Thus, these results support the notion that both microbial richness and composition are needed to accurately estimate the consequences of losses in microbial diversity (from global environmental changes such as climate change and land use intensification) on ecosystem functioning.

Interestingly, observational data were consistent with what was observed in the Microcosm study, providing insights into the main microbial pattern controlling

multifunctionality in terrestrial ecosystems, and demonstrating the value of using each of these approaches. For example, in both the field and microcosm studies, an increase in taxa richness was positively related to multifunctionality. This result suggests that observational data can be useful for predicting microbial community shifts and their consequences for ecosystem functioning under global change, but also that this observational data will be useful in developing generic algorithms to be included in global biogeochemical models.

3.6. Conclusions

In conclusion, these findings provide strong evidence, from two independent approaches, that bacterial richness is important and independent drivers of multiple ecosystem functions related to organic matter decomposition and nutrient cycling. Greater microbial richness was critical drivers of multifunctionality in both field and microcosm studies. These findings advance our understanding of the mechanisms underpinning relationships between biodiversity and ecosystem functionality in terrestrial ecosystems, and reinforce the need to develop approaches and policies to protect soil microbial diversity and their positive effects for multiple ecosystems functions.

Chapter 4

Experimentally testing the link between microbial diversity and the resistance of ecosystem functions to global change scenarios

4.1. Abstract

Soil microbial communities play fundamental roles for many ecosystem processes; however, little empirical evidence is available on the role of microbial biodiversity in driving the responses of soil functions to global change. This is a significant gap in our understanding on the biodiversity-function relationship, as the stability of microbial communities, defined as a community's ability to resist and recover from disturbances, will have huge implications on the sustainability of ecosystem functioning in future climate change scenarios. Here I addressed the ecological insurance hypothesis by examining the effect of loss of microbial diversity on the stability of broad (CO_2 production) and narrow (CH_4 oxidation) functions under different climate change drivers (elevated temperature, nitrogen deposition, and wetting-drying cycles). I used the dilution- to- extinction approach to create microbial diversity gradients in soil microcosms from samples collected from sites with differences in soil properties. Among the three disturbances, the resistance was lowest in the elevated temperature treatment. Most importantly this study identified that diversity/richness and community composition are likely to be important drivers of resistance of both broad and specific function to global change drivers; however, the results varied with soil types and type of disturbances. The loss of microbial diversity may have significant negative effects on the performance of both specialised and broad functions. These findings further suggest that microbial diversity and community measure of relative abundance of r- and k- strategists can contribute to explaining the response of microbial community composition to climate-related disturbances in most cases. Together, our work demonstrates links between microbial communities and resistance of soils to perform ecosystem processes, and provides insights into the importance of soil microbial communities for buffering effects of global climate change in terrestrial ecosystems.

Keywords: Ecosystem function; Resistance; Copiotrophs-Oligotrophs; Climate change; Soil microbes

4.2. Introduction

The functioning and stability of natural ecosystems arebeing largely impacted by various anthropogenic and climatic disturbances including nitrogen deposition, global warming, and extreme climatic events (e.g., drought). These disturbances alter both the biodiversity of plants and microbes and the overall functioning of terrestrial ecosystems. The role of biodiversity as a major driver of ecosystem functioning is largely recognized (Hooper *et al.*, 2005; Cardinale *et al.*, 2006; Loreau, 2010; Reich *et al.*, 2012). Biodiversity provides a larger genetic pool of species that can respond differently to disturbances and provide an ecosystem insurance by reducing the likelihood of large changes in ecosystem functioning in response to environmental perturbations (Ecological Insurance theory; Yachi and Loreau, 1999; McCann, 2000). In the last decade, many studies have explicitly demonstrated that global change can alter ecosystem functions indirectly by altering above-ground biodiversity (Walther *et al.*, 2002; Gottfried *et al.*, 2012; Langley and Hungate 2014). Much less is known on the role of soil microbes, and microbial diversity in particular, in regulating the responses of ecosystem functions to global change drivers, including climate change and over-fertilization (Schimel *et al.*, 2007; de Vries *et al.*, 2012). Soil microbial communities are the most divergent and abundant organisms on the planet Earth and they perform multiple functions that determine the sustainable delivery of ecosystem services including primary production, and climate regulation (van der Heijden *et al.*, 2008; Wagg *et al.*, 2014; Bardgett and van der Putten, 2014; Bodelier, 2011). Therefore, understanding whether soil microbial communities can help regulating the resistance of ecosystem functions to global change drivers is crucial for developing sustainable ecosystem management and conservation policies.

In recent years, an increasing number of studies provided evidence of positive linkage between microbial diversity and ecosystem functions both in observational (Delgado- Baquerizo *et al.*, 2016; 2017) and experimental (Philippot *et al.*, 2016; Crawford *et al.*, 2012; Chapter 2) studies. However, role of microbial biodiversity in driving the responses of soil functions to global change remains largely unexplored and poorly understood (de Vries and Shade, 2013). Limited studies that attempted to decipher the role of soil microbial communities towards ecosystem resistance have provided contrasting results on the importance of various components of biodiversity including

richness, abundance and community structure (Tardy *et al.*, 2014; Girvan *et al.,* 2005; Awasthi *et al.,* 2014; Zhang and Zhang 2016). For example, a recent study in global dryland reported that resistance of multiple ecosystem functions (multifunctionality resistance) is linked to microbial community compositions but not diversity or abundance (Delgado-Baquerizo *et al.*, 2017a). Contrastingly, other studies reported that microbial diversity promotes functional resistance after environmental perturbation (Girvan *et al.*, 2005; Awasthi *et al.*, 2014). Resistance of soil functions is clearly related to their biodiversity, at least from a conceptual point of view (Wardle, 2002), however, empirical evidence for such a link is lacking. Soil composition is also expected to be a key driver of soil functional resistance to global change drivers. For example, some microbial mediated functions such as methane (CH_4) production/consumption defined as "narrow functions" carried out by specialised phylogenetic conserved groups of microbes (Hol *et al.*, 2010) will show differential trends in BEF relationship and functional resistance as compared to "broad/aggregate" functions such as CO_2 production widely distributed among microorganisms (Schimel and Schaeffer 2012; de Vries and Shade, 2013; Wood *et al.*, 2015).

The aim of this chapter was to evaluate the role of microbial diversity and community composition in driving the responses of soil broad and specialised functions to global change drivers, named warming, drying-wetting cycles and N fertilization. To achieve this aim, I used dilution- to- extinction approach to create microbial diversity gradients in soil microcosms from samples collected from sites with differences in soil properties (details provided in Chapter 2, Table 2.1). These microcosms differed both in microbial diversity (measured in terms of Shannon diversity and richness) and community composition whereas the abundance of various microbial groups (including total bacteria, fungi, methanotrophs as measured by qPCR) was similar within microcosms from individual sites (refer to chapter 2 section 2.3.3.2). Soil microcosms were subjected to treatments relevant to global change scenarios, via elevated temperature, nitrogen addition and wetting and drying cycle. Considered soil functions include "broad" (soil respiration measured as CO_2 production) and "narrow" soil (oxidation of CH_4) functions. I hypothesized that: a) loss of diversity will have a stronger

effect on the resistance of the specialised function, given the lower redundancy of specialised microbes, which have relatively low abundance in soil; b) differences in the resistance of measured functions between two soils would be related to the different components of soil biodiversity (e.g. community composition, diversity, richness).

4.3. Material and Methods

4.3.1. Site description and microcosm preparation

Soil samples were collected from the top 10 cm from Goolgowi mallee (site A; NSW 33.9667° S, 145.7000° E) and Warraderry State Forest (site B; NSW, 33.7035° S, 148.2612° E), New South Wales, Australia; both of them dominated by *Eucalyptus spp.* Site characteristics and soil properties for both the soils are presented in Table 2.1. Both the soil types differed in various soil properties (Table 2.1), microbial abundance (Supplementary Figure 2.1), microbial community composition (Supplementary Figure 2.2), and diversity of various groups of microbes (including total bacteria, fungi, and methanotrophs; Figure 2.2; 2.3)

I used a dilution to extinction approach, involving the inoculation of sterile soil microcosms with different dilutions of a soil microbial suspension, was used to manipulate microbial diversity (Peter *et al.,* 2011; Philippot *et al.,* 2013; Delgado-Baquerizo *et al.,* 2016). Briefly for each soil (soils A and B), 5 dilutions were used as the microbial inoculum to create a diversity gradient; these dilutions were undiluted (10x; DX); 1/10 dilution (D1); $1/10^3$ dilution (D3); $1/10^6$ dilution (D6); and $1/10^{10}$ dilution (D10). Details of microcosm assembly is provided in Chapter 2 (also see figure 2.1).

4.3.2. Disturbance treatments

A total of total of 160 (2 sites x 5 dilution x 4 replicates x 4 treatments) were incubated under different treatments for 21 days. Twenty-five grams of soils from each microcosm was placed in four 500ml jar, one each for each global driver plus an environmental control. The lid of the jar was closed with polyethylene film allowing gas exchange, but avoiding water losses (except for wetting drying treatment which was kept without using polyethylene film). The levels of different treatment were selected to provide the realistic estimation of the response of ecosystem functioning to climate change, and land-use

intensification such as N fertilization from atmospheric N deposition (Liu et al., 2016; Delgado-Baquerizo et al., 2017). Environmental control was incubated at 25^0C, the average temperature for both sites, and 35% of water holding capacity (WHC). This amount of WHC is reported to ensure minimum microbial activity during the incubation period (Schwinning and Sala, 2004; Delgado-Baquerizo *et al.,* 2013a; b; 2017). The warming treatment has similar water conditions as the environmental control but with increased temperature (4.5^0C). The increment in the temperature was chosen to mimic global warming forecasts by the end of this century (A2 scenario from IPCC Climate Change 2013). The wetting drying treatment was incubated at the same temperature as the environmental control, but included three wetting-drying cycles. Each cycle involved wetting until a 35% WHC was achieved and a subsequent natural drying for 5 days. Soil samples were watered the first day of incubation. Finally, the N fertilization treatment included the same temperature and water conditions as the environmental controls plus the equivalent of 20 kg N ha^{-1} $year^{-1}$ which was added in the form of NH_4NO_3 during the first watering. The amount was selected to simulate artificial N loads from N deposition in global drylands (Eldridge and Delgado-Baquerizo, 2017). Moisture content was adjusted and maintained at 35% WHC during the duration of the experiment for all treatment other than the wetting-drying treatment.

4.3.3. Determination of microbial community composition

Please refer to section 2.3.3.1. for DNA extraction and PCR analysis, section 2.4.1 for biomass recovery, section 2.4.1 and Fig 2.3 for effect of dilution on diversity and richness, section 2.4.2 and Supplementary Figure 2.2a-2.2b for Miseq analysis of Bacterial and fungal community for details.

4.3.4. Gas flux measurement

Please refer to section 2.3.4.1.

4.3.5. Statistical and numerical analyses

Resistance index was determined using following equation as described Orwin & Wardle (2004).

$$RS = 1 - \frac{2|D0|}{(C0 + |D0|)}$$

Where D0 is the difference between the control and the disturbed (Treatment) Site At the end of the disturbance. Resistance index is bounded by -1 and +1, with a resistance index value of +1 showing that the disturbance had no effect (maximal resistance), and lower values showing stronger effects (less resistance) (Orwin and Wardle 2004). This index has the advantage of being standardized by the control.

All the differences between dilution treatments were assesses by means of one-way analysis of variance (ANOVA), dependent variables were tested for normality. Abundance were log transformed in order to mean the normality assumptions of the ANOVA. The relationship between Shannon diversity, richness, abundance, community structure and function was carried out by means of linear regressions. A one-way ANOVA was conducted to compare the effect of diversity on gas flux production and to compare the response among diversity, pairwise comparison of means was conducted using Tukey's HSD. All these analyses were carried out in SPSS 24.

4.4. Results

4.4.1. Function resistance of CO_2 (Broad function)

4.4.1.1. Elevated temperature treatment: The values of CO_2 resistance index were lower in both the soil types as compared to the two other treatments, named N deposition treatment and wetting and drying treatment. N deposition and wetting drying (Figure 4.1). No effect of diversity dilution on function resistance was observed for soil A. For soil B, the resistance index was significantly higher in highest diversity treatment (No dilution, DX) as compared to D1, D3 and D6 dilution and reduced sharply at D10 dilution. There was approximately 6-fold decrease in the resistance index between DX and D10 dilutions wherein the values decreased from 0.57±0.23 (DX) to -0.04±0.10 (D10). In site A, no significant relationship was observed between Shannon diversity, richness, community

composition and abundance of bacteria and fungi with CO_2 resistance index. In contrast, in samples from the site B a significant positive correlation was observed between bacteria community composition ($P<0.01$), Shannon diversity ($P<0.01$) and richness and CO_2 resistance index (Table 4.1).

***4.4.1.2. Nitrogen deposition treatment*:** Soils from both sites showed significant decrease in resistance index of CO_2 in soils with lower diversity under the N deposition treatment**.** In site A, the highest diverse soils (DX) had an average resistance index of 0.71±0.05 which decreased to 0.42±0.08 in the sample with lowest diversity (D10). Similarly, in site B, soils with highest diversity had an average resistance index of 0.76±0.08 which decreased to 0.25±0.34 in soils with lowest diversity (D10). The CO_2 Resistance index in this treatment was significantly linked to Shannon diversity ($P<0.02$) and richness ($P<0.05$) in Soils from site A. However, in site B only bacterial richness was statistically linked to CO_2 resistant index ($P<0.05$). (Table 4.1)

***4.4.1.3. Wetting and drying treatment*:** Soils from both sites showed significant decrease in resistance index of CO_2 in soils with lower diversity under the wetting and drying treatment. In soils from the site A, soil with the highest diversity (DX) have an average CO_2 resistance index of 0.71±0.06 and decreased to 0.58±0.01 in soils with lowest microbial diversity (D10). Similar trend was observed in soils from the site B. The highest diversity soils had an average CO_2 resistance index of 0.79±0.06 which decreased to an average resistance index of 0.3±0.36. There was strong positive and significant correlation was observed between CO_2 resistance index with Shannon diversity ($P<0.04$) in soils from the site A. No significant correlation between community composition, richness and abundance of bacteria, fungi and CO_2 resistance index was observed in soils from the site B (Table 4.1).

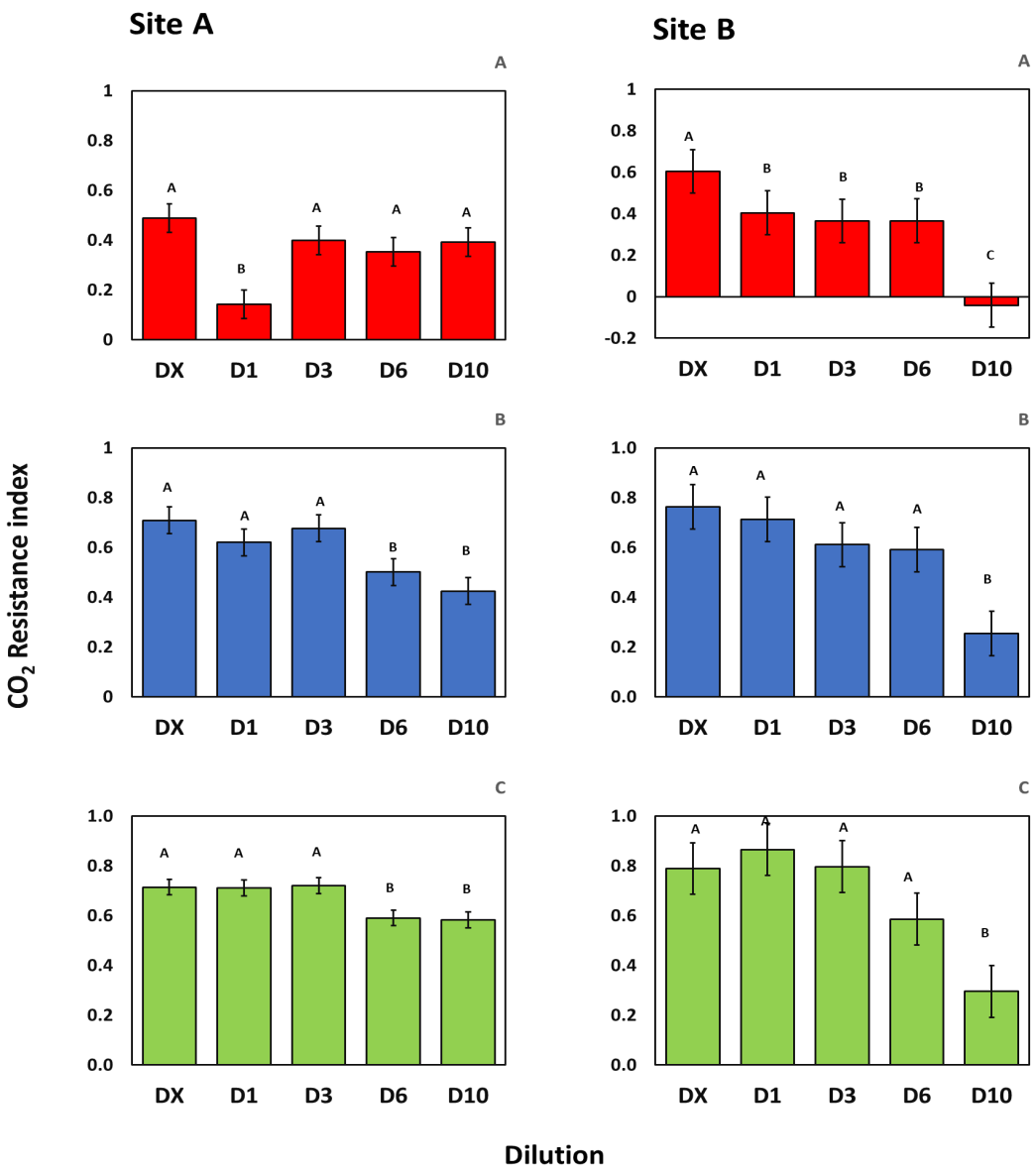


Figure 4.1. Resistance index calculated on CO_2 oxidation rate along with the dilution gradients with in different treatments [Elevated temperature (a), Nitrogen deposition (b), Wetting drying (c)]. Error bar indicate ± one standard error (n=4). Different letters indicate significant differences between the dilution.

Table 4.1. Relationship community composition (NMDS axis 1), Shannon diversity, richness and abundance of bacteria and fungi with CO_2 resistance in different treatment (n=4) in both site. Significant differences at *P<0.05* values are in bold letter.

Microbial community attributes	**Microbial groups**	**Soil A**			**Soil B**		
		Elevated temperature	**Nitrogen deposition**	**Wetting and drying**	**Elevated temperature**	**Nitrogen deposition**	**Wetting and drying**
Community composition	**Bacteria**	-0.01(0.957)	**-0.56(0.01)**	**-0.49(0.028)**	**0.56(0.01)**	0.4(0.082)	0.37(0.112)
	Fungi	0.06(0.803)	0.11(0.636)	0.09(0.719)	-0.34(0.148)	0 (1)	-0.12(0.618)
Shannon diversity	**Bacteria**	0.32(0.176)	**0.5(0.024)**	**0.46(0.041)**	**0.56(0.01)**	0.43(0.061)	0.35(0.131)
	Fungi	0.2(0.39)	0.39(0.086)	0.32(0.164)	0.31(0.188)	0.22(0.352)	0.14(0.565)
Richness	**Bacteria**	0.22(0.347)	**0.44(0.055)**	0.32(0.167)	**0.5(0.026)**	**0.45(0.045)**	0.35(0.136)
	Fungi	-0.06(0.801)	0.25(0.292)	0.2(0.405)	**0.52(0.02)**	0.39(0.086)	0.36(0.118)
Abundance	**Bacteria**	-0.04(0.853)	0.15(0.516)	-0.01(0.957)	0.19(0.425)	0.4(0.084)	0.23(0.327)
	Fungi	0.05(0.821)	-0.24(0.3)	-0.23(0.326)	0.14(0.563)	-0.09(0.712)	-0.14(0.554)

4.4.2. Function resistance of CH_4 (Narrow function)

***4.4.2.1. Elevated temperature treatment*:** The loss of diversity had very strong and negative impact on the resistance index of CH_4. Soils with the highest diversity (DX) had an average resistance index of 0.56±0.09, which linearly decreased to an average resistance index of 0.08±0.03 in soils with lowest microbial diversity (D10). Similar but comparatively weak trend was found in soils form site B (Figure 4.2). In soils from the site A, significant positive relationship observed between community composition of bacteria and fungi, however, community composition of *pmoA* was observed negatively significant with CH_4 resistance index (P<0.005). Bacterial fungal and *pmoA* richness were also significantly correlated with CH_4 resistance index (P<0.001). In soils from the site B, no significant correlation was observed between CH_4 resistant index, community composition, Shannon diversity, richness and abundance of bacteria fungi and *pmoA*. (Table 4.2).

4.4.2.2. Nitrogen deposition treatment: In soils from the site A, the highest diverse soil (DX) had an average of 0.68±0.05 which decreased to average 0.47±0.13 in soil with lowest microbial diversity (D10). Similarly, in soils from the site B, the resistance index of resistant index of CH_4 in the highest diverse soils of 0.69±0.08 decreased to resistant index of 0.33±0.13 in soils with the lowest diversity (Figure 4.2). In this treatment community composition of *pmoA* was observed negatively linked with CH_4 resistance index (P<0.01). A positive significant relationship was observed between Shannon diversity of bacteria fungi and *pmoA* (P<0.02) in soils from both sites. Richness of bacteria and *pmoA* were also found significant lined to CH_4 resistance index. (Table 4.2).

***4.4.2.3. Wetting and drying treatment*:** I observed a trend towards decrease in the resistance index with the lower diversity treatments (D3, D6, D10) as compared to higher diversity treatment (DX, D1) in soil A but no consistent effect was found in soils from the site B. The resistance index of CH_4 oxidation data had the highest value of 0.73+0.11 in the soils with highest diversity (DX) significantly decreased in samples with lower diversity with an average value of 0.49 in soils with the lowest diversity (D10). The impact of diversity on resistance index of CH_4 oxidation in soils from the site B was inconsistent (Figure 4.2). In soils from the site A, only fungal diversity and richness was

significant linked with the CH_4 resistance index. Additionally, bacteria abundance was found positively significant with CH_4 resistance index. However, no significant relationship was observed between resistance index and community composition, diversity, richness and abundance in soils from the site B. (Table 4.2).

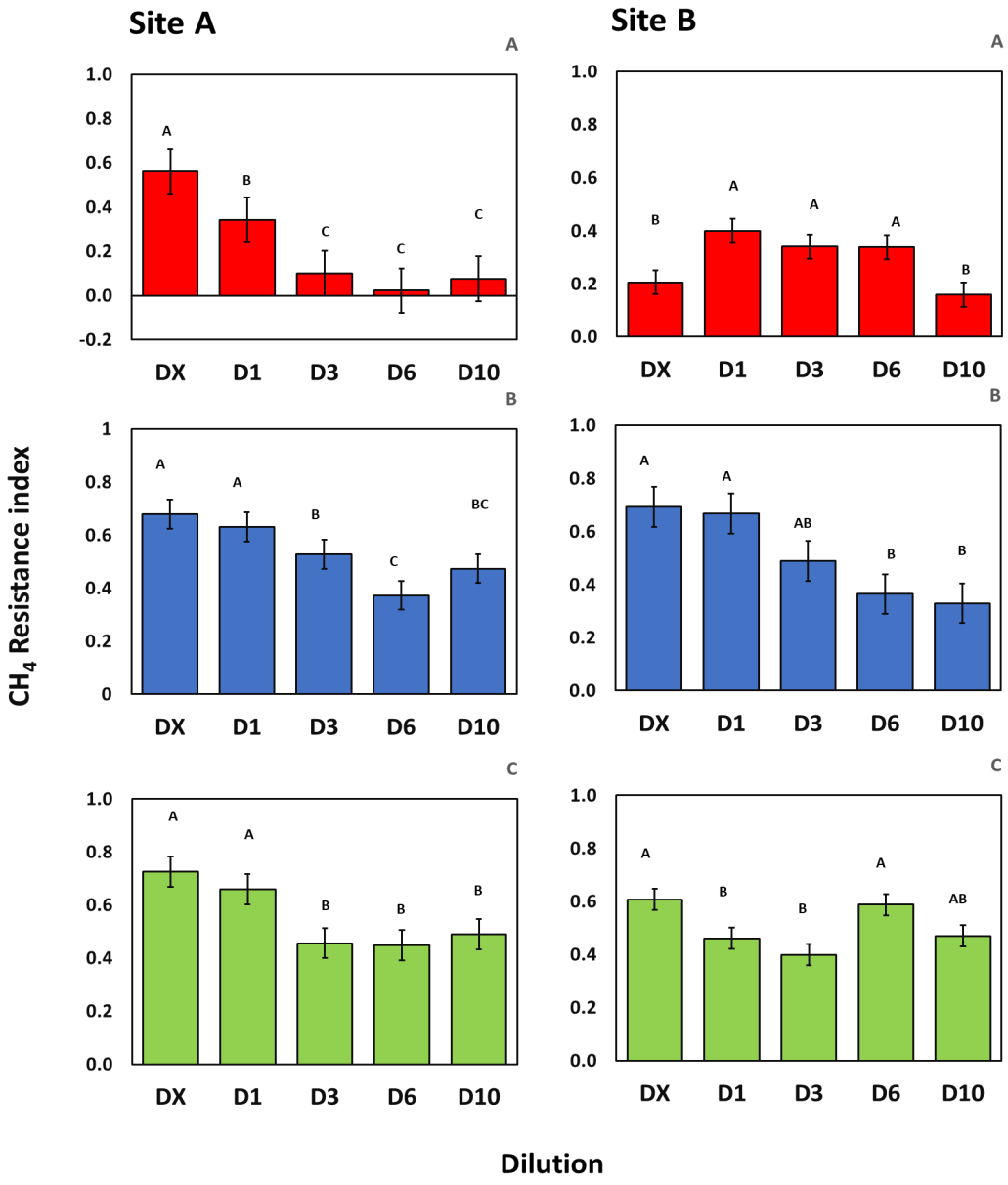


Figure 4.2. Resistance index calculated on CH_4 oxidation rate along with the dilution gradients with in different treatments [Elevated temperature (a), Nitrogen deposition (b), Wetting drying (c)]. Error bar indicate ± one standard error (n=4). Different letters indicate significant differences between the dilution.

Table 4.2. Relationship community composition (NMDS axis 1), Shannon diversity, richness and abundance of bacteria and fungi with CH_4 Resistance in different treatment (n=4) in both site. Significant differences at $P<0.05$ values are in bold letter.

Community		**Soil A**			**Soil B**		
		Elevated temperature	**Nitrogen deposition**	**Wetting and drying**	**Elevated temperature**	**Nitrogen deposition**	**Wetting and drying**
Community composition	**Bacteria**	-0.21(0.38)	-0.24(0.313)	0.09(0.7)	0.17(0.486)	**0.48(0.031**)	0.05(0.838)
	Fungi	**0.52(0.018)**	-0.36(0.123)	0.2(0.392)	-0.38(0.095)	-0.19(0.423)	0.08(0.726)
	pmoA	**-0.61(0.005)**	**-0.56(0.011)**	-0.23(0.321)	0.15(0.527)	0.41(0.077)	0.03(0.915)
Shannon diversity	**Bacteria**	**0.6(0.006)**	**0.5(0.026)**	0.35(0.125)	0.13(0.582)	**0.56(0.01)**	0.06(0.811)
	Fungi	**0.74(0.001)**	**0.51(0.021)**	**0.52(0.018)**	-0.04(0.875)	**0.64(0.002)**	0.18(0.462)
	pmoA	**0.77(0.001)**	**0.54(0.013)**	0.33(0.155)	0.17(0.464)	**0.59(0.006)**	0.17(0.484)
Richness	**Bacteria**	**0.68(0.001)**	**0.43(0.058)**	0.34(0.147)	0.11(0.647)	**0.55(0.011)**	0.01(0.952)
	Fungi	**0.72(0.001)**	0.26(0.278)	**0.54(0.013)**	0.19(0.421)	**0.61(0.004)**	0.06(0.803)
	pmoA	**0.71(0.001)**	**0.58(0.007)**	0.33(0.151)	0(0.986)	**0.59(0.006)**	-0.01(0.976)
Abundance	**Bacteria**	0.3(0.193)	**0.49(0.03)**	**0.44(0.054)**	0.23(0.321)	0.21(0.375)	0.28(0.226)
	Fungi	-0.39(0.089)	0.13(0.601)	-0.26(0.27)	-0.15(0.518)	-0.14(0.548)	0.36(0.119)
	pmoA	0.19(0.412)	-0.12(0.618)	-0.31(0.179)	-0.42(0.064)	-0.27(0.256)	-0.07(0.767)

4.4.3. Relationship of resistance index with relative abundance of various phyla (class in Proteobacteria)

I categorized the bacterial groups as oligotrophs/copiotrophs based on the previous studies (Table 4.3 and 4.4). The rational of this differentiation is based on the fact that these functional groupings in bacteria were reported to be related to the resistance in soil microbial communities in previous studies (de Varies and Shade 2014; Delgado-Baquerizo *et al.*, 2017a). Our results showed differences in the relative abundances of both the groups in two soil types and among various dilutions within each soil (Figure 4.3)

4.4.3.1. CO_2 resistance index (Broad function, Table 4.3):

In this analysis, none of the groups was statistically correlated with CO2 resistance index under the elevated temperature treatment in soils from the site A. In soils from the site B, resistance index was positively correlated ($P<0.05$) with various oligotrophic groups including Acidobacteria ($P<0.02$); Chloroflexi ($P<0.02$); Gemmatimonadetes ($P<0.02$); Planctomycetes ($P<0.02$); and Deltaproteobacteria ($P<0.03$). For copiotrophs the resistance index was positively and negatively correlated with the relative abundance of Alphaproteobacteria ($P<0.02$) and Bacteroidetes ($P<0.02$).

In soils from the both sites A and B, resistance index under the nitrogen deposition treatment, was significantly and positively correlated with Deltaproteobacteria ($P<0.002$ and $P<0.05$ for soil A and B, respectively). Resistance index was negatively correlated with Actinobacteria ($P<0.05$) and Bacteroidetes (copiotroph; $P<0.04$) for soil A and B, respectively. The resistance index was significantly and positively correlated with various oligotrophic groups in soils from sites A and B under the wetting and drying treatment. Significant positive correlations were observed for Chloroflexi ($P<0.02$); Gemmatimonadetes ($P<0.03$); and Deltaproteobacteria ($P<0.008$) for soils from the site A and Planctomycetes ($P<0.05$) was significantly correlated with resistance index both for soil A ($P<0.05$) for soil B.

Table 4.3. Relationship between main bacteria phylum (class for Proteobacteria) with CO_2 resistance index in different treatment (n=4). Green and red colour in phylum represent copiotrophs and oligotrophs, respectively. * shows the references that have classified bacteria phyla/class into copiotroph/oligotrophy grouping. Significant differences at $P<0.05$ values are shown in bold letter.

Phylum/Class*	Soil A			Soil B		
	Elevated temperature	Nitrogen deposition	Wetting and drying	Elevated temperature	Nitrogen deposition	Wetting and drying
Acidobacteria[1, 2]	0.27(0.259)	0.1(0.668)	0.35(0.128)	**0.52(0.02)**	0.33(0.151)	0.34(0.14)
Actinobacteria	0(0.987)	**-0.44(0.053)**	-0.11(0.647)	**0.43(0.051)**	0.26(0.263)	0.41(0.071)
Bacteroidetes[1]	-0.32(0.169)	0.18(0.437)	-0.18(0.452)	**-0.49(0.027)**	**-0.45(0.046)**	-0.39(0.092)
Chloroflexi[2,3]	0.35(0.127)	0.2(0.41)	**0.51(0.022)**	**0.5(0.026)**	0.34(0.14)	0.42(0.068)
Firmicutes	0.34(0.149)	0.17(0.468)	0.05(0.848)	-0.04(0.872)	-0.01(0.977)	0.33(0.161)
Gemmatimonadetes[4]	0.27(0.255)	0.38(0.096)	**0.48(0.034)**	**0.49(0.028)**	0.17(0.468)	0.08(0.741)
Planctomycetes[5]	0.22(0.363)	0.14(0.547)	0.19(0.434)	**0.51(0.022)**	0.31(0.184)	**0.44(0.05)**
Verrucomicrobia[4]	0.22(0.36)	0(0.992)	0.24(0.301)	-0.25(0.293)	0.03(0.902)	-0.2(0.4)
Alphaproteobacteria[1,2]	-0.05(0.843)	0.18(0.444)	0.24(0.311)	**0.5(0.026)**	0.29(0.217)	0.07(0.762)
Betaproteobacteria[1,2]	-0.12(0.618)	0.27(0.245)	-0.04(0.88)	-0.2(0.4)	-0.38(0.094)	-0.4(0.077)
Deltaproteobacteria[1,2]	0.04(0.88)	**0.65(0.002)**	**0.57(0.008)**	**0.49(0.03)**	**0.42(0.05)**	0.34(0.142)
Gammaproteobacteria[1,2]	-0.03(0.912)	0.13(0.598)	-0.13(0.576)	-0.31(0.19)	-0.09(0.7)	-0.1(0.686)

*[1] Fierer *et al.*, (2005); [2] Trivedi *et al.*, (2013); [3] Trivedi *et al.*, (2013); [4] Portillo *et al.*, (2013); [5] Fuerst and Sagulenko, (2011)

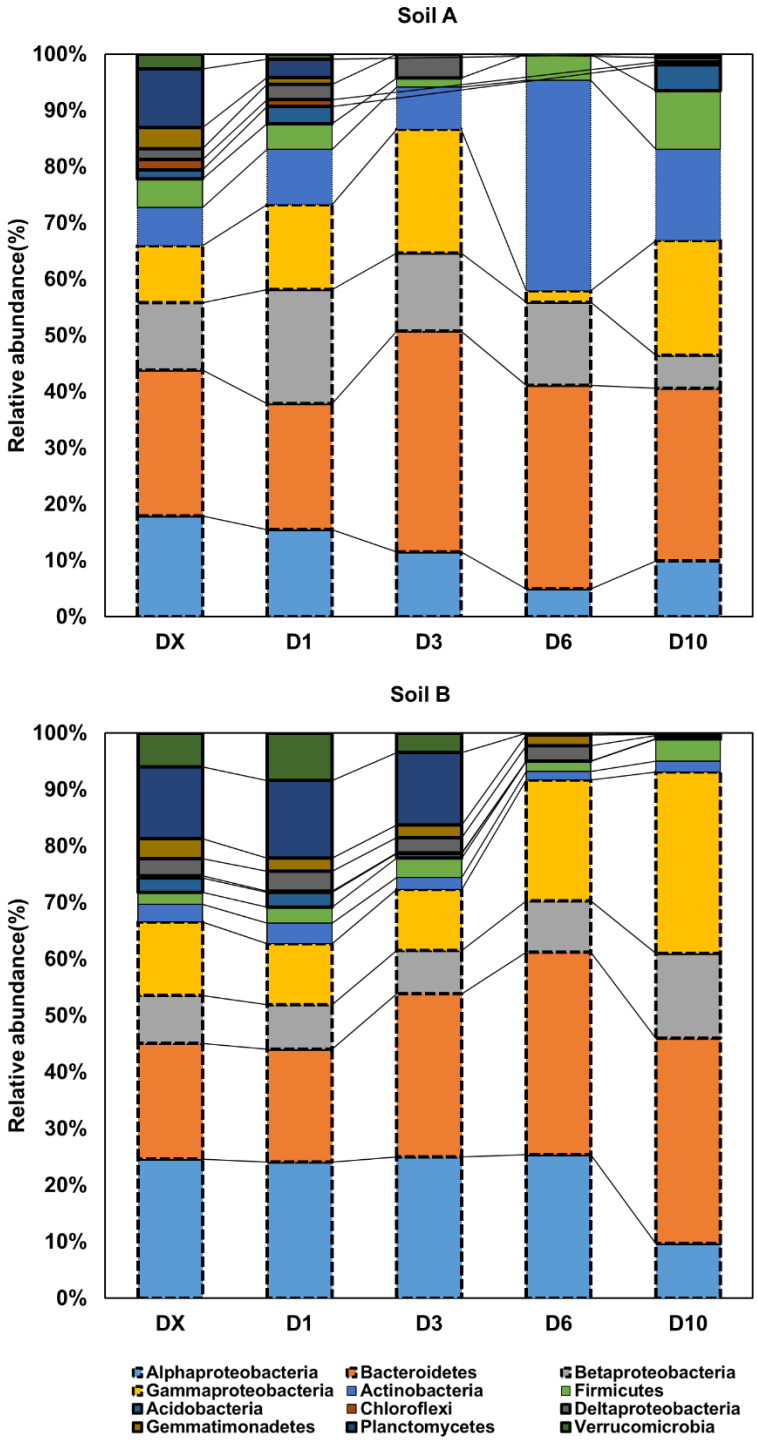


Figure 4.3. Relative abundance of soil bacterial community composition at phylum level in soil A and B. The proteobacterial classes (e.g., Alpha-, Beta-, Gamma-and Deltaproteobacteria) were also included at the phylum-level analysis to provide more detailed information. Broad dotted and plain borders represent copiotrophs and oligotrophs, respectively.

Table 4.4. Relationship between main bacteria phylum (class for Proteobacteria) with CH_4 resistance index in different treatment (n=4). Green and red colour in phylum represent copiotrophs and oligotrophs, respectively. * shows the references that have classified bacteria phyla/class into copiotroph/oligotrophy grouping. Significant differences at $P<0.05$ values are shown in bold letter.

Phylum/Class*	Soil A			Soil B		
	Elevated temperature	Nitrogen deposition	Wetting and drying	Elevated temperature	Nitrogen deposition	Wetting and drying
Acidobacteria[1, 2]	0.37(0.113)	0.38(0.104)	0.39(0.086)	0.01(0.955)	**0.62(0.003)**	-0.03(0.9)
Actinobacteria	**-0.5(0.026)**	-0.4(0.077)	0.05(0.843)	0.11(0.636)	**0.52(0.018)**	0.23(0.329)
Bacteroidetes[1]	-0.18(0.456)	**-0.44(0.05)**	-0.01(0.967)	-0.08(0.731)	-0.37(0.114)	-0.23(0.337)
Chloroflexi[2,3]	0.42(0.066)	0.37(0.107)	0.42(0.063)	0.21(0.376)	0.37(0.104)	0.05(0.82)
Firmicutes	0(0.992)	0.21(0.364)	-0.21(0.378)	0.19(0.435)	-0.07(0.757)	-0.26(0.276)
Gemmatimonadetes[4]	**0.66(0.001)**	**0.45(0.046)**	**0.59(0.006)**	0.22(0.356)	0.2(0.409)	0.03(0.9)
Planctomycetes[5]	**0.64(0.002)**	0.31(0.187)	**0.57(0.009)**	0.26(0.268)	**0.46(0.043)**	-0.19(0.432)
Verrucomicrobia[4]	**0.53(0.016)**	0.28(0.225)	**0.55(0.012)**	0.04(0.86)	**0.59(0.006)**	-0.1(0.691)
Alphaproteobacteria[1,2]	**0.56(0.01)**	**0.83(0.001)**	0.33(0.157)	-0.05(0.828)	0.29(0.221)	0(0.992)
Betaproteobacteria[1,2]	0(0.987)	0.1(0.684)	-0.15(0.537)	-0.1(0.682)	-0.08(0.738)	-0.04(0.875)
Deltaproteobacteria[1,2]	**0.65(0.002)**	0.3(0.197)	0.42(0.067)	0.24(0.308)	0.34(0.145)	0.13(0.585)
Gammaproteobacteria[1,2]	0.34(0.141)	0(0.995)	-0.06(0.811)	0.18(0.45)	-0.32(0.166)	0.44(0.064)

*[1] Fierer *et al.*, (2005); [2] Trivedi *et al.*, (2013); [3] Trivedi *et al.*, 2013; [4] Portillo *et al.*, 2013; [5] Fuerst and Sagulenko 2011

4.4.3.2. CH_4 resistance index (Narrow function; Table 4.4)

In soils from the site A, under elevated temperature resistance index of CH_4 was positively correlated (P<0.05) with various oligotrophic groups including Gemmatimonadetes (P<0.001); Planctomycetes (P<0.002); Verrucomicrobia (P<0.01); and Deltaproteobacteria (P<0.002). For other groups, resistance index was negatively correlated with Actinobacteria (P<0.02) and positively correlated with the relative abundance of Alphaproteobacteria (copiotroph; P<0.01). None of the groups was correlated with CH_4 resistance index under the elevated temperature in soils from the site B. For the nitrogen deposition treatment, oligotrophic groups belonging to Gemmatimonadetes (P<0.04) and Planctomycetes (P<0.04), Verrucomicrobia (P<0.006), Acidobacteria (P<0.03) were significantly and positively correlated with CH_4 resistance index in soils from the site A and B, respectively. In soils from the site A, copiotrophic groups belonging to Bacteroidetes (P<0.05) and Alphaproteobacteria (P<0.001) showed significant negative and positive correlations with resistance index, respectively. Oligotrophic groups belonging to Gemmatimonadetes (P<0.001); Planctomycetes (P<0.009); and Verrucomicrobia (P<0.01) showed significant positive correlation with CH_4 resistance index under the wetting and drying treatment. None of the groups was correlated with CH_4 resistance index under wetting-drying treatment in soils form the site B.

4.5. Discussion

The relationship between microbial diversity and functionality in the context of perturbation has been addressed in a few studies that used serial dilutions of soil microbial communities as models (Griffiths *et al.*, 2000, 2004; Wertz *et al.*, 2007). These studies have provided contrasting results on the relationship between biodiversity and the stability of various functions under environmental perturbations. For example, a few studies found no consistent linkage between biodiversity and the stability resistance for both broad and specialised functions, attributing that to the high functional redundancy of the microbial communities Griffiths *et al.,* (2000; 2004) and Wertz *et al.,* (2007). On the other hand, Tardy *et al.,* (2014) reported that increased diversity in soil microbial communities provides stability towards environmental disturbances. In our study, I found that that the overall patterns on the effect of microbial diversity on the stability of measured functions are consistent in many cases

suggesting a general biodiversity effect. Comparison of our results with previous studies (Griffiths *et al*., 2000; 2004; Wertz *et al*., 2007; Tardy *et al*., 2014) that have used experimental approaches, suggest that the importance of microbial biodiversity towards functional resistance is context dependent and may rely on the type of soil; studied process; and component of soil biodiversity (Figure 4.1-4.2; Table 4.1). Therefore, caution must be applied in formulating universal trends on the contribution of soil microbes in ecosystem functioning particularly under global climate change.

Results from this study showed that the role of microbial diversity and composition in driving the responses of soil functions to global change drivers is highly contextual and it depents on the studied soil and function (Hypothesis 1 and 2). Even so, it is also clear that the role of biodiversity is more noticeable in the case of specialised than broad functions. Thus, although I observed an accelerated declining curve across diversity levels for CO_2 resistance index in most of the treatments (except for elevated temperature treatment in soil A) these trends were not significant at higher dilutions (from DX to D6 dilutions). Previous studies have suggested that as respiration is a universal function among soil microbes, the loss of diversity or changes in the community composition that the disturbance might have caused did not impair the ability of the species that survived the disturbance to perform essential functions (Wertz *et al.*, 2006). It was suggested that the resistance of the processes wherein many species are equally efficient, it matters marginally which species is dominating in the environment (Langenheder *et al.*, 2005). However, Zhang and Zhang (2016) showed significant impact of microbial diversity loss on soil respiration. Our resulted showed that various components of soil biodiversity are related to CO_2 resistance and a significant decline in the resistance index was observed at lower dilutions. It can be argued that as respiration is the cumulative total of the degradation of various resources, identity of species with different functional potentials might be important for stability of this broad function in natural conditions under long-term disturbances (Trivedi *et al.*, 2016). Based on the knowledge gained from several studies, it can be postulated that functional diversity of soil microorganisms with respect to the utilization of C sources might be important for resource acquisition and will influence the stability of CO_2 respiration in natural systems. These results also suggested the importance of these functional groups in mediating the stability of CO_2 respiration.

In the present study, a rapid decline in the resistance of CH_4 flux under various perturbations were observed from higher to lower diversity in both the soil types for all the three treatments (except for CH_4 oxidation resistance in elevated temperature in soils from the site B). These findings are in lines with earlier studies that have reported that functions performed by only a few taxa (e.g. in situations of ecological coherence of closely related taxa, Philippot *et al.*, 2010), the sensitivity of the resistance of these narrow functions may closely follow changes in the microbial biodiversity patterns (Tardy *et al.,* 2013). Also, the functions driven by these specialised microbial guilds are not only dependent on their own diversity but also on the richness of other microbial groups (Ho *et al.*, 2014). The second point is important because the ability of CH_4 oxidation is currently only known in members of Alpha-, and Delta-Proteobacteria and Verrucmicrobia. Therefore, even if some members of specific function of microbes are more resistant than others (Levin *et al.*, 2011), diversity loss and shifts in composition of overall soil microbial community may have important ecological implication in the specialised microbial community and their associated function. However, some studies have not found significant relationships between the resistance to narrow functions and microbial diversity (e.g. Wertz *et al.*, 2007). These differences may arise because of choice of function and the way the function was determined. Most of the earlier studies have used the enzymatic activities as a proxy of the ecosystem function. In this study, the gas flux was directly measured from our soil samples that provide an exact evaluation of the function. Furthermore, functional resistance of soil microbial communities also depends on the soil type and its microbial community composition (Griffiths *et al.*, 2008).

Both CO_2 and CH_4 resistance to warming was lower as compared to N deposition and wetting-drying treatment. This result agrees with other studies that have implied that the relevance of ecological insurance to maintain a function in response to environmental perturbation depends on the type of stress (Bressan *et al.*, 2008; Baho *et al.*, 2012). It was expected these results as samples were collected from drylands wherein the wetting drying cycles are the disturbances that these soils are exposed on regular basis and are more likely to be adapted. Also, the soils used are relatively poor in resources and therefore I postulate that addition of N might not have changed the microbial community structure (Trivedi *et al.,* 2016a) and their response to environmental perturbations resulting in higher degree of resistance. A fundamental

trade-off between growth rate and resource efficiency (Hall *et al.*, 2009) may underline the capacity of microbial communities to respond to disturbances (Schimel *et al.*, 2007; Wallenstein and Hall, 2012) (see next paragraphs for discussion on the importance of various functional groups of soil microbes towards stability of soil functions). Wallenstein and Hall (2012) have posited that resource availability might constrain the rate of soil microbial community adaptation and recovery wherein in low resource environments shifts in the microbial community structure will be slow, whereas in high resource conditions, communities will respond rapidly. Indeed, resource availability has been linked to system stability in various studies (Orwin *et al.*, 2006; De Vries *et al.*, 2012b; Tardy *et al.*, 2014).

Among the three disturbances, the resistance was lowest in the temperature treatment. Previous studies have reported that temperature had a more significant effect on the stability of ecosystem process as compared to other perturbations such as wetting-drying (Tabor-Kaplon *et al.*, 2005; 2006). Temperature increases has a uniform positive effect on the physiology, growth rate, and metabolic response of all the microbial groups albeit at different rates (Allison *et al.*, 2010; Schindlbacher *et al.*, 2011; Andersen *et al.*, 2014). Increased in microbial growth rates influence resource utilization patterns and can create several new ecological niches that influences interactions between species and their functional potential (Hibbing *et al.*, 2010; Shade *et al.*, 2012; Litchman *et al.*, 2015).

The present study provides evidence for a link between diversity, richness, community composition and the resistance of soil functions to elevated temperature, nitrogen deposition and wetting drying in two different soils. The experimental set up provided a unique opportunity to tease apart various components of microbial biodiversity (community structure and diversity) controlling for the microbial abundances (all the microcosms have similar numbers of total bacteria, fungi, and methanotrophs) in driving resistance of ecosystem processes under perturbations. Most importantly this study identified that diversity/richness and community are likely to be important drivers of resistance of broad and specific function to scenarios that are relevant to global climate change, however the results varied with soil types and type of disturbances. Bacterial richness was significantly correlated with CO_2 and CH_4 resistance indexes in both the soils under N deposition treatments. On the other

hand, bacterial community composition was correlated with CO_2 resistance in soil A but not in B and with CH_4 resistance in soil B but not in soil A. The importance of the soil microbial communities as drivers of ecosystem functions is supported by a number of experiments evidence that total abundance of microbes controls the resistance of particular functions such as soil respiration or N mineralization to drought (Downing and Leibold, 2010; de Vries *et al.*, 2012; de Vries and Shade, 2013). Recently, Delgado-Baquerizo *et al.*, (2017a) have provided first empirical evidence that microbial community composition and resistance of multifunctionality index are linked at global scale. In lines with the findings from global study (Delgado-Baquerizo *et al.*, 2017a) our study indicated that although microbial diversity is an important driver of community resistance for both broad and specialised functions in response to specific global change drivers, however, other factors may also contribute towards resistance of ecosystem functioning under different environmental perturbances. I also found the microbial community contributions towards functional resistance depends on the type of stress and soil types. Changes in microbial communities resulting from land use intensification (Gossner *et al.*, 2016; Trivedi *et al.*, 2016a) or climate change (Maestre *et al.*, 2015) will likely alter the resistance of critical ecosystem functions to global change drivers. However, a holistic approach that takes into account multiple components of microbial diversity and their interactions with the environment will be required to understand the effect of disturbances on functional resistance.

de Vries and Shade (2013) proposed that community level measure might have a theoretical relationship with a functional trait of microbial groups. A framework has been proposed that links the response of soil microbial community to disturbances associated with climate change to fluctuations in the community composition of functional microbial groups differentiated as copiotroph (r-strategists, high growth rate, low resource efficiency) and oligotroph (k-strategists, low growth rate, high resource efficiency) (Fierer *et al.*, 2007; de Vries and Shade, 2013; Trivedi *et al.*, 2013). A fundamental trade-off between growth rate and resource efficiency (Hall *et al.*, 2009) may underline the capacity of microbial communities to respond to disturbances (Schimel *et al.*, 2007; Wallenstein and Hall, 2012). Indirect impacts of global change on soil functions indirectly driven by oligotroph-dominated microbial communities are expected to be much lower than those in copiotroph-dominated

communities. The reason is that oligotroph are slow growers and their effect on soil function will take longer to be detectable (Grime 2001; Haddad *et al.*, 2008; Bapiri *et al.*, 2010; De Vries *et al.*, 2012a; Lennon *et al.*, 2012; Leff *et al.*, 2015).

This study found some support for copiotroph-oligotroph theory in explaining the resistance of soil microbes to various disturbances albeit it didn't hold true for all the scenarios tested in this study. For example, CO_2 and CH_4 resistance indexes in soils from both sites under N deposition and Wetting-Drying disturbances, were positively correlated with the relative abundance of various microbial groups belonging to oligotrophs (except for CH_4 resistance index in soil B) (Table 4.2). Similar results were found for CO_2 and CH_4 resistant index in soil B and A, respectively. Recently, Delgado-Baquerizo *et al.,* (2017a) have also reported strong relationship of oligotrophs with multifunctional resistance at global scale. Although it is possible that within the categories and distinctions there will be exceptions that will not respond as suggested in this or earlier studies (Trivedi *et al.*, 2013; de Vries and Shade, 2014). It needs to be mentioned here that CH_4 oxidation ability is currently known to be restricted in members of Alpha-, and Gamma-Proteobacteria and Verrucomicrobia. Strong-linkage between the relative abundance in these taxa and the CH_4 resistance index provide further confidence in the data. Relationship between other taxa (e.g. Planctomycetes, Gemmatimonadetes) can be explained by previous works which reported that narrow functions driven by specialised microbial guilds were not only dependent on their own diversity but also on the richness of other microbial groups (Ho *et al.*, 2014). Alternatively, it is simply a reflection of statistical collinearity between overall diversity decline and diversity of different taxa. Further study is needed distinguish the dominating mechanism that modulates the differential resistance mechanisms of soil microbial communities under environmental disturbances. I propose that genomic, metabolic, and physiological information on the oligotrophy dichotomy will link the resistance of microbial community to global change drivers.

4.6. Conclusions

Altogether, this chapter demonstrates that the loss of microbial biodiversity may have significant negative effects not only on the performance of specialised functions but also on broad functions. These findings further suggest that microbial diversity and

community measure of relative abundance of r- and k- strategists can contribute to explaining the response of microbial community composition to climate-related disturbances in most cases (if not all). Whether the results of this laboratory study will match the dynamics of "real-world ecosystems" needs to be verified by means of field experiments, taking into account the role played by soil structure, types and spatial distribution of microorganisms at a micro-scale.

Chapter 5*

Niche differentiation mediated by vegetation is the major regulator of nitrifying microbial communities across the global drylands

* The chapter will be submitted to **Ecology Letter** for publication soon.

5.1. Abstract

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are the main players driving nitrification in terrestrial ecosystems, a key step of the N cycle. Despite their importance, the environmental factors driving the attributes of AOA and AOB communities are largely unexplored and poorly understood at the global scale. Using a field survey conducted in 80 dryland ecosystems from six continents, this study identified the major environmental predictors (geolocation, human impacts, climate, soil properties, nutrient availability and vegetation attributes) of the richness, abundance and the interaction network of AOA and AOB communities. Findings from this study provide novel evidence that vegetation presence is the most important regulator of the richness, abundance and relative abundance of ecological clusters within the network of interactions of nitrifying microbial communities across the globe. Samples collected under plant canopies always had lower richness and abundance of AOA, but higher richness and abundance of AOB, than samples collected in open areas between plant canopies. Further analyses suggested that plant traits such as growth form, ability to fix N, and root type, and the diversity of the plant community also influence the attributes of AOA and AOB communities. Abundance of AOB and ecological groups of AOA were significantly linked to potential nitrification rates in bare soil, but not in vegetated areas, suggesting a micro-habitat driven decoupling in the biodiversity and function relationship. Together, these results suggest that reductions in plant cover derived from increases in aridity will influence AOA and AOB communities, with important implications for ecosystem functioning (e.g. nutrient cycling and food production)

Keywords: Ammonia-oxidizing archaea (AOA); Ammonia-oxidizing bacteria (AOB); Nitrification; Dryland; Human Impacts; Network analysis; Structural equation modelling

5.2. Introduction

The availability of nitrogen (N) for plants and microbes is largely mediated by the functional attributes of soil microbial communities (Bengtsson *et al*., 2003; Balser and Firestone 2005; Hogberg *et al*., 2013). A clear example of this is the case of autotrophic nitrification, a central biological pathway in global N budget and soil productivity, which is mainly driven by ammonia-oxidizing bacteria (AOB) and

archaea (AOA) communities (Nicol *et al*., 2008; Verhamme *et al*., 2011). Although both AOA and AOB co-habit diverse soils, there has been considerable debate about the major regulators of the diversity, composition and abundance of these organisms (Schleper 2010; Hatzenpichler 2012; Prosser and Nicol, 2012; Hu *et al*., 2015b; 2016; Delgado-Baquerizo *et al*., 2016b). These differential responses to environmental and climatic factors (Tourna *et al*., 2008; Yao *et al*., 2013) might be the consequence of the cellular, genomic, and physiological differences between AOA and AOB (He *et al*., 2012). The potential different drivers of AOA and AOB might ultimately lead to a strong niche differentiation for these nitrifiers in terrestrial environments; an important gap of knowledge to be fulfilled.

Previous studies have illustrated that abiotic factors (edaphic and climatic variables) such as soil pH (Gubry-Rangin *et al*., 2011; Hu *et al*., 2013; Oton *et al*., 2015), soil type (Chen *et al*., 2010), moisture contents (Hu *et al*., 2015b), temperature (Tourna *et al*., 2008), C/N ratios (Bates *et al*., 2011), salinity (Bernhard *et al*., 2010), and geographical distance (Hu *et al*., 2015a), play important roles in determining the relative abundance of AOA and AOB at the regional scale. For example, increases in moisture content and nutrient availability may facilitate the growth of AOB over AOA (Hu *et al*., 2015a; b). Additionally, there is growing evidence suggesting the global drivers such as N deposition and fertilization linked to human activities may lead to variations in structure and abundance of nitrifiers at the regional and global scales (Nelson *et al*., 2016). Much less is known, however, about the role of biotic factors, especially the influence of vegetation structure and function, in regulating the microbial attributes of AOA and AOB communities in terrestrial ecosystems. Recent studies do suggest that plants might play an essential role in driving the distribution of ammonia oxidizers in soil (Moreau *et al*., 2015; Thion *et al*., 2016) and niche differentiation linked to different vegetation types can strongly influence the within plot distribution of the abundance of AOA and AOB at the regional scale (Delgado-Baquerizo *et al*., 2013). Identifying the relative importance of plants in predicting the distribution of AOA and AOB communities is of paramount importance as plant communities are highly sensitive to on-going global environmental change (Franklin *et al*., 2016). The ecological drivers of diversity attributes for AOA and AOB remain largely unknown. This knowledge gaps persist because of several reasons. First, although previous studies have significantly

advanced our understanding on the patterns and drivers of the diversity, composition and abundance of soil bacteria, fungi and archaea in terrestrial ecosystems worldwide (Maestre et al 2015), these studies have not specifically targeted global distribution of ammonia oxidizers. Second, although prior work has improved our understanding of the major predictors for the abundance of AOA and AOB (Processer and Nicol, 2012), the network of interactions of AOA and AOB and its controls remain largely unexplored. Recent studies suggest that soil microorganisms often exhibit strong positive co-occurrence, pattern also called 'modules' (Menezes *et al.,* 2015). These modules provide the opportunity to identify the environmental preferences of highly connected taxa by integrating highly dimensional data into predictable clusters of taxa (Shi *et al*., 2016). Therefore, a system-level understanding on the major predictors of the community richness, abundance, interaction networks and functioning of AOA and AOB in terrestrial ecosystems at the global scale is required to improve nutrient cycling modelling, including the prediction of the responses of nitrification to global change.

Drylands occupy approximately 45% of the Earth's land surface representing the largest terrestrial biome on the planet (Safriel and Adeel, 2005; Hu *et al*., 2017) and provide a variety of essential ecosystem services (e.g. production of food fiber and fuel and maintenance of biodiversity) to more than 38% of the global population (Reynolds *et al.,* 2007; Schimel, 2010; Maestre *et al.,* 2012; Delgado- Baquerizo *et al.,* 2013a). Importantly, the extent of drylands is expected to expand up to 23% during the next century (Huang *et al*., 2016). Despite the extent of drylands, and their significance to the global biogeochemical cycles, we have a very limited understanding on the key modulators of AOA and AOB communities in these ecosystems (Hu *et al*., 2015; 2017; Delgado-Baquerizo *et al*., 2013; 2016a) as most previous studies have been conducted in mesic ecosystems (e.g. Fierer *et al*, 2009; Yao *et al.,* 2013). Indeed, and to the best of my knowledge, no study has ever identified the major environmental predictors of the abundance, diversity and network of interactions of AOA and AOB communities in global drylands, where N availability is often a primary limitation to ecosystem productivity (Radin, 1981; Kelly *et al.,* 1998). In this chapter, I used a survey conducted in 80 dryland ecosystems from six continents to identify the major environmental predictors of the abundance, diversity and network of interactions of soil AOA and AOB communities. This dataset

includes a wide range of environmental variables including vegetation attributes, geolocation, climate, human influence and multiple soil properties. I posit that among these predictors, plant canopies strongly influence the attributes of AOA and AOB communities in drylands. The reason is that AOA are better adapted to the harsher environmental conditions found outside plant canopies (Tourna *et al*., 2011; He et al., 2012), while AOB are expected to be copiotroph organisms that might take advantage of the higher nutrient availability typically found under plant canopies in drylands worldwide (Delgado-Baquerizo *et al*., 2016a). Also, it can be postulated that AOA organisms might benefit from increases in aridity, while AOB might benefit from increases in ammonium derived from human activities.

5.3. Materials and Methods

5.3.1. Field site description and soil data sampling

Field data and soil samples were collected from 80 dryland sites located in 12 countries from all continents except Antarctica (Maestre *et al*., 2015; Figure 5.1). These sites cover a good range of the climatic, soil and vegetation conditions found in drylands worldwide. Data collection was carried out between 2006 and 2012 according to a standardized sampling protocol as described in Maestre *et al*., (2012). At each site, 20 contiguous 1.5 m × 1.5 m quadrats located along four 30-m long transects, each separated 8 m apart (80 quadrats per site) were surveyed. Within these quadrats, the number of species present to quantify species richness were counted. The cover of perennial vegetation was measured using the line-intercept method along the four transects. Replicated soil samples (0-7.5 cm depth) were randomly taken under the canopy of the dominant perennial plant species and in open areas devoid of perennial vegetation (10-15 samples per site). After field collection, a fraction of the soil samples was immediately frozen at −20 ºC for microbial analyses; the other was air-dried for one month for physical and chemical analyses.

5.3.2. Molecular analysis

5.3.2.1. DNA extraction

Soil microbiological analyses were conducted on composite samples of each microsite (open and vegetated areas) and site. Soil DNA was extracted from 0.5 g of defrosted soil samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories,

Carlsbad, CA, USA) per the manufacturer's instructions. See details description in Chapter 2 section 2.3.3.1.

5.3.2.2. Quantitative PCR (qPCR) analysis

Archaeal and bacterial *amoA* genes were quantified using a Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, Herculers, CA, USA). See detail description in Chapter 2 Section 2.3.3.2.

5.3.2.3. Terminal-restriction fragment length polymorphism (T-RFLP) analysis

The community structure of ammonia oxidizers was characterized by T-RFLP analysis using the fluorescently labelled primers FAM-CrenamoA23f/ CrenamoA616r and VIC-amoA-1F/amoA-2R for AOA and AOB, respectively. For detail see description in chapter 2 section 2.3.3.4 and Supplementary Table 2.1 for thermal cycling and primer information.

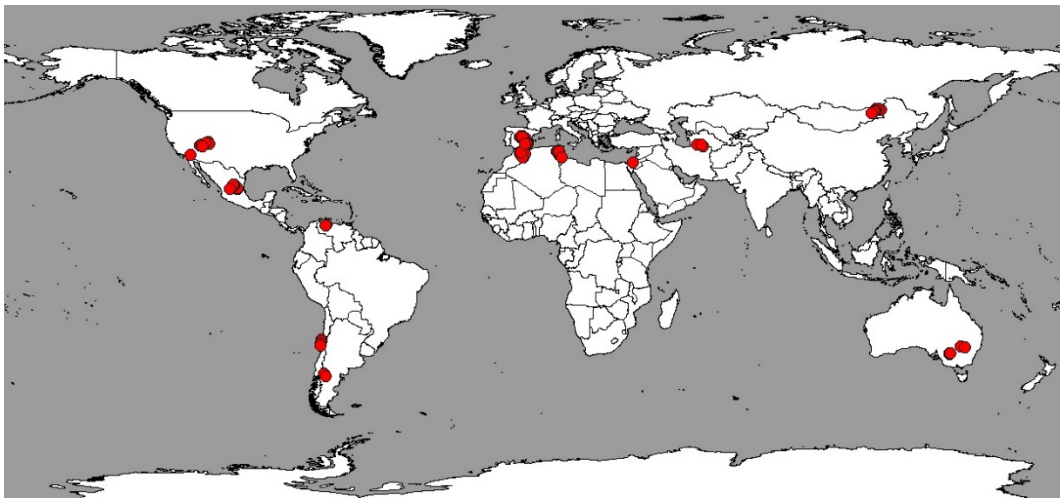


Figure 5.1. Locations of the study sites.

5.3.3. Climate and human influence index

Aridity (1-aridity index) was estimated using the Global Aridity Index dataset (http://www.cgiar-csi.org/data/global-aridity-and-pet-database; Zomer *et al.,* 2008). Information on mean annual temperature and rainfall and temperature seasonality for all our sites were obtained from the Worldclim database (http://www.worldclim.org; Hijmans *et al.,* 2005). Information on the Human Influence Index (HII; Sanderson *et*

al., 2002) for each site, a metric of its closeness to "civilization" vs. "pristine wildness" was derived by methods described by Delgado-Baquerizo *et al.,* 2016c. This index is based on eight measures of human presence: population density (km^{-2}), score of railroads, score of major roads, score of navigable rivers, score of coastlines, score of night time stable lights, and values of urban polygons and cover categories (urban areas, irrigated agriculture, rain-fed agriculture, other cover types including forests, tundra, and deserts). Similar indexes have been successfully used in the past to evaluate the role of human impacts on single ecosystems functions, including N cycling, at the global scale (e.g. Delgado-Baquerizo *et al.,* 2016c; Crowther *et al.,* 2015). More importantly, this index is a good predictor for global change drivers such as N deposition and N fertilization due to human activities, which are well-known to alter AOA and AOB communities (Delgado-Baquerizo *et al.,* 2016c; Supplementary Figure 5.1).

5.3.4. Soil properties, N cycling functions and functional plant trait

Soil organic carbon (C), total N, total phosphorous (P), C:N ratio and pH were measured in all soil samples as explained in Maestre *et al.,* (2012). In brief, soil pH was measured with a pH meter in a 1:2.5 mass: volume soil and water suspension. Total N was obtained using a CN analyzer (LECO CHN628 Series, LECO Corporation, St Joseph, MI, USA). The concentration of total organic C was determined as described in Anderson and Ingram (1993). Total P was obtained using a SKALAR San++ Analyzer (Skalar, Breda, The Netherlands) after digestion with sulphuric acid (three hours at 415ºC) as described in Anderson and Ingram, (1993). The concentration of ammonium main N source for AOA and AOB was measured as explained in Delgado-Baquerizo and Gallardo, (2011).

Potential nitrification rates were calculated as explained in Delgado-Baquerizo and Gallardo (2011). In brief, the potential net N nitrification rate was estimated as the difference between initial and final nitrate concentrations (Delgado-Baquerizo and Gallardo, 2011) by rewetting air-dried soils to reach 80% of the water holding capacity followed by the laboratory incubation for 14 days at 30 °C (Allen *et al.,* 1986).

For 69 of the 80 plots, I also had information on the plant growth form (grass, shrub or tree), ability to fix N (N-fixer vs. no N-fixer) and root morphology (lateral

vs. tap) for the dominant plant species under which soil samples were collected. These analyses were done only for (1) vegetated microsite and (2) those microbial attributes for which I found significant differences between plant canopy and bare soil –this is, they were regulated by vegetation.

5.3.5. Network analyses

To identify modules of strongly associated soil AOA or AOB organisms, a correlation network (aka co-occurrence network) was first built using the following protocol – analyses were done independently for AOA and AOB organisms. First, all pairwise Spearman's rank correlations (ρ) between all soil AOA and AOB taxa were calculated. A co-occurrence was considered robust if the Spearman's correlation coefficient was $P < 0.01$. I focused exclusively on positive correlations as they provide information on microbial taxa that may respond similarly to environmental conditions (Barberan *et al*., 2012). The network was visualized with the interactive platform Gephi (Bastian *et al*., 2009). Finally, default parameters from the interactive platform Gephi was used to identify modules of soil AOA or AOB organisms strongly interacting with each other. The relative abundance of each module was then calculated by averaging the standardized relative abundances (z score) of the AOA or AOB taxa that belong to each module.

5.3.6. Statistical analysis

A one-way ANOVA was used to explore the differences between plant and open areas microsites in community diversity (richness as number of T-RFs), abundance and the relative abundance of modules for AOA and AOB. Microsite was a fixed factor in these analyses. Abundance data for both genes were $\log_{10}$ transformed to normalize them prior to regression analyses. Non-metric multidimensional scaling (NMDS) analysis was carried out using the default programme settings to obtain an ordination diagram. All multivariate statistical analyses of T-RFLP data were conducted using PRIMER v 6113 and PERMANOVA^{+} (PRIMER-E, Plymouth, UK) (Clarke and Gorley, 2006), data underwent standardization and log+1 transformation, taxonomic distinctness was assessed using the Euclidean dissimilarity measure. Relative T-RFLP abundance data were log transformed prior to these analyses.

A classification Random Forest analysis (Grace, 2006) was used as explained in Delgado-Baquerizo *et al*., (2016) and Trivedi *et al*., (2016), was used to identify

which were the main drivers of diversity (richness), abundance and the abundance of modules within the network of interaction of AOA and AOB. Our models included the next environmental predictors: plant canopy influence (vegetation = 1 and bare soil = 0), distance from equator (absolute latitude), longitude, aridity (1 – aridity index), mean annual temperature, temperature and rainfall seasonality, human influence, pH, soil C, soil P, ammonium, electrical conductivity, and C: N ratio. These analyses were conducted using the rf Permute package (Archer, 2017) of the R statistical software, version 3.0.2 (http://cran.r-project.org/).

The Structural equation modelling (SEM- Grace, 2006) was employed to investigate the direct and indirect effects of multiple environmental predictors (those included in our Random Forest analyses) on the richness, abundance and the relative abundance of modules within the network of interactions for AOA and AOB. Unlike regression or analysis of variance, SEM offers the ability to separate multiple pathways of influence and view them as a system (Shipley, 2002; Grace, 2006; Delgado-Baquerizo *et al.,* 2016). Another important capability of SEM is its ability to partition direct and indirect effects that one variable may have on another and estimate the strengths of these multiple effects (Shipley, 2002; Grace, 2006; Delgado-Baquerizo *et al.,* 2016). When data manipulations were complete, *a priori* model (Supplementary Figure 5.2) was parameterized using our dataset and tested its overall goodness of fit. There is no single universally accepted test of overall goodness of fit for SE models. Thus, the Chi-square test ($\chi 2$; the model has a good fit when $0 \leq \chi 2 \leq 2$ and $0.05 < P \leq 1.00$) and the root mean square error of approximation (RMSEA; the model has a good fit when RMSEA $0 \leq$ RMSEA ≤ 0.05 and $0.10 < P \leq 1.00$; Schermelleh-Engel *et al*., 2003) were used. Additionally, because some variables were not normally distributed, the fit of the model was confirmed using the Bollen-Stine bootstrap test (the model had a good fit when $0.10 <$ bootstrap $P \leq 1.00$; Schermelleh-Engel *et al*., 2003). The standardized total effect (direct plus indirect effects from the SEM) of the different predictors on AOA and AOB abundance, richness and community composition were also calculated. All SEM analyses were conducted using the software AMOS 20 (IBM SPSS Inc., Chicago, IL, USA).

I also used correlations (Pearson) to examine the relationship between AOA and AOB abundance, richness and relative abundance of modules to the net potential

nitrification rates for our soils. Significance was accepted at the ($P < 0.05$) level of probability.

Finally, for the plots in which had plant traits information, I evaluated the effect of plant growth form, ability of fix N, root shape and plant richness on the abundance, richness and the relative abundance of modules within the network of interaction of AOA and AOB organisms. To do so, I used ANOVA or Pearson correlations.

5.4. Results

The data show a strong role for plant canopy influence in driving the niche partitioning of AOA and AOB at the global scale (Figures 5.2 and 5.3.). In particular, the NMDS analysis revealed strong differences in the composition of AOA and AOB communities between vegetated and open microsites (Figures 5.2a, and 5.2b). Moreover, the abundance and especially richness of AOA was found significantly lower in plants vs. bare soil microsites (Figure 5.2c), with reverse true for AOB (Figure 5.2d).

The T-RFLP analysis of AOA communities yielded 12 distinct T-RFs, of which 54, 98, and 294 bp fragments were the most dominant genotypes that accounted for 43, 21, and 8% of the total AOA T-RFs (Figure 5.3). Twelve distinct T-RFs were obtained for AOB communities and were dominated by 60, 61 and 92 bp fragments that accounted for 35, 33, and 11% of the total AOB T-RFs in global drylands. Taxonomic information for each T-RF is available in the Supplementary Table 5.1. The dominant AOB TRFs (60, 61, and 92) were reported previously to be related to cluster 3 of *Nitrosospira* belonging to β-proteobacteria. The dominant AOA T-RFs 98/294 and 54 were related to *Nitrososphaera* and *Nitrosotalea*, respectively (Yao *et al*., 2013; Hu *et al*., 2015). Finally, three modules of AOA and three modules of AOB taxa were found to be strongly co-occurring with each other within these *amoA* groups. In general, I found strong differences in the relative abundance of these modules between vegetation and bare microsites for both AOA and AOB communities (Figure 5.4).

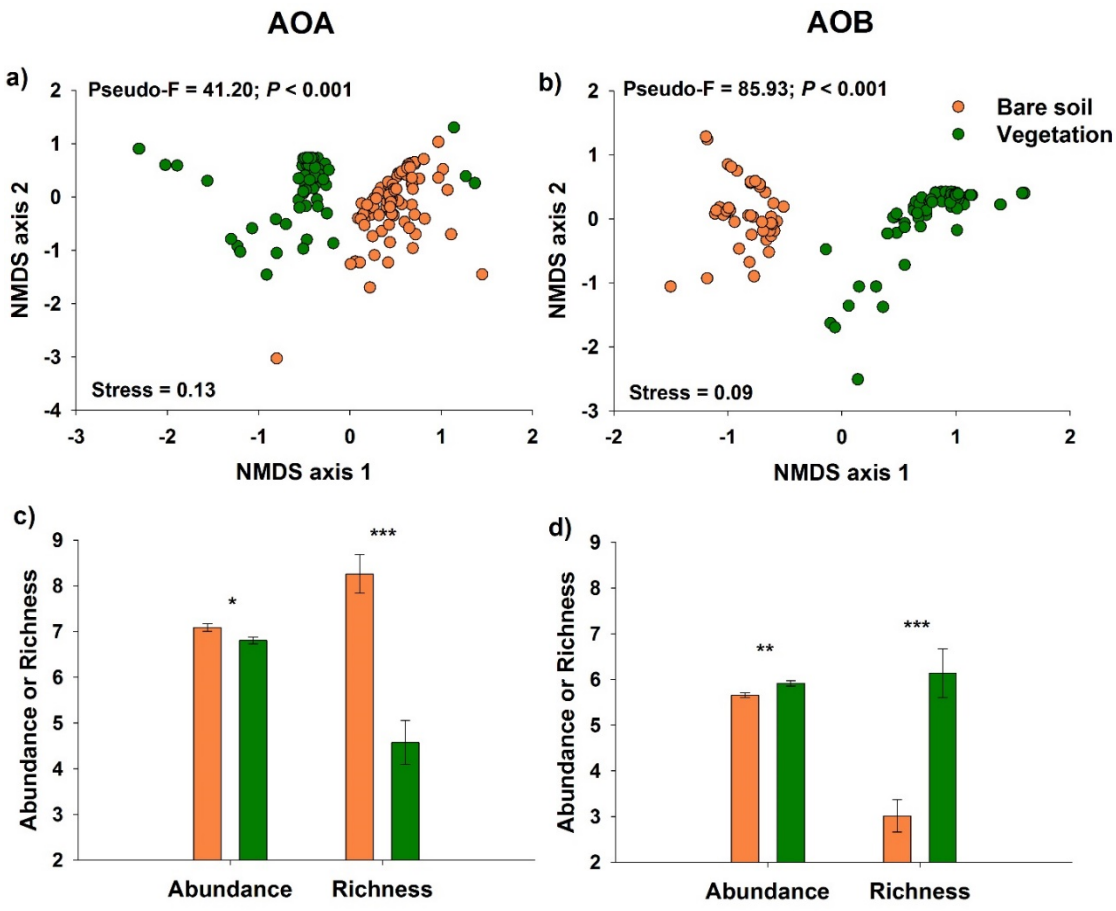


Figure 5.2. AOA and AOB composition, richness and abundance in bare soil and vegetated microsites. Panels A and B show 2-D nonmetric multidimensional scaling (NMDS) analysis ordination of AOA (A) and AOB (B). Panels (C) and (D) show the abundance and richness of AOA (C) and AOB (D) in two microsites of global drylands. P values as follows: ***P < 0.001; **P < 0.01; *P < 0.05.

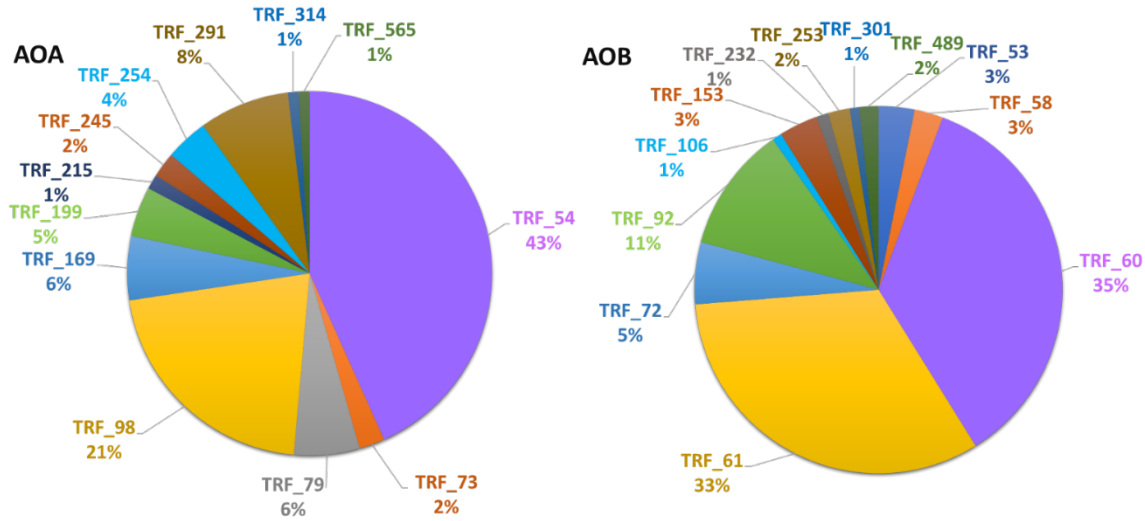


Figure 5.3. Relative abundance of dominant T-RFs (>1%) for AOA and AOB in vegetated and bare soil areas.

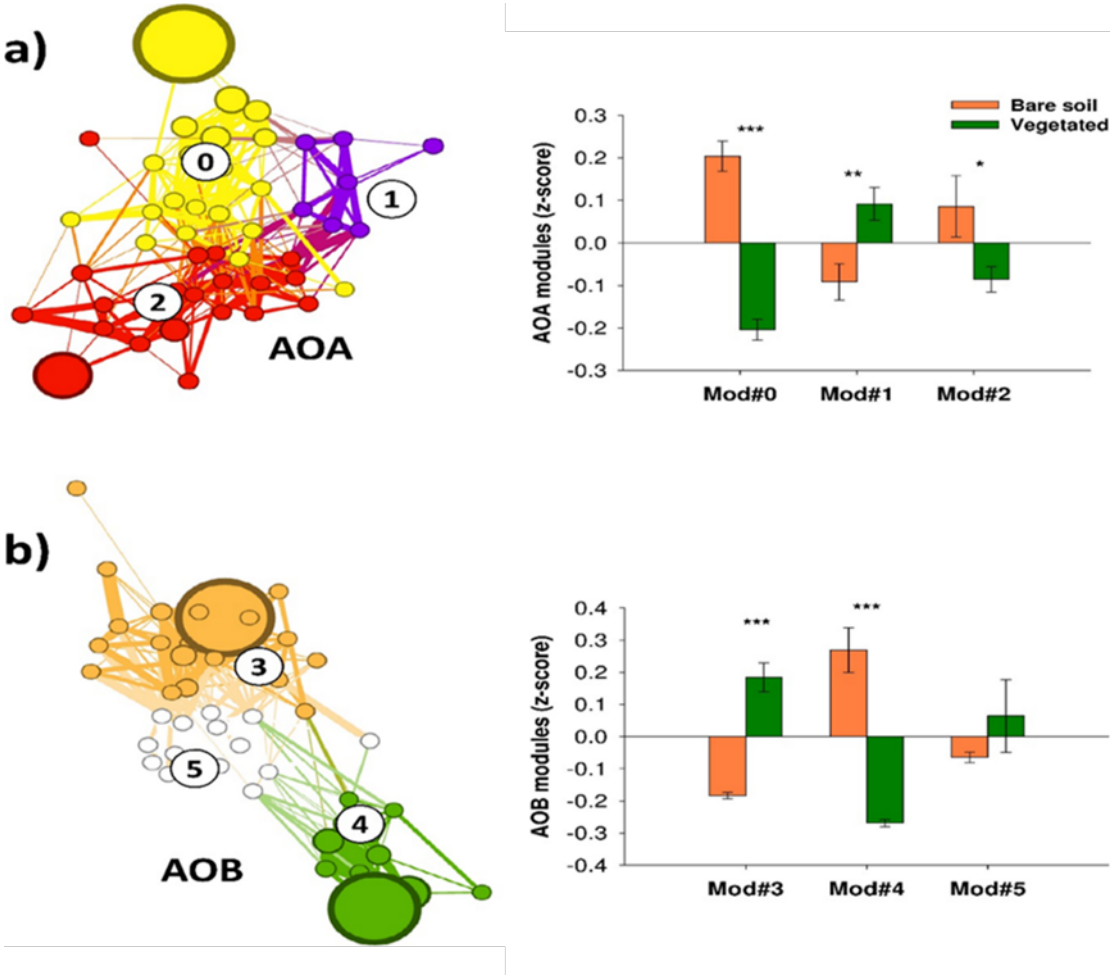


Figure 5.4. Soil network of interactions for AOA (a) and AOB (b) communities based on correlation analysis (left panel). A connection stands for a strong (Spearman's ρ>0.6) and significant (P- values <0.01) correlation. The size of each node is proportional to the number of connections (that is, degree). Different colours within AOA and AOB networks represent highly connected T-RFs structured among densely connected group of nodes (that is, modules) that form a clustered topology. A characterization of the taxa within each module is available in Supplementary Table 5.1. Right panel shows correlation values obtained for different modules under bare and vegetated samples compared using z-scores. P values as follows: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

5.4.1. Drivers of the ammonia-oxidizing community in global drylands

5.4.1.1. Random forest analysis

Out of 14 environmental predictors evaluated, the RF analysis showed that vegetation was the single most important variable for the richness, abundance and relative abundance of modules of both AOA and AOB; this response was especially noticeable for modules #0 to 4 and for AOA and AOB richness (Figure 5.5). Soil pH and longitude were also important predictors of richness and abundance of AOA and AOB in global drylands. Aridity was a major predictor of AOA. MAT and temperature and rainfall seasonality were important predictors of AOA and AOB. Salinity (electrical

conductivity) was a major driver of the abundance of AOA and human impact for both AOA and AOB. Soil C and P were important predictors of the abundance of AOB and AOA, respectively. Ammonium was an important predictor of AOB abundance but not AOB.

5.4.1.2. Structure equation modelling

This SEM models were satisfactorily fitted to this data, as suggested by non-significant $\chi 2$ values, non-parametric Bootstrap P-values, and by values of RMSEA. These models indicated that vegetation was a major driver of the richness, abundance and network of interactions of AOA and AOB (Figure 5.6 and 5.7). This is evident from the multiple direct effects of plant canopy influence on the richness, abundance and relative abundance of modules for AOA and AOB (Figure 5.6) and from the total standardized effects (sum of direct and indirect effects) presented in Figure 5.7. Moreover, vegetation influence had multiple indirect effects on the richness, abundance and modules for AOA and AOB via their positive effects on soil C and C:N ratio, which in turn positively affected the amount of ammonium in soil (Figure 5.6). After vegetation, temperature seasonality and annual temperature had the highest total effect on AOA and AOB attributes (Figure 5.6).

Other factors directly and indirectly influenced the richness, abundance and network of interactions of AOA and AOB. For example, human influence had a direct positive effect on the abundance of AOB and an indirect positive (AOB) or negative (AOA) effect on the abundance of these organisms via increases in ammonium (Figure 5.6). Aridity had a strong indirect impact on the abundance of AOA and AOB via reductions in soil C (Figure 5.6). Aridity also had a direct positive effect on the richness of AOA and in the relative abundance of modules #1 and 2 (AOA). Temperature and rainfall seasonality had a direct positive effect on the abundance of AOB, and multiple indirect effects on the richness and network of interactions of AOA and AOB that were mediated by reductions in soil P caused by these climatic features.

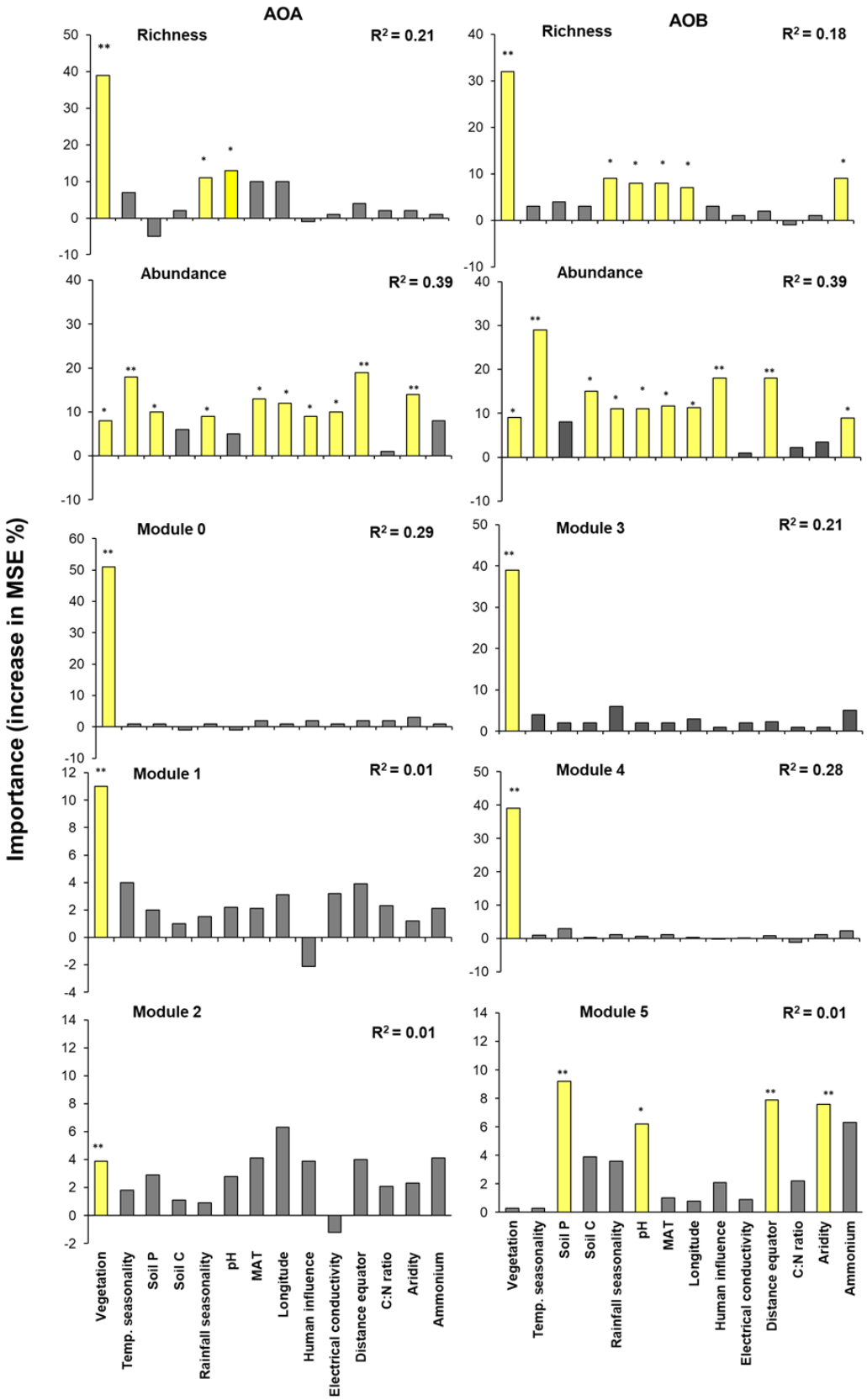


Figure. 5.5. Random forest mean predictor importance (percentage of increase of mean square error) of AOA and AOB main T-RFs, abundance and richness. Yellow coloured bar shows significant differences wherein * and ** represent significance levels of $P<0.05$ and $P<0.001$, respectively. MAT mean annual temperature.

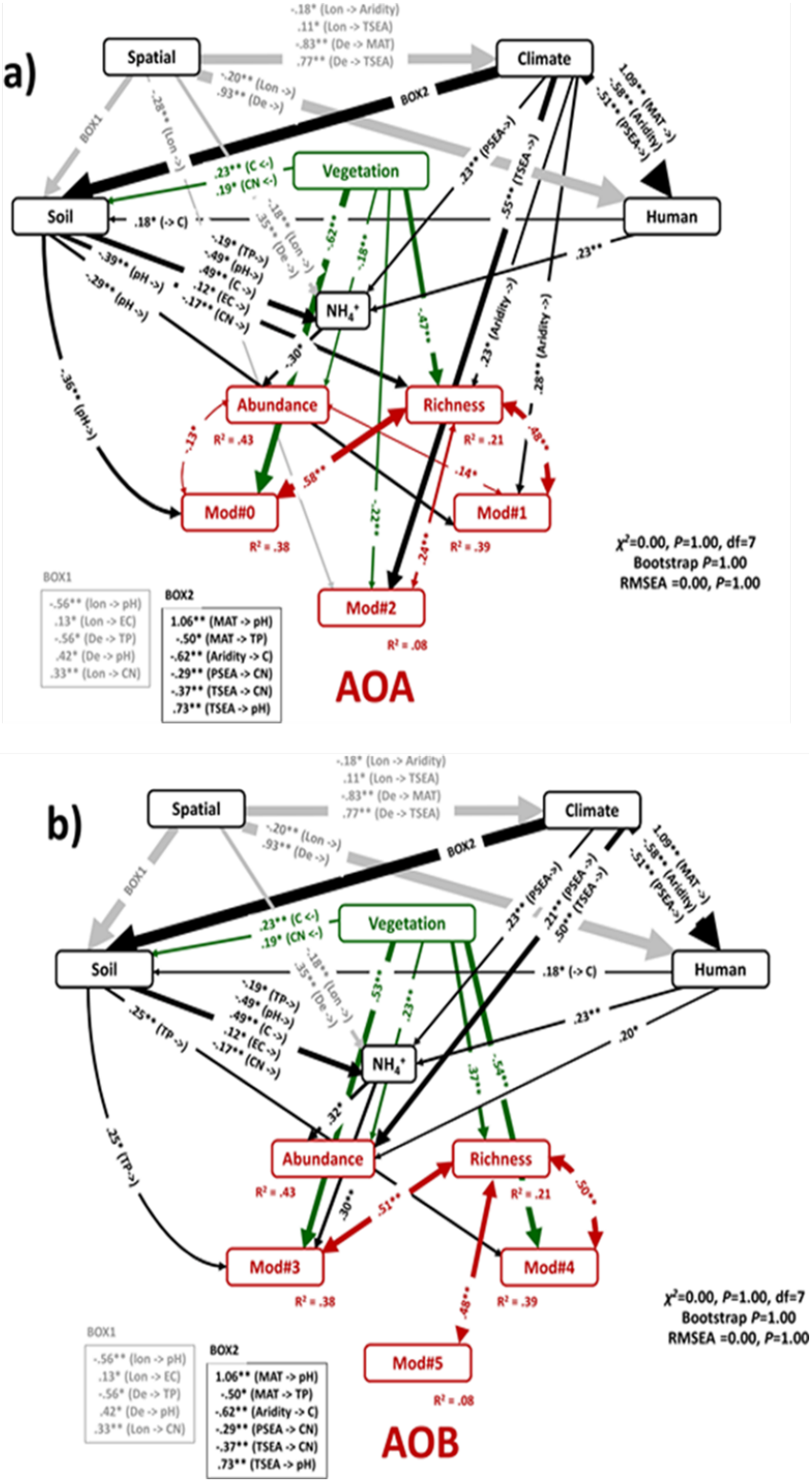


Figure 5.6. Mechanistic modelling for the distribution of AOA and AOB across the global drylands. Numbers adjacent to arrows are indicative of the effect- size bootstrap P value of the relationship. Continuous red colour arrow indicate relationship between richness, abundance and modules for AOA (a) and AOB (b). Green colour indicates the relationship between vegetation influences (vegetation vs. bare soil). Soil includes soil C, pH, C:N ratio, electrical conductivity (EC), and total P.

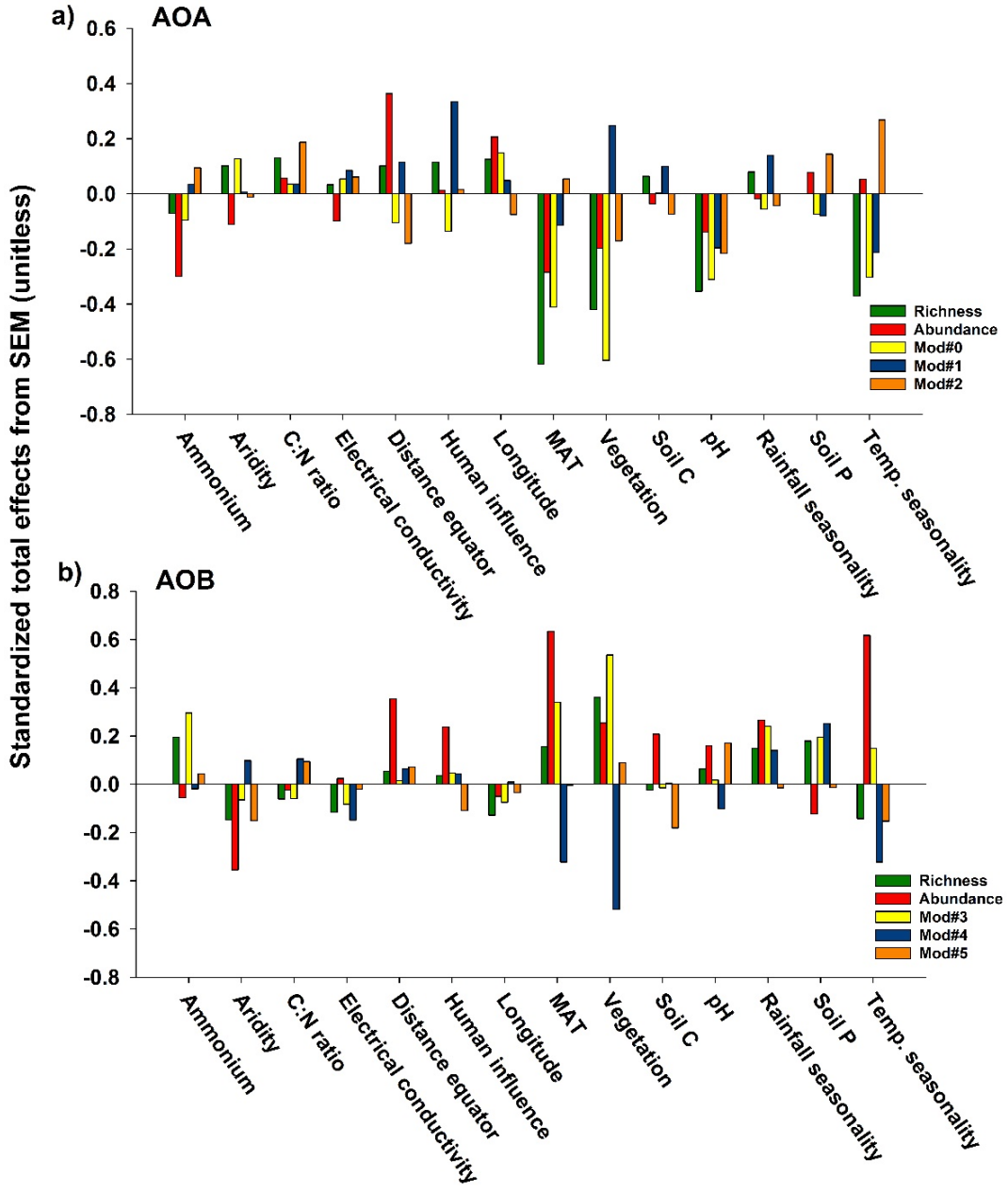


Figure 5.7. Standardized total effects (direct plus indirect effects) derived from the structural equation modelling, including the effects of vegetation, human (Human impact index), climate, soil properties and geolocation on the abundance, richness and relative abundance of modules of AOA and AOB.

5.4.1.3. Functional plant effects

Plant functional group composition had multiple effects on the attributes of AOA and AOB communities. For example, abundance and richness of AOA and relative abundance of modules #0 and 1 peaked under trees for a vegetated microsite (Figure

5.8). Abundance of AOB peaked under grasses and shrubs (Figure 5.8). Richness of AOB and abundance of module #3 peaked under no N fixer plants. Abundance of AOA, richness of AOA and the relative abundance of modules #0 and 1 peaked under tap roots (vs. lateral roots; Figure 5.8). Abundance of AOB was slightly higher under plants having lateral rather than tap roots (Figure 5.8). Finally, abundance of AOA was positively related to plant richness. Statistical analyses are available in supplementary Table 5.2.

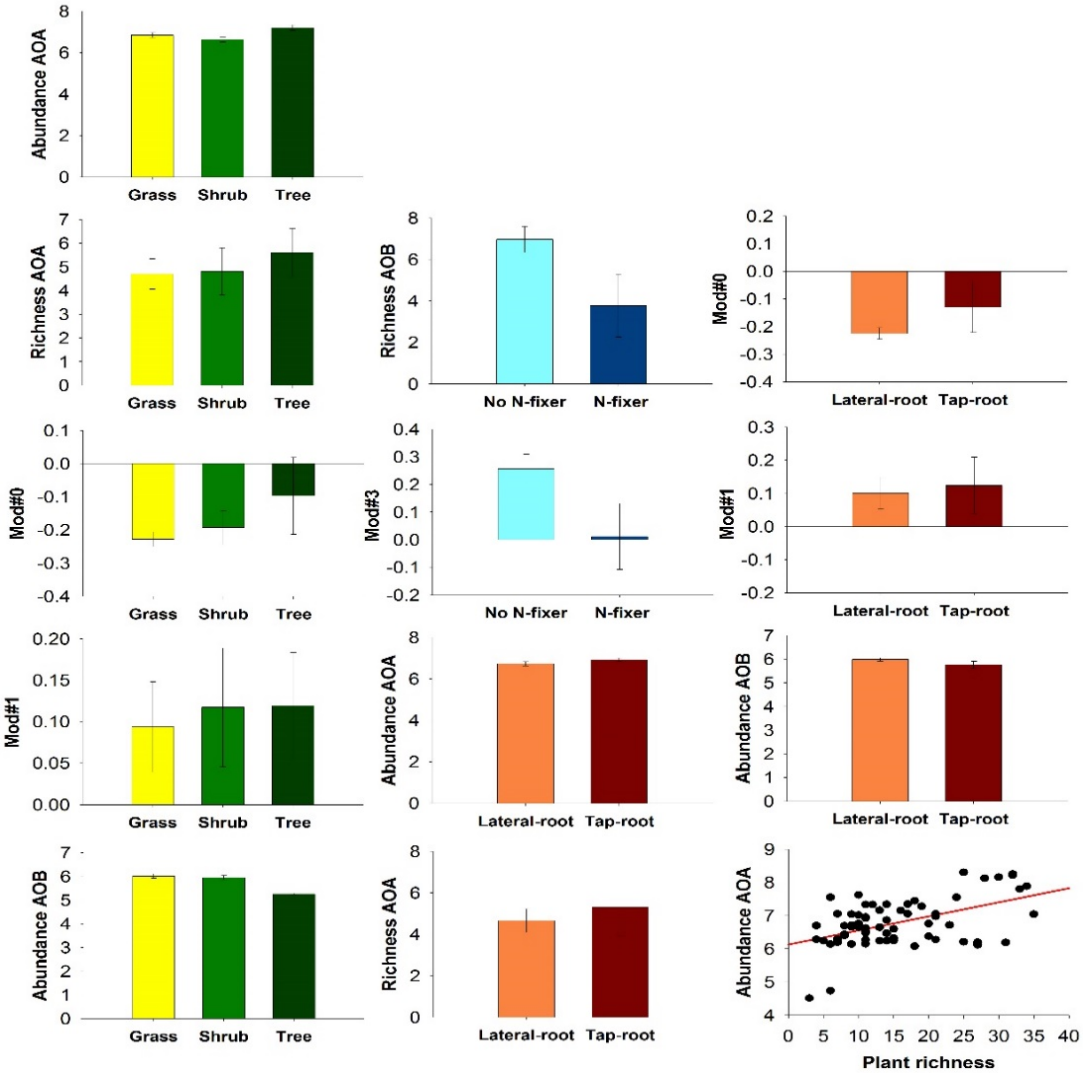


Figure 5.8. Plant traits effects on the multiple attributes of AOA and AOB. Statistical analyses are available in Supplementary Table 5.2.

5.4.1.4. Linking AOA and AOB attributes to net potential nitrification

A strong micro-habitat driven impact on the linkage between AOA and AOB organisms with nitrification rates was observed. I found positive correlations between the richness of AOA and the relative abundance of modules #4 and #5 (both AOB) with the net potential nitrification rates (Table 5.1) for the bare soil microsite. However, I did not find any significant correlation between any of the studied attributes for AOA and AOB and the net potential nitrification rates under vegetated areas (Table 5.1).

Table 5.1. Correlation (Pearson) between AOA and AOB attributes and potential net nitrification rates.

			Bare soil	Vegetation
AOA	**Abundance**	Spearman ρ	-0.006	-0.089
		P-value	0.955	0.434
	Richness	Spearman ρ	0.081	0.165
		P-value	0.475	0.144
	Mod#0	Spearman ρ	-0.076	0.125
		P-value	0.501	0.271
	Mod#1	Spearman ρ	0.277	0.127
		P-value	0.013	0.262
	Mod#2	Spearman ρ	-0.012	0.051
		P-value	0.916	0.654
AOB	**Abundance**	Spearman ρ	0.271	0.138
		P-value	0.015	0.223
	Richness	Spearman ρ	-0.022	0.188
		P-value	0.844	0.094
	Mod#3	Spearman ρ	-0.162	0.207
		P-value	0.151	0.066
	Mod#4	Spearman ρ	-0.025	-0.05
		P-value	0.823	0.659
	Mod#5	Spearman ρ	0.105	0.015
		P-value	0.354	0.897

5.5. Discussion

This study provides evidence that the presence and characteristics of plant patches largely drives the abundance, richness and interaction networks of AOA and AOB communities in drylands across the globe. This is especially true when comparing the predictive capacity of vegetation influence to those of previously proposed predictors of AOA and AOB organisms, including geolocation, human influence, climate and multiple soil properties. In particular, the diversity and abundance of copiotrophic

AOB was observed to be heightened under plant canopies, while that of oligotrophic AOA was dominant in open areas devoid of perennial vegetation. Similarly, plant canopies have a strong influence on the composition and the network of interactions of AOA and AOB communities. Multiple functional plant attributes including plant growth form, capacity to fix N and root shape, and species richness regulated AOA and AOB communities in vegetated microsites. Climate was, after vegetation, the major driver of AOA and AOB. In particular, annual temperature and temperature seasonality had a large impact on the multiple attributes of AOA and AOB, whereas increases in aridity indirectly promoted the abundance of AOA and reduced that one of AOB. Greater human influence promoted that one of AOB and reduced that one of AOA via positive effects on ammonium. Aridity and human influence are well-known to be decoupled in drylands meaning that the most arid places suffer the lowest human influence. Because of this, AOA and AOB are also expected to be decoupled under future global change scenarios as discussed below. Interestingly, the link between AOA and AOB attributes and nitrification rates is also limited to bare soil areas, suggesting that any changes in the relative abundance of plant vs. bare soil can have profound implications for N cycling in these ecosystems. Overall, these results suggest that niche differentiation mediated by vegetation is the major regulator of bacterial and archaeal nitrifying communities in drylands worldwide. Together, these findings significantly advance our understanding of the patterns and mechanisms driving N cycling in drylands and demonstrate the important role of plant communities in regulating soil nitrifiers.

As revealed by our SEM analyses, plant canopies had both direct and indirect (via positive effects on soil C organic matter, C:N ratio and ammonium under the canopy) effects on all the attributes of AOA and AOB communities. The differences in the ammonia-oxidizing community structure at the different sampling locations and the spatial distribution patterns for AOA and AOB suggest that micro-sites are the major factor contributing to community structure of both groups of ammonia oxidizers in drylands. The NMDS ordination of the AOA/AOB communities shows a clear separation between vegetative and bare samples. It was surprising that at such a large geographical scale I did not observe any separation based on other environmental variables studied in our study, indicating that possible effects of spatial heterogeneity were overridden by effects of within plot drivers such as the effect of vegetation vs.

bare soil. In line with previous reports, these results suggest that plants may provide different niches to physiologically contrasting groups of microorganisms such as AOA and AOB, which may be involved in diverse processes that affect the overall functioning of these ecosystems (Delgado-Baquerizo *et al*., 2013; 2016b). These results suggest that reductions in plant cover linked due to increases in aridity (Delgado-Baquerizo *et al*., 2013 Supplementary Figure 5.3), which are forecasted for drylands globally (Huang *et al.,* 2016) will promote the preferred niches for AOA and will likely reduce the abundance and richness of AOB communities. Such changes might have strong consequences for the ecosystem functioning linked to nutrient cycling in these ecosystems. There were multiple ways that the plant community influenced the abundance, richness and relative abundance of modules within the network of interactions of AOA and AOB. Of special interest was the highest abundance of AOA in woodland ecosystems and of AOB in grasslands, even when as demonstrated in all cases, AOA was, on average, always more dominant in open areas than in vegetated microsites.

The indirect effects of plants on the multiple attributes of AOA and AOB were not completely unexpected. Plant patches can affect the ammonia-oxidizing community by altering the abiotic conditions of soils including resource inputs higher litter content, soil C and ammonium, low temperature, and high moisture content compared to open areas forming "islands of fertility" (Maestre *et al.*, 2001, 2003). It has been reported that AOA is outcompeted by AOB under vegetation canopies (Delgado-Baquerizo *et al.,* 2013; Yang *et al*., 2017). Earlier studies have reported that substrate availability and physiological adaptation as the principal determinants of the differential growth, metabolic divergence, and ecological niches of AOA and AOB (He *et al*., 2012; Prosser and Nicol, 2012). AOA have a higher substrate affinity and efficiency of metabolism that suits their oligotrophic lifestyle, making them better competitors in nutrient-limited and warmer environments (He *et al*., 2012; Lehtovirta-Morley *et al*., 2011; Hu *et al*., 2016). The greater abundance and richness of AOA in open (higher temperature and low nutrients) as compared to vegetated microsites could be attributed to their intrinsic physiological adaptation to warming and/or significant divergence to substrate affinity (Martens-Habbena *et al*., 2009; Urakawa *et al*., 2011; Tourna *et al*., 2011). On the other hand, AOB are more abundant under vegetation microsites due to their lower temperature optimum and substrate efficiency

(Hu *et al*., 2015). This study also found multiple direct effects of plants on the abundance, richness and relative abundance of modules for AOA and AOB. These findings are in agreement with recent studies that ammonia oxidizers are highly sensitive to changes in community plant traits (Moreau *et al*., 2015; Thion *et al*., 2016). For example, AOB abundance is significantly higher in vegetated sediments compared to bulk sediments, whereas it is not the case for AOA in the *Kandelia obovate* rhizosphere (Wang *et al*., 2015); plant species effects on ammonia-oxidizing community structures were only limited to AOB (Ruiz-Rueda *et al*., 2009). In this respect, plant communities might be playing a key role in driving both AOA and AOB communities and also mineralization rates by "encouraging" soil microbes via release of key substrates.

These results indicate that, in spatially heterogeneous ecosystems such as drylands, plant canopy influence plays a critical role in controlling the populations of soil nitrifiers, although the magnitude of their effects is likely to be regulated by particular plant traits. The link between nitrifiers attributes (clusters of AOB taxa and richness of AOA) and nitrification rates is only significant in open areas between plant canopies. The reported link between particular modules of AOB and nitrification rates in bare soil areas is especially interesting as nitrifiers are not a highly phylogenetic diverse group of organisms. Drylands, due to their harsh climatic conditions, seem to select for specific ecotypes within ammonia-oxidizing communities that show metabolic complementarity towards resource use and functional complementarity towards process rates. In the case of AOA, diversity was a major driver of nitrification rates. These results are supported by previous studies showing a strong link between microbial community and soil function (e.g. Philippot *et al*., 2013; Wagg *et al*., 2014; Delgado-Baquerizo *et al*., 2016a). Therefore, any losses in the diversity of these organisms, or alterations on the composition of AOB, might result in reductions in the nitrification rates from these ecosystems. The lack of relationship between AOA and AOB and nitrification rates in vegetated areas might be related to several factors. For instance, plant microsites might promote soil processes driven by heterotrophic organisms such as litter decomposition, organic matter de-polymerization and heterotrophic nitrification inhibiting autotrophic nitrification (driven by AOA and AOB) in these environments. Similarly, the higher C:N ratio of soils in these environments is also known to inhibit autotrophic nitrification explaining the lack of

relationship between AOA and AOB in these environments (Robertson and Goffman *et al.,* 2015). In addition, plant roots of many species are known to release biological nitrification inhibitors which can negatively affect the activity of ammonia monooxygenase and lead to decoupling of ammonia oxidizers and nitrification rates (Coskun *et al*., 2017).

Given the strong negative effect of aridity on human influence reported here and by others (Supplementary Figure S7 in Delgado-Baquerizo *et al*., 2016c), these results suggest that global environmental changes will lead to an increasingly stronger spatial separation of AOA and AOB communities across the globe. In particular, global change might result in dryland assemblies completely dominated by AOA communities those under the most arid conditions and lowest human influence and plant coverage and assemblies of drylands largely dominated by AOB communities those under the less arid conditions and largest human influence and plant coverage. Moreover, it is predicted that aridity will increase in global drylands in late 21st century (Dai, 2013; Feng and Fu, 2013) and will negatively impact the cover of vascular vegetation in most drylands (Maestre *et al*., 2012; Vicente-Serrano *et al*., 2012). Thus, the decreased plant cover and the increase of open areas will likely increase the abundance of AOA at the expenses of AOB due to the high resistance to water and nutrient stresses of the former (Adair and Schwartz, 2008; Verhamme *et al*., 2011). In fact, previous studies found positive relationships between aridity and AOA abundance at the regional scale (Delgado-Baquerizo *et al*., 2013; 2016). For example, increases in aridity can have indirect negative effects on the abundance of AOB via reductions in soil C organic matter and ammonium. In contrast, human impacts directly and indirectly via positive effects on the amount of soil ammonium will likely increase the dominance of AOB vs. AOA in locations with high human influence but lower aridity. Human impacts such as those studied here are known to promote the accumulation of N in the dryland soils world-wide (Delgado-Baquerizo *et al*., 2016b; c). N deposition is known to cause fluctuations in the structure and function of soil microbial communities including AOB and AOA (Leff *et al*., 2015). Both the groups of ammonia oxidizers showed differential response wherein the abundance of AOA decreased while that of AOB increased in response to N addition (Zhang *et al*., 2013). N deposition can foster the abundance of AOB because it increases the availability of NH_4^+-N, which is used as energy source by these

organisms (Sterngren *et al*., 2015). This study provides evidence that both aridity and human impacts are major predictors of AOA and AOB abundance and considering their relative importance of performing key functions related to N turnover in drylands, there is a crucial need to perform detailed studies on these functional groups of microbes under varying environmental conditions. Thus, as human impacts and aridity increase during this century, our results strongly suggest that they will have strong effects on the composition, abundance and diversity of AOA and AOB with implications for ecosystem functioning.

Other drivers such as soil P, C:N ratio and pH also affected the populations of AOA and AOB in the drylands studied. As the productivity of drylands is limited by both N and P availability (Delgado-Baquerizo *et al*., 2013a), the interactive effects of N and P on the abundance of AOB warrants further detailed investigation. As found by previous studies (Nielsen *et al*., 2010; Bates *et al*., 2011; Hu *et al*., 2015; Lu *et al*., 2015), the C:N ratio was another important driver of the abundance of AOA and AOB. It is postulated that increase in C inputs can intensify interspecific competition between ammonia-oxidizing microbes and heterotrophic bacteria, however, it is equally probable that these populations are being influenced by an increasing availability of N (Bates *et al*., 2011).

5.6. Conclusions

In conclusion, the presence and attributes of plant canopy patches have strong regulating impact on the diversity, abundance, and the network of interaction of AOA and AOB in global drylands. In particular, soils beneath plant canopies always had higher abundance and richness of AOB, but lower abundance and richness of AOA suggesting that AOB are better fit for copiotrophic and AOA for oligotrophic environments. These findings advance our understanding about how biotic and abiotic features control fine-scale variations in microbial abundance and associated ecosystem processes in highly heterogeneous ecosystems such as drylands. They also help to refine our predictions of the impacts of global environmental change on N cycling. These findings support the argument that niche separation and adaptation of the microbial community can have important consequences for biogeochemical cycling at global scales, and therefore these factors should be explicitly considered in models predicting ecosystem functioning under global change scenarios.

Chapter 6

General conclusions

Soil microbial communities play essential role in maintaining ecosystem functions including primary productivity, water and climate regulations. However, their control over the process rate and mechanism of microbial regulation of ecosystem functions is not fully known. While significant progress has been made in understanding the link between aboveground biodiversity and ecosystem functions (BEF) and stability, relationship between microbial biodiversity and ecosystem functions (microbial BEF) remains poorly described. Assessing and identifying linkage and shape of linkage for microbial BEF is critical to predict the ecosystem response to microbial diversity loss because of predicted global change.

Previous studies provided some evidence in support of positive relationship between microbial BEF in observational study from plot and global scales (Delgado-Singh *et al.,* 2014; Baquerizo *et al.,* 2016a; 2017; 2017a; Colombo *et al.,* 2016; Jing *et al.,* 2016). However, observational links have been questioned because of their inability to conclusively establish a cause and effect relationship between diversity and process outcomes. Evidence from manipulative works have been widely inconsistent which hinder developing a unifying concept of microbial BEF. Nonetheless, there is some evidence supporting a positive microbial BEF relationship from freshwater ecosystems (e.g. Peter *et al*., 2011; Delgado-Baquerizo *et al*., 2016). It was argued that evidence from freshwater, where microbial diversity is presumably lower, may not be applicable in terrestrial ecosystems because high microbial diversity and abundance results in functional redundancies (Miki *et al.,* 2014). The lack of evidence on microbial BEF and shape of the BEF are critical knowledge gaps and addressing these issues can substantially improve prediction of ecosystem and climate models and our ability to better manage natural resources for sustainability of ecosystem functional and services (Bardgett and van der Putten, 2016).

The overarching aim of my research was to systematically evaluate the relationship between soil microbial BEF and resistance. To achieve this, I used a novel theoretical and experimental framework. First, I applied theoretical principle used in plant ecology and aboveground BEF, but with explicit consideration of diversity (structural vs functional) and function (broad vs narrow) types. Second, I combined both observational (from regional to global scales) and manipulative experimental design to provide robust evidence for the microbial BEF. My project aim was achieved

using controlled microcosm experiments (**Chapters 2 - 4**) and observational (**Chapter 3, 5**) studies to identify patterns and causal mechanisms with environmental influences.

Following are key steps and important findings from my project.

- Microcosm experiments were conducted to test the link between taxonomic and functional microbial diversity and ecosystem functioning, using the dilution-to-extinction approach for two different soil types (Chapters 2-4). Result from this study suggested that any loss in microbial diversity significantly compromises the rate of key specialised functions e.g. CH_4 consumption, N_2O emission (carried out by specialised microbial taxa), and multiple ecosystem functions related to C, N and P cycles simultaneously (i.e. multifunctionality). Statistical modelling provided evidence that loss in the microbial diversity led to the proportional, and in some cases exponential, decline in the rate of key ecosystem processes and multifunctionality and, therefore, indicate lack of any significant functional redundancy. These findings indicate that any loss of microbial diversity can influence functionality of an ecosystem (depending on the function analysed). In this work, the BEF relationship followed a positive linear trend, indicating that losses of bacterial species would have proportional effects on soil functioning (**Chapter 2**).

- In Chapter 3, I assessed the relative importance of soil microbial richness and community composition for ecosystem functions using a combined manipulative and observational study. This study provided evidence that both microbial richness and community compositions are important but independent drivers of ecosystem multifunctionality. Positive effects of bacterial richness on individual functions (i.e. enzymatic activity and carbon degradation) were also observed. The multi-model approach supported the evidence that microbial diversity provided both independent and complementary impact on ecosystem processes. The results also indicated that microbial community relationship with ecosystem function, in some case, are soil dependent, and that soil pH and C were also important predictors of soil multifunctionality **(Chapter 3).**

- In Chapter 4, I explored the relationship between microbial diversity and resistance of ecosystem functions to global change treatments. Soils with low microbial diversity

has demonstrated lower functional resistance to global change treatments (e.g. elevated temperature, N deposition, wetting and drying cycling), thereby providing evidence that a loss of diversity in soil has the potential to affect specific functional resistance. However, results were not consistent in some cases, for two soils and function types. For example, the relationship between microbial diversity and functional resistance index of broad function (soil respiration) was soil and treatment dependent. Further, my study indicated that multiple aspects of microbial community (e.g. diversity, richness and composition) could affect broad and narrow functions and their resistance to perturbation. An interesting finding of my project was that soil microbes performing various functions respond differently to the same climate stressors, in two soil types (**Chapter 4**).

- In Chapter 5, I found that the most important driver of nitrifying communities in global dryland was plant canopy. This study also identified several ecological clusters within the network of the interactions occurring in the nitrifying microbial communities across the global drylands**.** Plant canopies always had higher abundance and richness of AOB, suggesting that AOB and AOA bacterial communities prefer copiotrophic and oligotrophic environments, respectively. Strong linkage between nitrifying communities and nitrification rate was observed in bare soils. The work presented in chapter five is the first to investigate the main drivers of the nitrifying bacterial community in global dryland ecosystems, and suggest that with predicted decline in vegetation under projected climate change, will alter the nitrifying composition in such a way which could compromise the rate of nitrification, a crucial step in nutrient nitrogen cycling, with unknown consequences **(Chapter 5).**

6.1. Conclusions and future work

Over my work demonstrated that the loss of microbial diversity will have at least proportional impact on ecosystem multifunctionality and suggest that the functional redundancies in microbial communities is limited only in few cases of broad functions. These results suggest that any loss in microbial diversity due to management practices or climate change (Maestre *et al*., 2015) will likely reduce soil processes and multifunctionality. These findings have significant implication beyond academic research and suggest that microbial diversity should be protected, conserved and protected just like animal and plant diversity for ecosystem sustainability. For

example, conventional agriculture practice and land-use change are known to negatively impact soil microbial communities and findings from this study suggest that for the sustainable provision of ecosystem services, a better approach of farming may be needed which could minimize the loss of microbial diversity. Based on findings from this project, I argue that microbial diversity should be explicitly considered in future conservation debate and policy decision for ecosystem sustainability when considering consequences of global change.

No scientific work is without a limitation and mine is not an exception. For example, I only used two soil types for my microcosm study and given microbial BEF for some functions was influenced by soil types, particularly functional resistance to global treatment (Chapter 4), future works need to include multiple soil types varying in nutritional, textural and other properties to confirm the unifying concept of microbial BEF. In my microcosm study, the plant community was deliberately excluded from the experimental design to avoid cofounding influence on microbial BEF. However, plant species and trait diversity are known to impact microbial diversity and functions (Delgado-Baquerizo *et al*., 2017; Leff *et al.,* 2017). Now that direct relationship between microbial diversity and ecosystem function is established, other factors (e.g. plant diversity) and their feedback response could now be examined in order to confirm the 'real- world' ecological interactions and consequences for ecosystem functions. I suggest that the future work should simultaneously manipulate above and below ground diversity to quantify the biotic interactions and consequences for ecosystem functions.

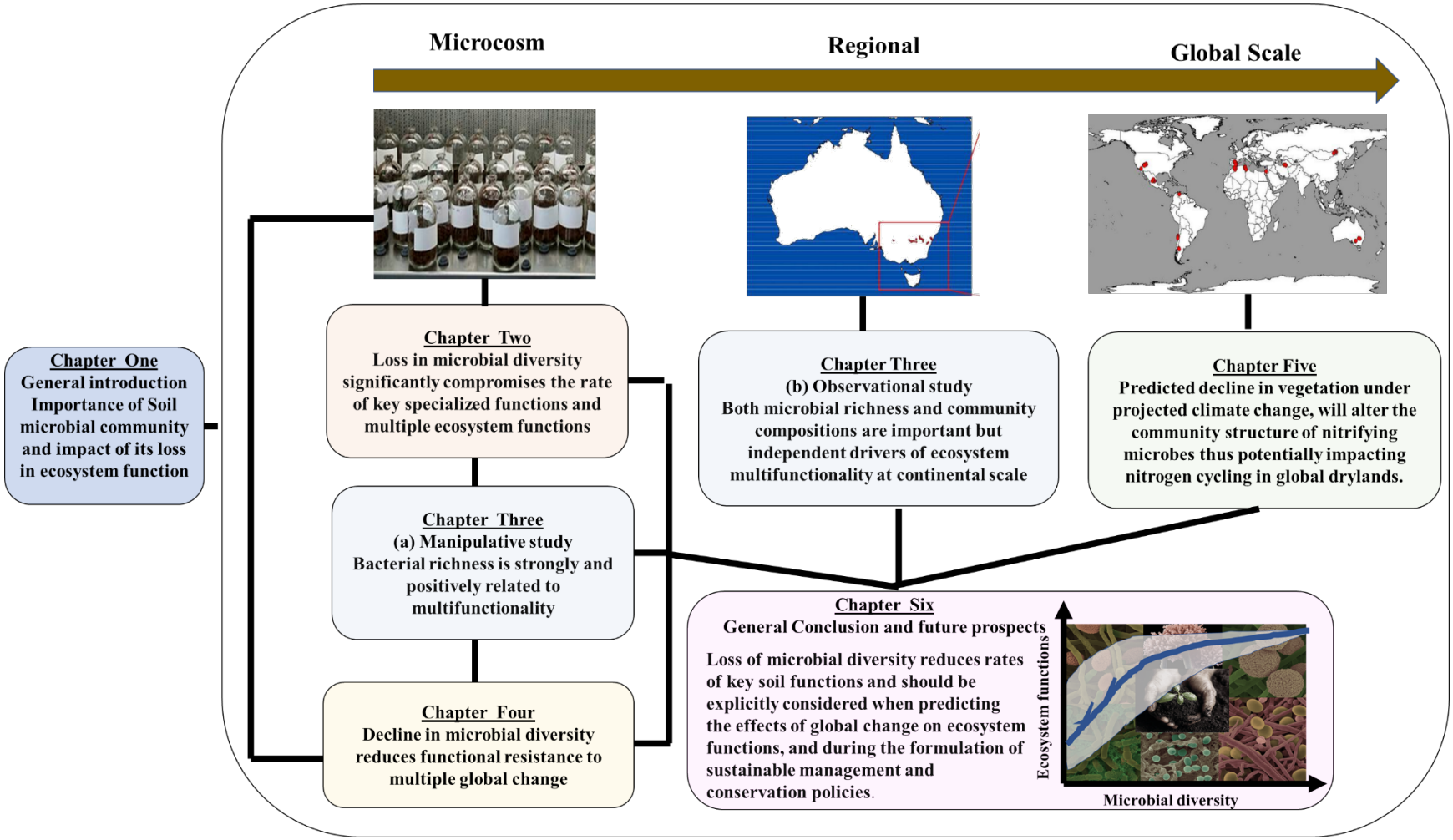
A major limitation to advance to the knowledge of the relationship between soil functions and microbial diversity, is the lack of field experiment and long-term facilities mainly due to technical issue. It is difficult to maintain the manipulated microbial diversity level in the field experiment. A concentrated approach and technological breakthrough is needed to overcome this challenge in order to establish a long-term field experiment to assess the consequences of the loss of microbial diversity. Additionally, my project focused on a limited number of functional measures and functional communities, therefore, I propose that other functional communities need to be considered, and that other terrestrial ecosystems such as grasslands and temperate forests be incorporated into a global study. The results of a

study of this scale would significantly increase our knowledge and understanding of the drivers in soil microbial communities, and soil ecosystem functionality at a global level. Only when the results from future studies described above, are consistent with findings from this work, it can be concluded that positive microbial BEF is unifying and universal principle.

Nonetheless, this study has significantly advanced our understanding of the impact of soil microbial diversity loss on ecosystem functioning, providing evidence from microcosm to global scale. My study highlights the relative importance of soil physico-chemical properties on microbial BEF. These findings have implication beyond the academic research in terms of sustainable use of natural resource for the provision of multiple ecosystem services. Therefore, I propose that along with multiple aspects of the soil ecosystem (e.g. functional potential and physico-chemical properties), microbial community should be explicitly considered when predicting the effects of global change on ecosystem functions, and during the formulation of sustainable management and conservation policies.

Summary of Research Findings

Microbial regulation of ecosystem process: Evidence from microcosm to global scale



Supplementary Information

Chapter 2

Supplementary Table 2.1. Details of QPCR primers and PCR cycling conditions used in this study.

Group/Gene	Primers	Sequence (5' –3')	Thermal conditions	References
qPCR				
Total bacteria	Eub338 Eub518	ACT CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GG	95°C, 15 min, 1 cycle 95°C for 60 s, 53°C for 30 s, 72°C for 60 s, 40 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1 cycle	Fierer *et al.,* 2005
Total Fungi	ITS1F 58R	TCC GTA GGT GAA CCT GCG G CGC TGC GTT CTT CAT CG	95°C, 15 min, 1 cycle 95°C for 60 s, 53°C for 30 s, 72°C for 60 s, 40 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1 cycle	Fierer *et al.,* 2005
nosZ	nosZ2F nosZ2R´	CGC RAC GGC AAS AAG GTS MSS GT CAK RTG CAK SGC RTG GCA GAA	95°C, 15 min, 1 cycle 95°C for 15 s, 65 to 60°C for 30 s (- 1°C by cycle), 72°C for 30 s, 80°C for 15 s, 6 cycles 95°C for 15 s, 60°C for 30 s, 72°C for 30 s, 80°C for 15 s, 40 cycles 95°C for 15 s, 60 to 95°C, 1 cycle	Hallin *et al.,* 2009
amoA (AOA)	19F CrenamoA616r48x	ATG GTC TGG CTW AGA CG GCC ATC CAB CKR TAN GTC CA	95°C, 10 min, 1 cycle 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, 40 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1 cycle	Hallin *et al.,* 2009
pmoA	pmoA-189F pmoA-650R	GGN GAC TGG GAC TTC TGG GAA SGC NGA GAA GAA SGC	95°C, 15 min, 1 cycle 95°C for 60 s, 55°C for 30 s, 72°C for 60 s, 40 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1cycle	Costello and Lidstrom (1999)
TRFLP				
nosZ	nosZ 121f nosZ 1917	CGC RAC GGC AAS AAG GTS MSS GT	95°C for 5 min, 1 cycle 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, 30 cycles	Kloos *et al.,* (2001)

		CAK RTG CAK SGC RTG GCA GAA	72°C for 10 min, 1 cycle	
amoA	CrenamoA23F CrenamoA616R	ATG GTC TGG CTW AGA CG GCCATCCATCTGTATGTCCA	95°C for 5 min, 1 cycle 94°C for 10 s, 55°C for 30 s, 72°C for 60 s, 30 cycles 72°C for 10 min, 1 cycle	Tourna *et al.,* (2008)
pmoA	pmoA-189F pmoA-650R	GGN GAC TGG GAC TTC TGG GAA SGC NGA GAA GAA SGC	95°C for 5 min, 1 cycle 94°C for 45 s, 62°C for 60 s, 72°C for 180 s, 30 cycles 72°C for 10 min, 1 cycle	Holmes *et al.,* (1995)

Supplementary Table 2.2. PERMANOVA results describing differences in the overall community composition of different microbial groups in two sites. Community composition was determined using MiSeq technique for total bacteria and fungi and by TRFLP technique for ammonia oxidizing archaea (*amoA* gene), N_20 reducing bacteria (*nosZ* gene) and methanotrophs (*pmoA* gene).

Bacteria	**Site A**	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
		Si	4	23578	5894.4	4.2896	0.0001	9889
		Res	15	20612	1374.1			
		Total	19	44189				
	Site B	Si	4	21681	5420.3	2.3339	0.0001	9804
		Res	15	34837	2322.5			
		Total	19	56518				
Fungi	**Site A**	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
		Si	4	22706	5676.5	1.5462	0.0021	9795
		Res	15	55068	3671.2			
		Total	19	77774				
	Site B	Si	4	24128	6032.1	2.1022	0.0001	9794
		Res	15	43042	2869.5			
		Total	19	67170				
amoA	**Site A**	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
		Si	4	11130.8	282.69	1.0867	0.3975	9947
		Res	15	41.62.3	260.14			
		Total	19	5293.1				
	Site B	Si	4	1770.5	442.63	2.3331	0.0172	9897
		Res	15	2845.8	189.72			
		Total	19	4616.3				
nosZ	**Site A**	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
		Si	4	3509.3	877.32	1.0074	0.4586	9870
		Res	15	13063	870.88			
		Total	19	16573				
	Site B	Si	4	3905	976.25	8.2849	0.0001	9915
		Res	15	1767.5	117.84			
		Total	19	5672.5				
pmoA	**Site A**	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
		Si	4	3978.2	994.56	13.682	0.0001	9943
		Res	16	1163	72.689			
		Total	20	5141.3				
	Site B	Si	4	738.83	184.71	5.4903	0.0001	9947
		Res	15	504.64	33.643			
		Total	19	1243.5				

Supplementary Table 2.3. PERMANOVA results describing pairwise differences the community composition of microbial groups in different dilutions. Community composition was determined using MiSeq technique for total bacteria and fungi and by TRFLP technique for ammonia oxidizing archaea (*amoA* gene), N_20 reducing bacteria (*nosZ* gene), and methanotrophs (*pmoA* gene).

	Site A				**Site B**			
	Groups	**t**	**P(perm)**	**Unique Perms**	**Groups**	**t**	**P(perm)**	**Unique perms**
Bacteria	**DX, D1**	1.5868	0.0293	35	DX, D1	1.3133	0.032	35
	DX, D3	2.4944	0.0279	35	DX, D3	1.9766	0.0289	35
	DX, D6	2.2512	0.0297	35	DX, D6	2.6285	0.0306	35
	DX, D10	2.0586	0.0324	35	DX, D10	2.4845	0.0296	35
	D1, D3	1.0762	0.1744	35	D1, D3	1.8176	0.0293	35
	D1, D6	1.2105	0.0277	35	D1, D6	2.5808	0.0308	35
	D1, D10	0.95855	0.5468	35	D1, D10	2.4544	0.0299	35
	D3, D6	1.489	0.025	35	D3, D6	1.7264	0.0267	35
	D3, D10	1.2977	0.0292	35	D3, D10	1.9078	0.0308	35
	D6, D10	1.2385	0.0273	35	D6, D10	1.2365	0.0275	35
Fungi	**DX, D1**	1.2882	0.0307	35	DX, D1	1.0634	0.1989	35
	DX, D3	1.6587	0.0289	35	DX, D3	1.0488	0.2911	35
	DX, D6	1.7347	0.0272	35	DX, D6	1.5626	0.0292	35
	DX, D10	1.5697	0.0293	35	DX, D10	1.8479	0.0278	35
	D1, D3	0.94804	0.7483	35	D1, D3	1.0468	0.2024	35
	D1, D6	1.1339	0.1988	35	D1, D6	1.42	0.0291	35
	D1, D10	1.0072	0.3195	35	D1, D10	1.7447	0.0279	35
	D3, D6	1.1972	0.1145	35	D3, D6	1.4028	0.0298	35
	D3, D10	1.0065	0.4755	35	D3, D10	1.7249	0.0295	35
	D6, D10	0.97326	0.5742	35	D6, D10	1.1578	0.092	35

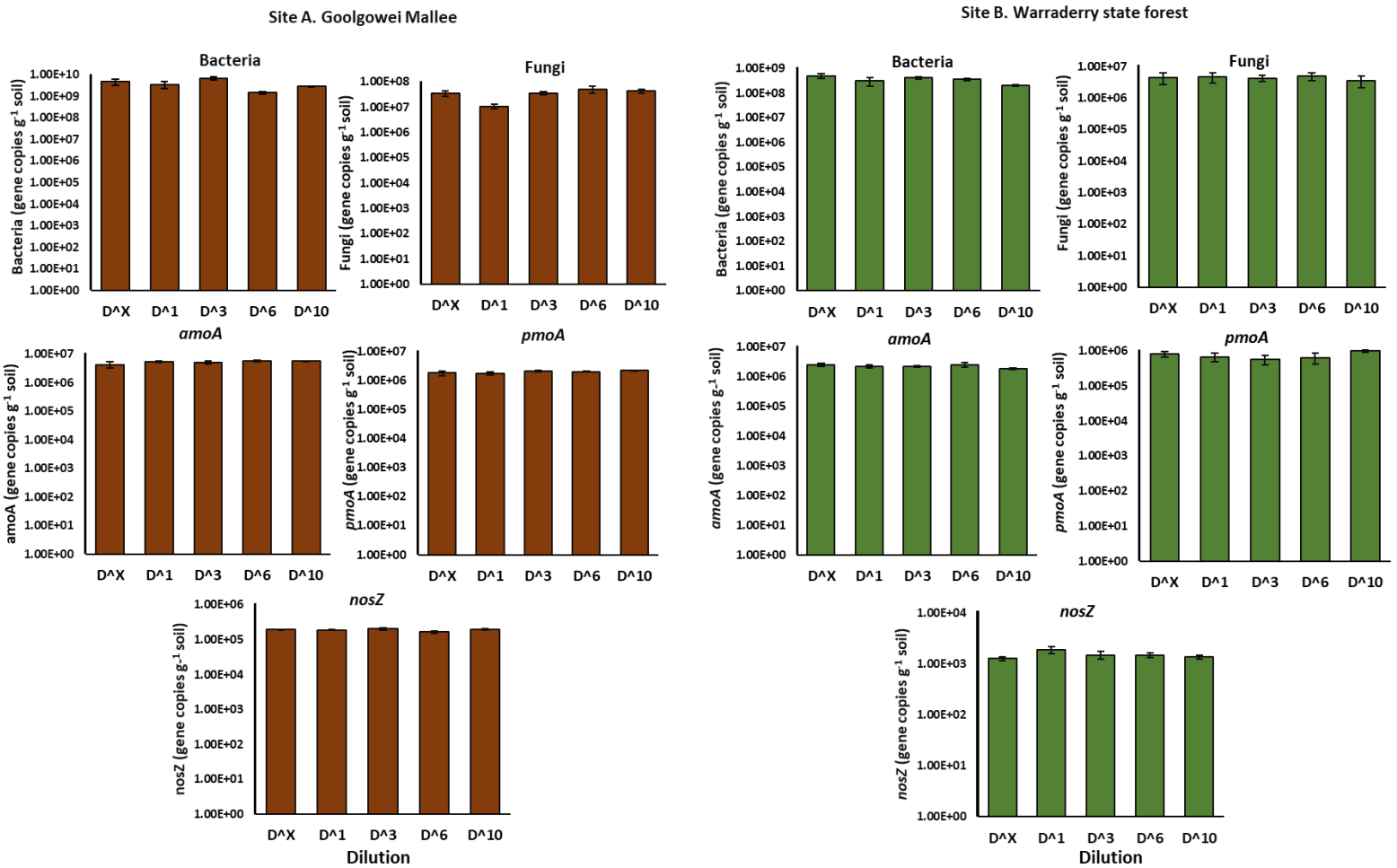
	Site A				Site B			
	Groups	**T**	**P(perm)**	**Unique Perms**	**Groups**	**t**	**P(perm)**	**Unique perms**
amoA	**DX, D1**	1.8156	0.0876	126	**DX, D1**	0.95516	0.607	35
	DX, D3	1.5621	0.1709	35	**DX, D3**	0.7763	0.7755	35
	DX, D6	1.393	0.2298	25	**DX, D6**	1.7439	0.0864	35
	DX, D10	0.38023	0.9103	35	**DX, D10**	1.5385	0.112	35
	D1, D3	1.0311	0.326	91	**D1, D3**	0.70459	0.8537	35
	D1, D6	0.48114	0.7656	91	**D1, D6**	1.53	0.0856	35
	D1, D10	1.094	0.3155	126	**D1, D10**	1.355	0.1435	35
	D3, D6	0.6379	0.6224	25	**D3, D6**	1.4273	0.168	35
	D3, D10	0.97202	0.4542	35	**D3, D10**	1.0119	0.41	35
	D6, D10	1.0476	0.3446	25	**D6, D10**	1.92	0.0579	35
nosZ	**DX, D1**	0.93826	0.6259	35	**DX, D1**	1.8792	0.0532	35
	DX, D3	1.2345	0.1744	35	**DX, D3**	1.7516	0.0282	35
	DX, D6	0.8495	0.8822	35	**DX, D6**	2.1704	0.0273	35
	DX, D10	1.2842	0.1123	35	**DX, D10**	3.954	0.0283	35
	D1, D3	0.77388	0.8234	35	**D1, D3**	1.7007	0.0545	35
	D1, D6	0.75406	0.9139	35	**D1, D6**	2.002	0.0291	35
	D1, D10	1.0537	0.2541	35	**D1, D10**	3.7289	0.0273	35
	D3, D6	0.90765	0.7161	35	**D3, D6**	1.3936	0.1156	35
	D3, D10	1.2615	0.0839	35	**D3, D10**	2.8449	0.0279	35
	D6, D10	0.95206	0.569	35	**D6, D10**	2.7096	0.0281	35
pmoA	**DX, D1**	2.1621	0.062	35	**DX, D1**	1.2035	0.2857	35
	DX, D3	4.2993	0.01	126	**DX, D3**	3.3559	0.0283	35
	DX, D6	4.4696	0.0256	35	**DX, D6**	2.6448	0.0281	35
	DX, D10	4.8847	0.0305	35	**DX, D10**	3.7004	0.0337	35
	D1, D3	3.0454	0.016	126	**D1, D3**	2.333	0.029	35
	D1, D6	3.7273	0.0289	35	**D1, D6**	2.0025	0.0308	35
	D1, D10	4.2845	0.0267	35	**D1, D10**	2.9242	0.0281	35
	D3, D6	1.6245	0.0919	126	**D3, D6**	1.4661	0.0293	35
	D3, D10	2.9045	0.0086	126	**D3, D10**	2.5166	0.0281	35
	D6, D10	1.7126	0.0592	35	**D6, D10**	0.69676	0.4533	35

Supplementary Table 2.4. Bacterial and fungal Chao1 and abundance-based coverage estimators (ACE) diversity indexes (±standard deviation) in diversity gradients microcosms as determined by MiSeq analysis for two sampling sites. Different letters for bacteria and fungi in two sites represent statistically significant differences ($P< 0.05$) between dilutions. Dx represent positive control.

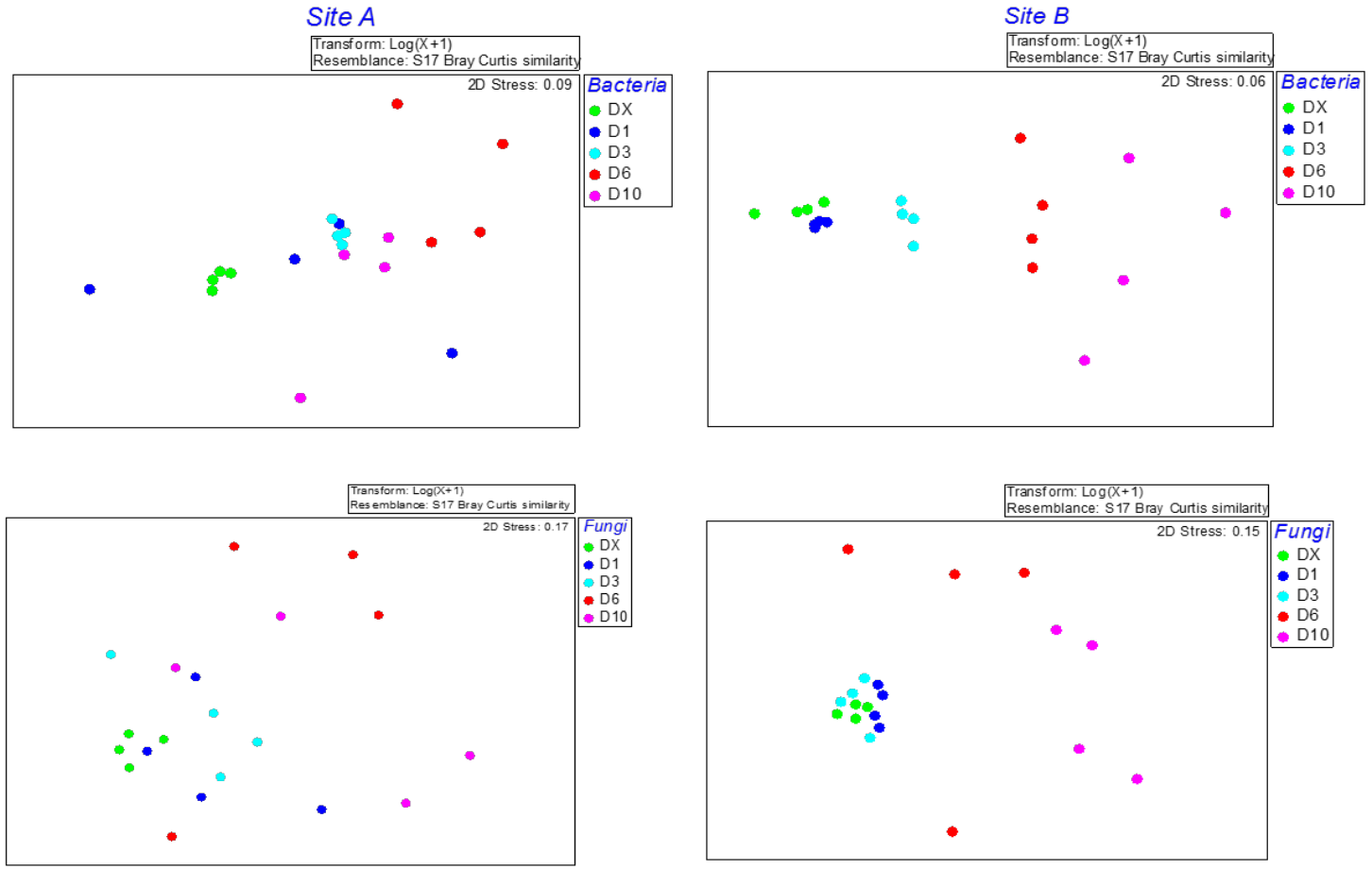
Microbial group	Diversity	Site A		Site B	
		Chao1	Ace	Chao1	Ace
Bacteria	**DX**	3081.37± 137.21a	3171.93± 121.53a	2672.99± 143.58a	2787.86± 119.17a
	D1	2426.84± 907.21b	2655.39± 100.53b	2559.8± 19.12b	2600.27± 41.83b
	D3	909.9± 28.01c	985.53± 28.27c	1427.65± 80.35c	1452.66± 67.25c
	D6	904.78± 73.99c	974.45± 88.15c	759.12± 23.43d	833.62± 23.74d
	D10	502.71± 73.93d	570.11± 62.64d	440.38± 49.56e	486.22± 56.68e
Fungi	**DX**	4776.65± 125.73a	5470.66± 102.62a	4915.4± 96.68a	5361.02± 217.5a
	D1	3652.95± 161.32b	3900.51± 297.27b	4431.97± 104.01b	5180.68± 229.35b
	D3	2599.2± 531.05c	2901.25± 683.06c	3949.59± 32.58c	4460.88± 77.69c
	D6	925.74± 193.76d	1053.45± 265.04d	1083.47± 182.62d	1200.57± 219d
	D10	844.45± 160.42e	893.64± 168.12e	1423.93± 250.05e	1623.32± 286.76e

Supplementary Table 2.5. Partial correlation (Pearson's R) between diversity of different microbial groups and their associated functions (multi-functionality for total bacteria and fungi) controlling for biomass (determined by qPCR using similar genes used for diversity analysis for different microbial groups) and community composition (1st axis of NMDS plots).

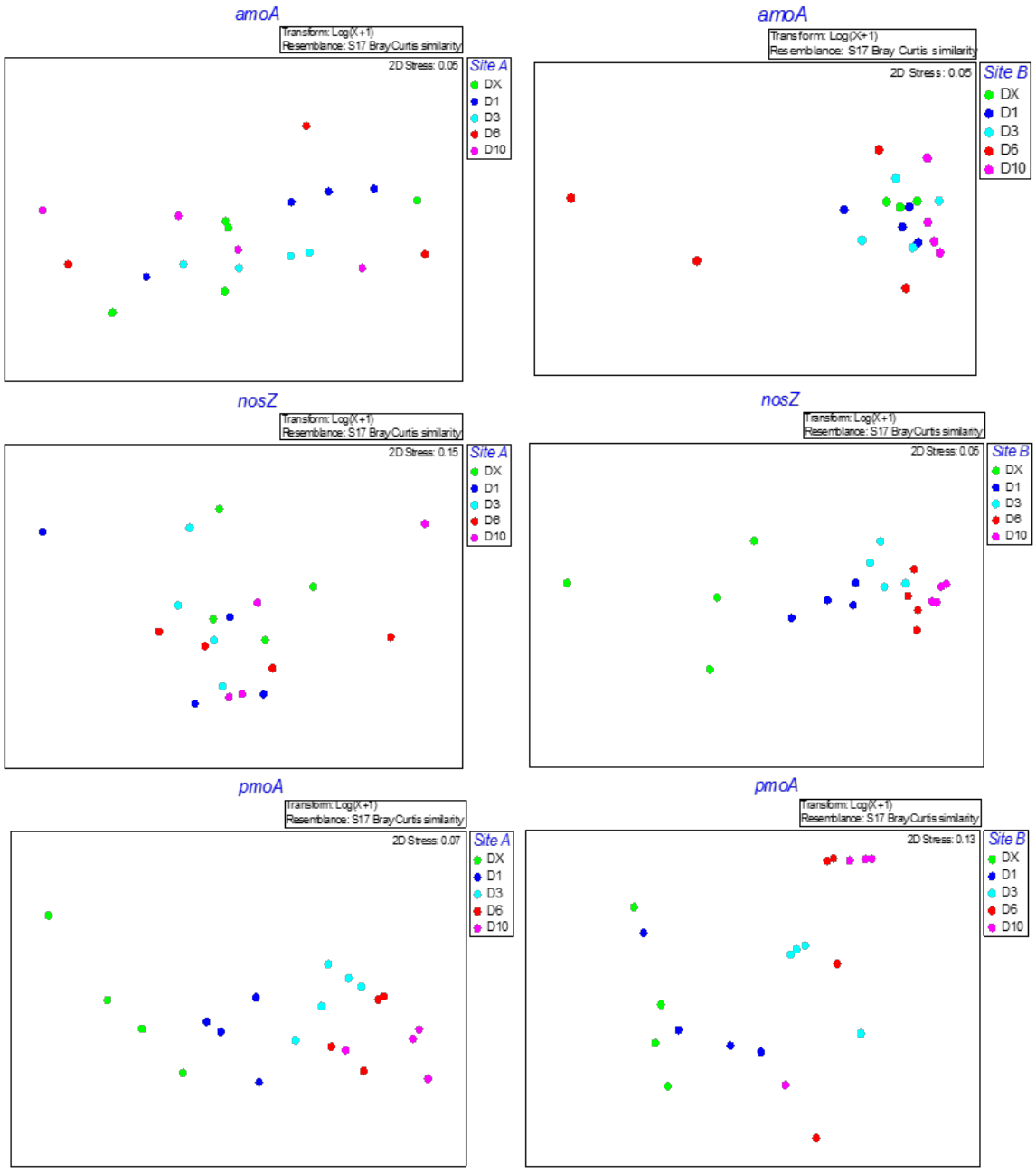
Microbial groups	**Functions**	**Site**		**Site**	
		A		**B**	
Community composition (NDMS Axis-1)		**Pearson's r**	**P-value***	**Pearson's r**	**P-value***
Total Bacteria	Multi-functionality	0.54	0.04	0.79	0.001
Total Fungi	Multi-functionality	0.63	0.002	0.73	0.003
N_2O reducing microbes	N_2O production	-0.85	0.001	-0.51	0.001
Ammonia oxidising archaea	NO_3 production	0.83	0.001	0.91	0.001
Methanotrophs	CH_4 consumption	-0.63	0.001	-0.86	0.001
Biomass(Abundance)					
Total Bacteria	Multi-functionality	0.83	0.001	0.86	0.001
Total Fungi	Multi-functionality	0.86	0.001	0.85	0.001
N_2O reducing microbes	N_2O production	-0.57	0.03	-0.84	0.001
Ammonia oxidising archaea	NO_3 production	0.81	0.001	0.88	0.001
Methanotrophs	CH_4 consumption	-0.85	0.001	-0.96	0.001



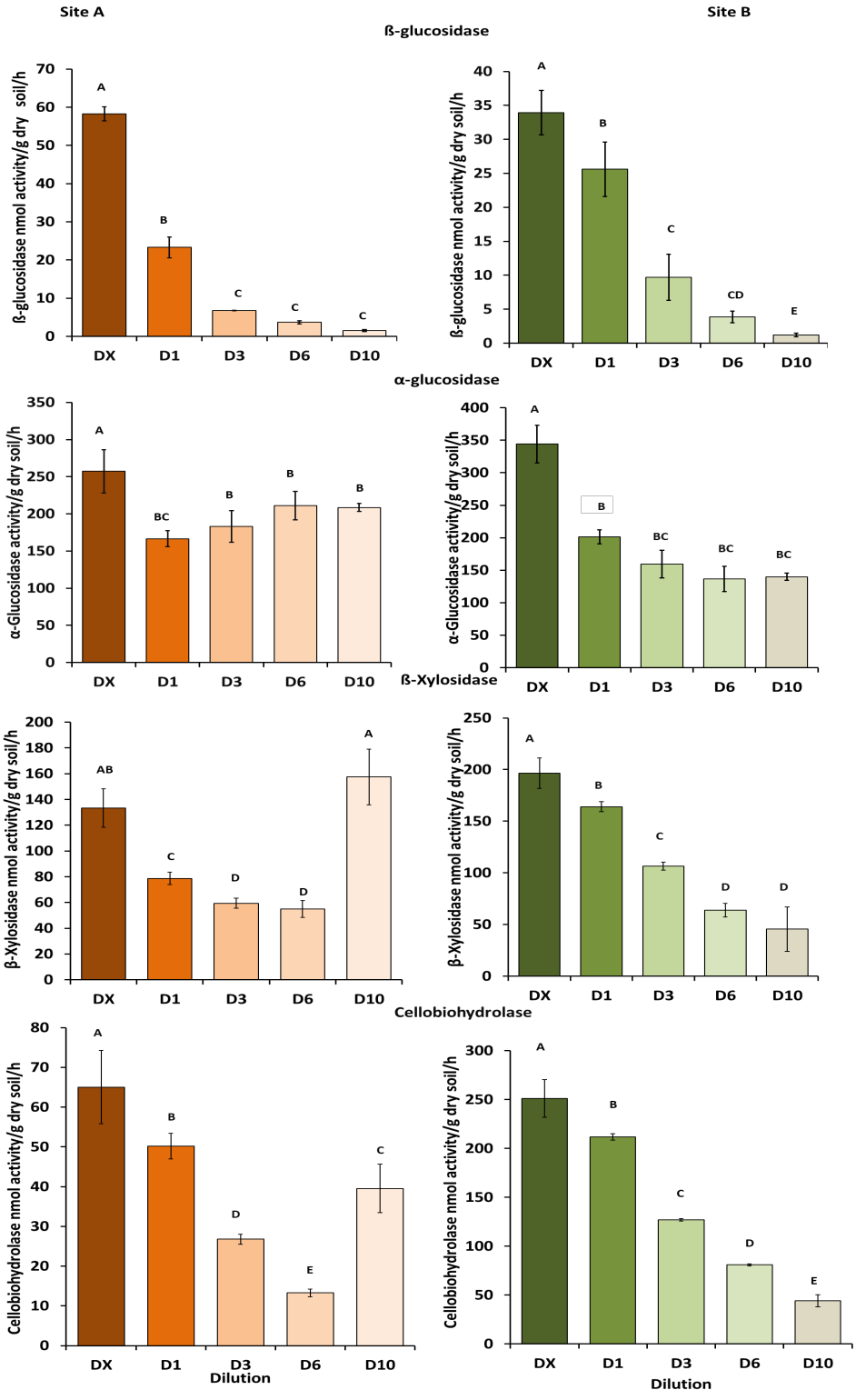
Supplementary Figure 2.1. Quantification (number of gene copies g^{-1} soil) of total bacteria, total fungi, a*amoA* gene, *pmoA* gene, and *nosZ* gene in dilution microcosms of two sites.



Supplementary Figure 2.2a. Nonmetric multidimensional scaling (NMDS) plots describing differences in the community composition of microbial groups in different dilutions from two sites. Community composition was determined using MiSeq for total bacteria and fungi.



Supplementary Figure 2.2b. Nonmetric multidimensional scaling (NMDS) plots describing differences in the community composition of microbial groups in different dilutions from two sites. Community composition was determined using TRFLP for ammonia oxidizing archaea (*amoA* gene), N_20 reducing bacteria (*nosZ* gene) and methanotrophs (*pmoA* gene).


Site A
Site B
ß-glucosidase
ß-glucosidase nmol activity/g dry soil/h
A
B
C
C
C
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D1
D3
D6
D10
ß-glucosidase nmol activity/g dry soil/h
A
B
C
CD
E
DX
D1
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D6
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α-glucosidase
α-Glucosidase activity/g dry soil/h
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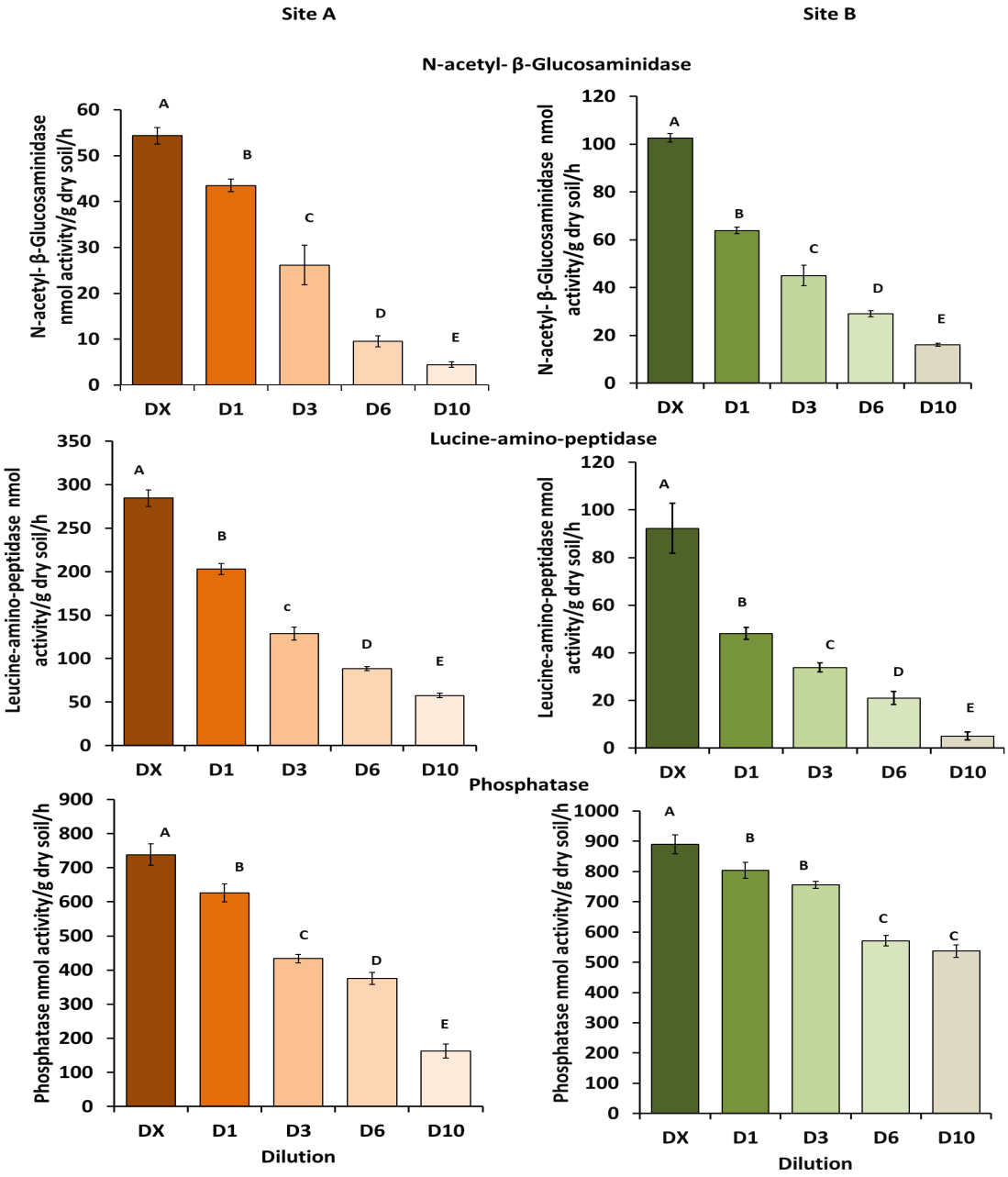
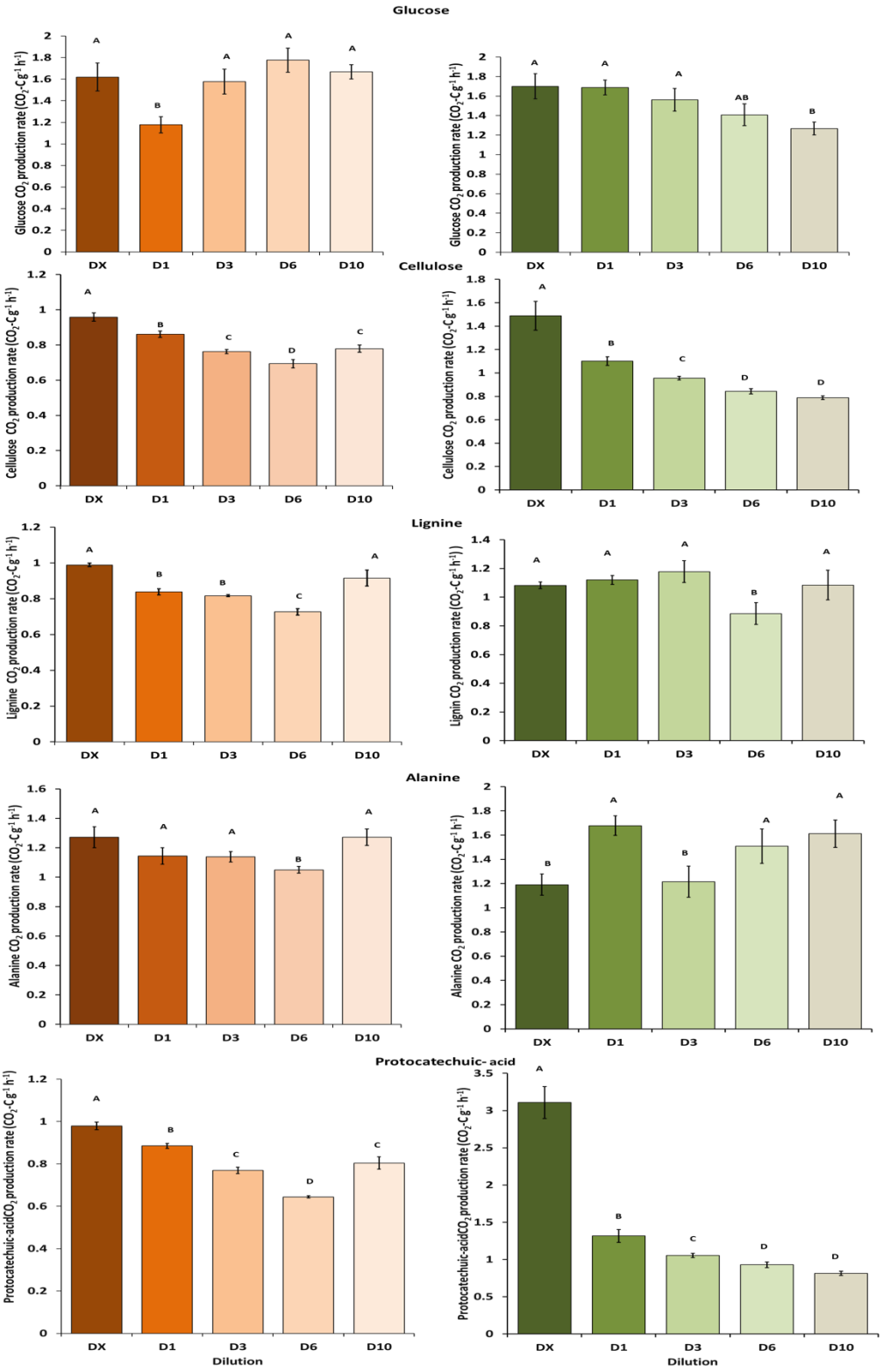
Dilution

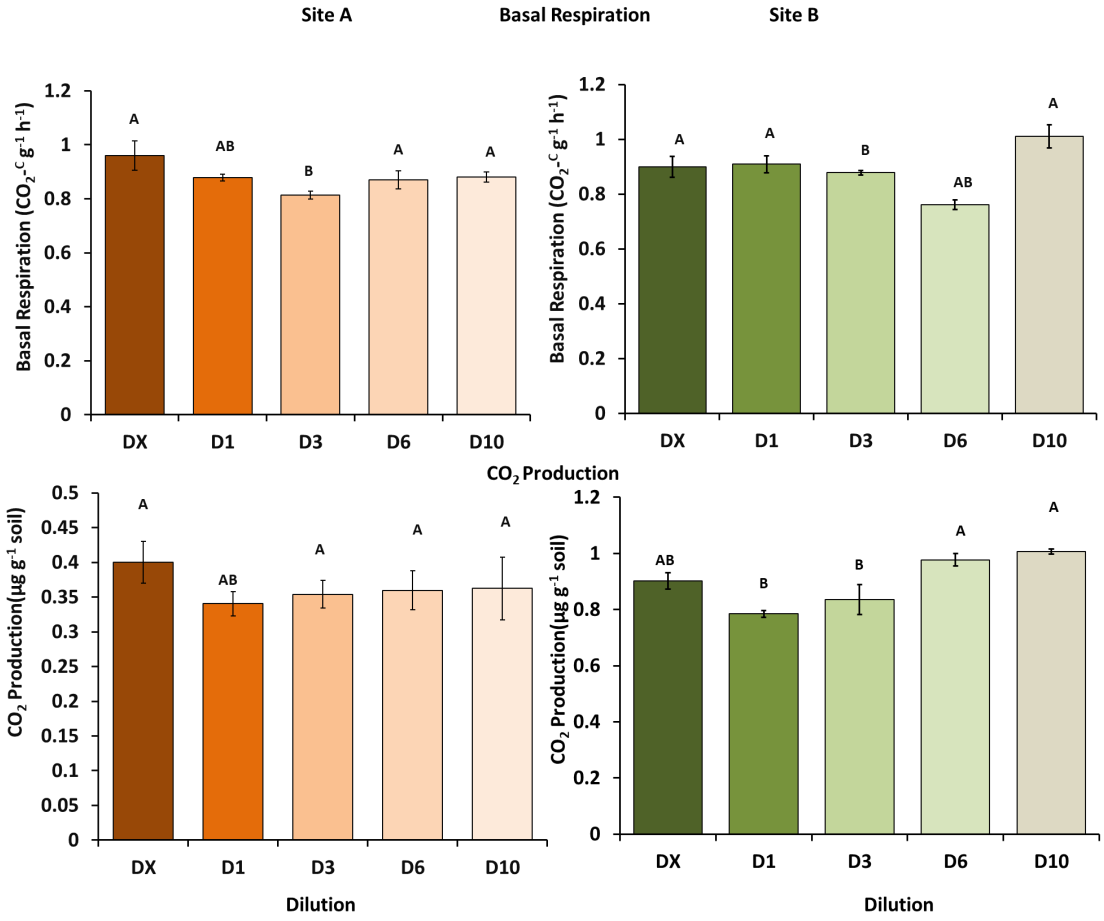
Continued……



Supplementary Figure 2.3. Quantification of enzymatic activities from different dilutions from two sites. Different letters represent significant differences at $P < 0.01$.



Supplementary Figure 2.4. Quantification of substrate utilization measured by Micro-Resp analysis from different dilutions from two sites. Different letters represent significant differences at P <0.01.



Supplementary Figure 2.5. Quantification of CO_2 production (gas flux analysis) and basal respiration (Micro-Resp analysis) from different dilutions from two sites. Different letters represent significant differences at P <0.01.

Chapter 3

Supplementary Table 3.1. Information on sampling sites.

Sample	Site - ID	Latitude	Longitude	Soil-pH	Soil-C	Microsite
1	CYP_028	-33.84	147.39	6.23	1.86	OPEN
2	CYP_028	-33.84	147.39	6.74	3.20	TREE
3	JM055	-34.36	146.21	6.91	2.21	OPEN
4	JM055	-34.36	146.21	8.47	4.16	TREE
5	JM057	-34.42	146.31	5.64	2.26	OPEN
6	JM057	-34.42	146.31	8.93	4.93	TREE
7	JM060	-34.28	146.58	5.85	0.84	OPEN
8	JM060	-34.28	146.58	6.44	2.42	TREE
9	JM061	-34.35	146.92	8.66	1.74	TREE
10	JM062	-34.44	147.42	5.51	4.49	OPEN
11	JM062	-34.44	147.42	7.07	9.66	TREE
12	JM065	-34.74	149.89	5.43	2.23	OPEN
13	JM065	-34.74	149.89	5.52	4.87	TREE
14	JM071	-33.65	150.86	5.23	1.96	OPEN
15	JM071	-33.65	150.86	5.46	3.46	TREE
16	JM072	-33.62	150.77	5.55	2.55	OPEN
17	JM072	-33.62	150.77	5.63	8.45	TREE
18	JM076	-34.36	148.92	5.61	2.58	OPEN
19	JM076	-34.36	148.92	5.12	3.87	TREE
20	JM077	-33.98	148.95	6.43	0.90	OPEN
21	JM077	-33.98	148.95	6.18	2.45	TREE
22	JM079	-33.83	148.61	5.86	1.73	OPEN
23	JM079	-33.83	148.61	7.24	7.32	TREE
24	JM081	-33.51	148.17	5.70	3.00	OPEN
25	JM081	-33.51	148.17	8.10	9.08	TREE
26	JM085	-33.73	148.20	5.91	1.39	OPEN
27	JM085	-33.73	148.20	7.35	4.74	TREE
28	JM092	-33.32	148.16	5.83	2.07	OPEN
29	JM092	-33.32	148.16	8.48	8.82	TREE
30	JM100	-34.51	150.24	6.64	2.35	OPEN
31	JM100	-34.51	150.24	5.93	2.88	TREE
32	Site 1	-34.00	145.73	6.28	0.89	OPEN
33	Site 1	-34.00	145.73	6.36	3.03	TREE
34	Site 2	-34.25	146.07	6.48	1.40	OPEN
35	Site 2	-34.25	146.07	8.90	2.53	TREE
36	Site 3	-33.91	150.99	5.97	7.69	OPEN
37	Site 3	-33.91	150.99	6.00	5.47	TREE

38	Site 4	-33.98	151.06	5.12	2.92	OPEN
39	Site 4	-33.98	151.06	4.83	12.32	TREE
40	BU1	-34.13	142.08	7.63	0.75	OPEN
41	BU1	-34.13	142.08	7.33	1.08	TREE
42	BU2	-34.16	142.20	8.97	0.97	OPEN
43	BU2	-34.16	142.20	9.00	1.55	TREE

Supplementary Table 3.2. Primers and conditions used for the quantification of total bacteria and various bacterial phylum/class by qPCR assay (Trivedi *et al.,* 2012).

Target group	**Forward primer (Sequence 5'-3')**	**Reverse primer**	**Annealing temp (°C)**	**Approx. amplicon length (bp)**
All *Bacteria*	Eub338 (ACT CCT ACG GGA GGC AGC AG)	Eub518 (ATT ACC GCG GCT GCT GG)	53	200
α-*Proteobacteria*	Eub338	Alf685 (TCT ACG RAT TTC ACC YCT AC)	60	365
β-*Proteobacteria*	Eub338	Bet680 (TCA CTG CTA CAC GYG)	60	360
Actinobacteria	Actino235 (CGC GGC CTA TCA GCT TGT TG)	Eub518	60	300
Firmicutes	Lgc353 (GCA GTA GGG AAT CTT CCG)	Eub518	60	180
Bacteroidetes	Cfb319 (GTA CTG AGA CAC GGA CCA)	Eub518	65	220
γ-Proteobacteria (Pseudomonas spp.)	PsF (TTA GCT CCA CCT CGC GGC)	PsR (GGT CTG AGA GGA TGA TCA GT)	65	300

Supplementary Table 3.3. Summary on the multiple microbial combinations used in this study including those for species richness one, two, four and six. A = α-Proteobacteria; B = β-Proteobacteria; C = γ-Proteobacteria; D = Firmicutes; E = Bacteroidetes; F = Actinobacteria.

Diversity	Combination	A	B	C	D	E	F
1	B	0	100	0	0	0	0
1	C	0	0	100	0	0	0
1	D	0	0	0	100	0	0
1	E	0	0	0	0	100	0
1	F	0	0	0	0	0	100
2	AB	50	50	0	0	0	0
2	AC	50	0	50	0	0	0
2	AD	50	0	0	50	0	0
2	AE	50	0	0	0	50	0
2	AF	50	0	0	0	0	50
2	BC	0	50	50	0	0	0
2	BD	0	50	0	50	0	0
2	BE	0	50	0	0	50	0
2	BF	0	50	0	0	0	50
2	CD	0	0	50	50	0	0
2	CE	0	0	50	0	50	0
2	CF	0	0	50	0	0	50
2	DE	0	0	0	50	50	0
2	DF	0	0	0	50	0	50
2	EF	0	0	0	0	50	50
2	AB	25	75	0	0	0	0
2	AC	25	0	75	0	0	0
2	AD	25	0	0	75	0	0
2	AE	25	0	0	0	75	0
2	AF	25	0	0	0	0	75
2	BC	0	25	75	0	0	0
2	BD	0	25	0	75	0	0
2	BE	0	25	0	0	75	0
2	CD	0	0	25	75	0	0
2	CE	0	0	25	0	75	0
2	CF	0	0	25	0	0	75
2	DE	0	0	0	25	75	0
2	DF	0	0	0	25	0	75

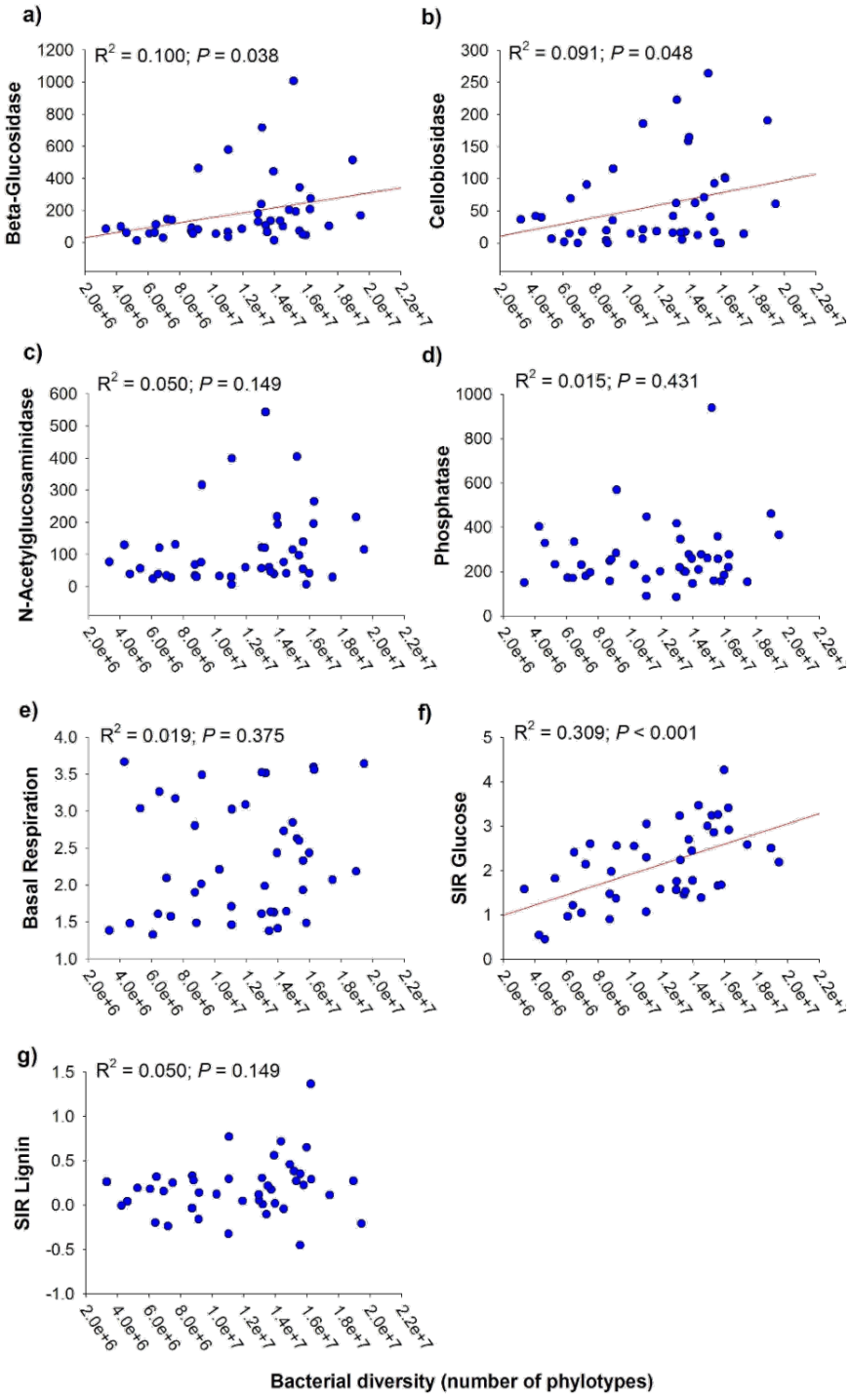
2	EF	0	0	0	0	25	75
2	AB	75	25	0	0	0	0
2	AC	75	0	25	0	0	0
2	AD	75	0	0	25	0	0
2	AE	75	0	0	0	25	0
2	AF	75	0	0	0	0	25
2	BC	0	75	25	0	0	0
2	BD	0	75	0	25	0	0
2	BE	0	75	0	0	25	0
2	BF	0	75	0	0	0	25
2	CD	0	0	75	25	0	0
2	CE	0	0	75	0	25	0
2	CF	0	0	75	0	0	25
2	DE	0	0	0	75	25	0
2	DF	0	0	0	75	0	25
2	EF	0	0	0	0	75	25
4	ABCD	25	25	25	25	0	0
4	ABCE	25	25	25	0	25	0
4	ABCF	25	25	25	0	0	25
4	ACDE	25	0	25	25	25	0
4	ACDF	25	0	25	25	0	25
4	ADEF	25	0	0	25	25	25
4	BCDE	0	25	25	25	25	0
4	BCDF	0	25	25	25	0	25
4	BDEF	0	25	0	25	25	25
4	CDEF	0	0	25	25	25	25
4	ABDE	25	25	0	25	25	0
4	ABDF	25	25	0	25	0	25
4	ABEF	25	25	0	0	25	25
4	ACEF	25	0	25	0	25	25
6	ABCDEF	16.6	16.6	16.6	16.6	16.6	16.6
6	ABCDEF	16.6	16.6	16.6	16.6	16.6	16.6

Supplementary Table 3.4. Correlations (Spearman) between averaging multifunctionality index (used in this study) and the number of functions at or above a threshold (25, 50 and 75%) of the maximum observed function (n = 43 for the Field study and n = 204 for each of the soils in the Microcosm study).

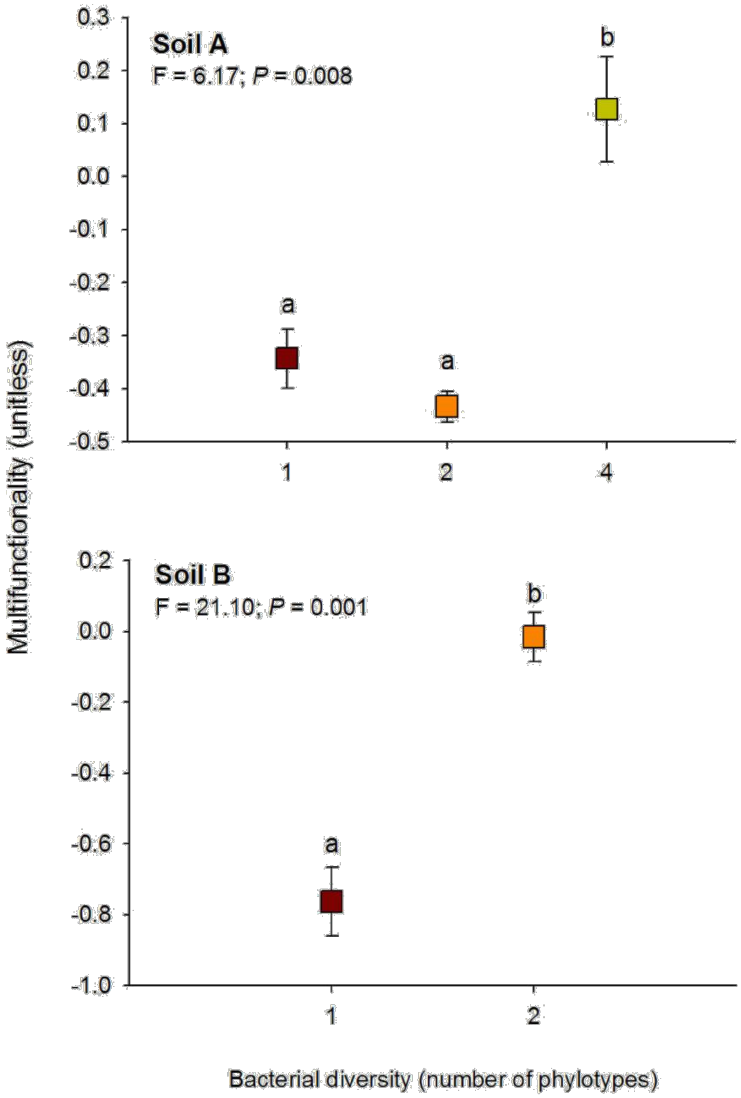
Approach	25%	50%	75%
I (Field study)	0.875	0.918	0.875
	<0.001	<0.001	<0.001
II (Soil A)	0.783	0.886	0.596
	<0.001	<0.001	<0.001
II (Soil B)	0.615	0.788	0.7
	<0.001	<0.001	<0.001

Supplementary Table 3.5. Correlations (Spearman) between bacterial richness and composition for field (n = 43) and microcosm (soils A and B) studies (n = 204). n = number of samples. A = α-Proteobacteria; B = β-Proteobacteria; C = γ-Proteobacteria; D = Firmicutes; E = Bacteroidetes; F = Actinobacteria.

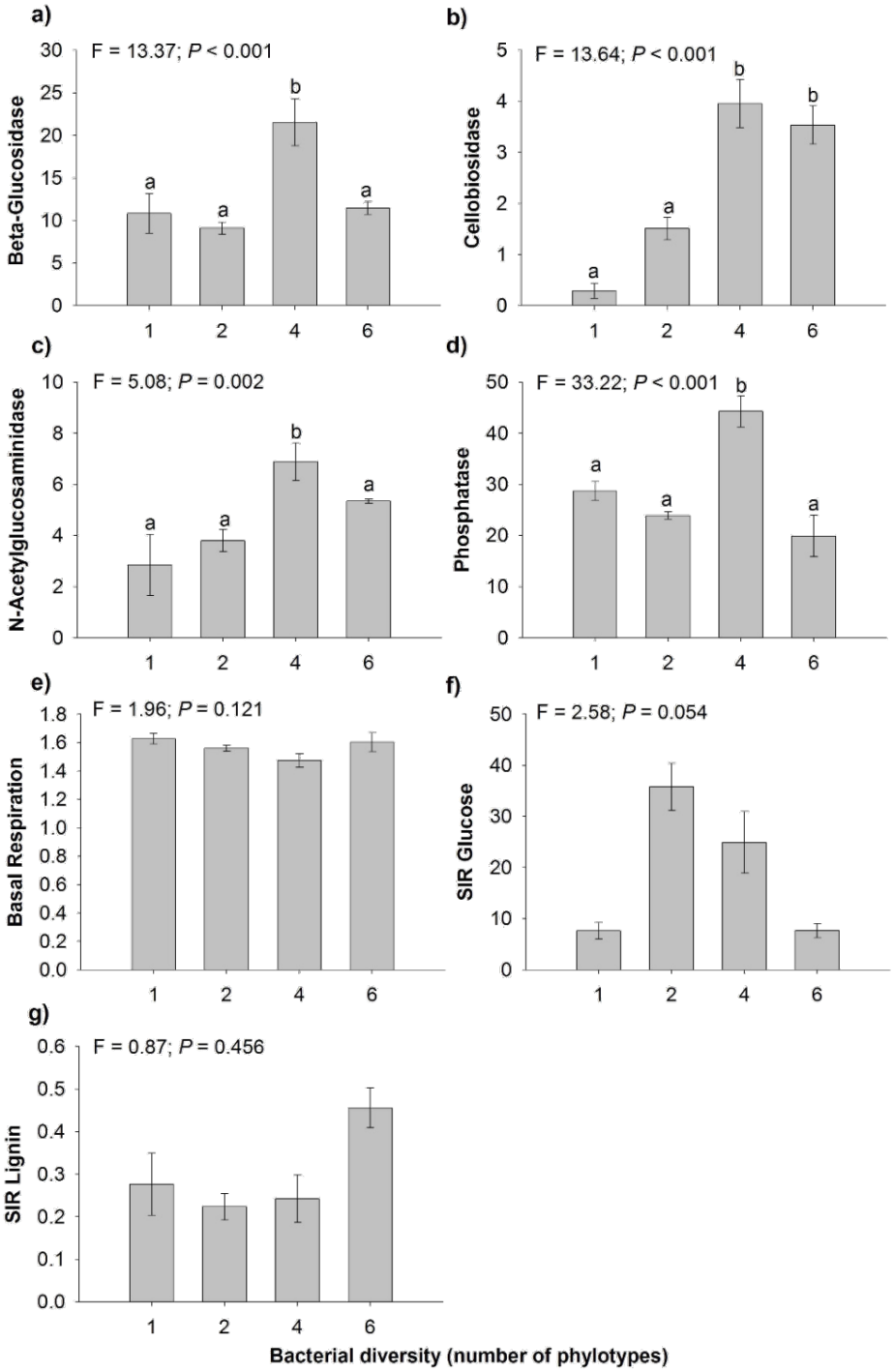
Study	Parameter	A	B	C	D	E	F
Field	ρ	-0.442	0.495	0.023	0.332	0.536	0.138
	P-value	0.003	0.001	0.883	0.03	<0.001	0.376
Microcosm (Soil A)	ρ	0.213	0.232	0.212	0.216	0.228	0.264
	P-value	0.002	0.001	0.002	0.002	0.001	<0.001
Microcosm (Soil B)	ρ	0.217	0.301	0.218	0.218	0.245	0.225
	P-value	0.002	<0.001	0.002	0.002	<0.001	0.001



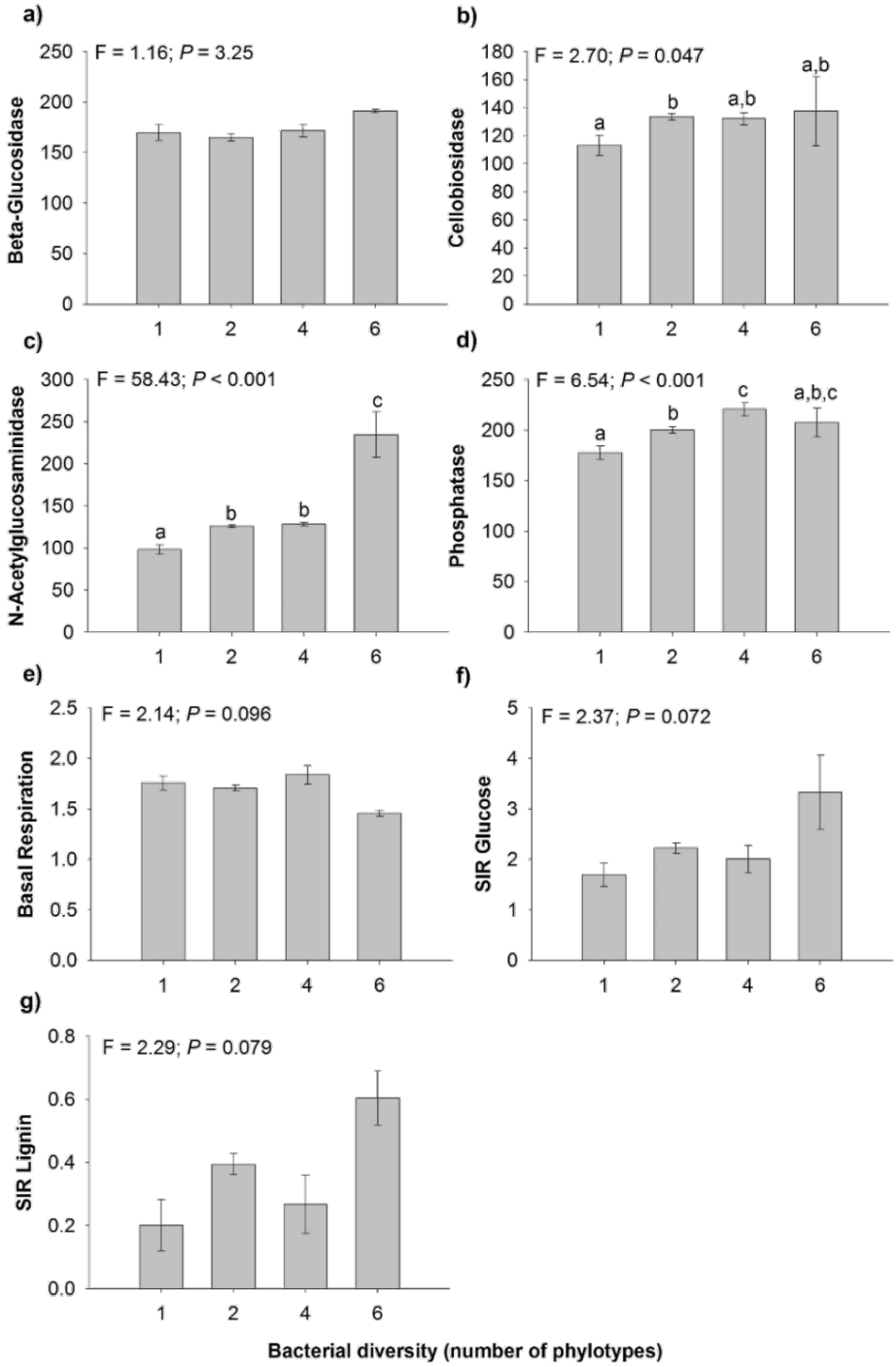
Supplementary Figure 3.1. Linear regressions between bacterial diversity (number of phylotypes) and single functions (n = 43). Bacterial richness in is calculated as the number of OTUs (97% similarity; x^2-transformed).



Supplementary Figure 3.2. Bacterial richness effects on multifunctionality after removing key species (Bacteroidetes/Actinobacteria and Proteobacteria classes for soils A and B, respectively) in Field and Microcosm (soils A and B) studies.



Supplementary Figure 3.3. Effects of bacterial richness on single functions for our soil A from the experimental approach. Bacterial diversity is the number of bacterial phyla/classes.



Supplementary Figure 3.4. Effects of bacterial richness on single functions for our soil B from the experimental approach. Bacterial diversity is the number of bacterial phyla/classes.

Chapter 5

Supplementary Table 5.1. AOA and AOB taxa within each of the modules in Figure 5.3.

amoA group	OTU ID	Module class	Taxonomy
AOA	TRF_53	0	Nitrosotalea
	TRF_54	0	Nitrosotalea
	TRF_73	0	Nitrososphaera
	TRF_79	0	Nitrososphaera
	TRF_153	0	Nitrososphaera
	TRF_163	0	Nitrososphaera
	TRF_191	0	Nitrosotalea
	TRF_199	0	Nitrosotalea
	TRF_215	0	Nitrososphaera
	TRF_245	0	Nitrosopumilus
	TRF_254	0	Nitrosotalea
	TRF_263	0	Nitrososphaera
	TRF_294	0	Nitrososphaera
	TRF_364	0	Nitrososphaera
	TRF_383	0	Not identified
	TRF_465	0	Nitrosotalea
	TRF_555	0	Nitrosotalea
	TRF_565	0	Nitrosotalea
	TRF_576	0	Nitrosopumilus
	TRF_585	0	Nitrososphaera
	TRF_50	1	Nitrososphaera
	TRF_98	1	Nitrososphaera
	TRF_126	1	Not identified
	TRF_169	1	Nitrosotalea
	TRF_196	1	Nitrososphaera
	TRF_272	1	Nitrososphaera
	TRF_278	1	Not identified
	TRF_290	1	Nitrososphaera

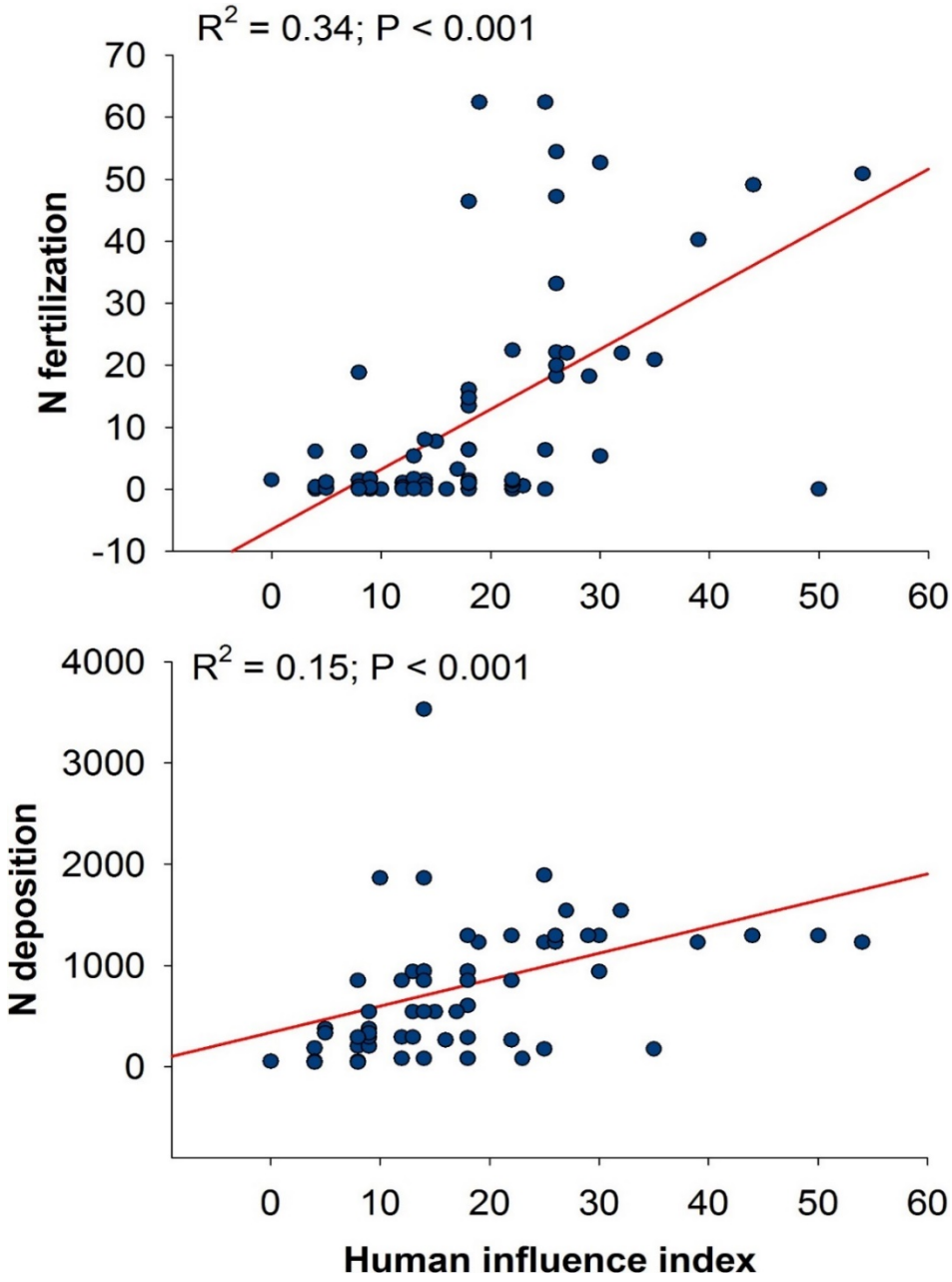
amoA group	OTU ID	Module class	Taxonomy
AOB	TRF_30	3	C. 7, Nitrosospira
	TRF_39	3	C. 4, Nitrosospira
	TRF_47	3	C. 3, Nitrosospira
	TRF_53	3	C. 3, Nitrosospira
	TRF_60	3	C. 3, Nitrosospira
	TRF_67	3	Not identified
	TRF_76	3	Not identified
	TRF_153	3	C. 3, Nitrosospira
	TRF_155	3	C. 3, Nitrosospira
	TRF_168	3	Not identified
	TRF_174	3	C. 3, Nitrosospira
	TRF_179	3	Not identified
	TRF_197	3	Not identified
	TRF_232	3	C. 3, Nitrosospira
	TRF_245	3	C. 4, Nitrosospira
	TRF_247	3	C. 3, Nitrosospira
	TRF_253	3	C. 3, Nitrosospira
	TRF_263	3	Not identified
	TRF_275	3	C. 3, Nitrosospira
	TRF_440	3	C. 3, Nitrosospira
	TRF_467	3	Not identified
	TRF_479	3	Not identified
	TRF_489	3	Not identified
	TRF_41	4	C. 3, Nitrosospira
	TRF_50	4	Not identified
	TRF_58	4	Not identified
	TRF_61	4	C. 3, Nitrosospira
	TRF_72	4	Not identified

	TRF_314	1	Nitrososphaera
	TRF_320	1	Nitrosotalea
	TRF_334	1	Nitrosotalea
	TRF_350	1	Nitrososphaera
	TRF_366	1	Not identified
	TRF_377	1	Not identified
	TRF_405	1	Not identified
	TRF_411	1	Not identified
	TRF_439	1	Nitrososphaera
	TRF_533	1	Nitrosotalea
	TRF_574	1	Nitrososphaera
	TRF_597	1	Nitrososphaera
	TRF_57	2	Nitrososphaera
	TRF_77	2	Nitrososphaera
	TRF_85	2	Nitrososphaera
	TRF_95	2	Nitrososphaera
	TRF_101	2	Not identified
	TRF_120	2	Not identified
	TRF_146	2	Nitrosotalea

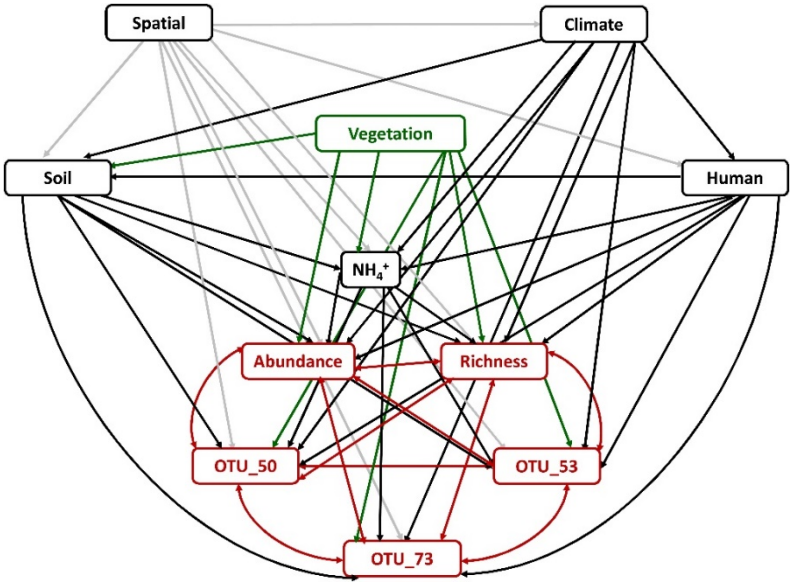
	TRF_78	4	C. 9, Nitrosospira
	TRF_82	4	Not identified
	TRF_84	4	Not identified
	TRF_88	4	Not identified
	TRF_92	4	C. 3, Nitrosospira
	TRF_106	4	Not identified
	TRF_35	5	C. 3, Nitrosospira
	TRF_63	5	Not identified
	TRF_95	5	Not identified
	TRF_99	5	Not identified
	TRF_109	5	C. 3, Nitrosospira
	TRF_125	5	C. 3, Nitrosospira
	TRF_160	5	Not identified
	TRF_182	5	Not identified
	TRF_201	5	Not identified
	TRF_206	5	C. 3, Nitrosospira
	TRF_320	5	Not identified
	TRF_409	5	C. 4, Nitrosospira
	TRF_431	5	Not identified
	TRF_472	5	Not identified
	TRF_485	5	Not identified
	TRF_499	5	Not identified

Supplementary Table 5.2. Plant traits effects on microbial attributes from AOA and AOB microbes. The table present summary results for ANOVA and Pearson correlation analyses.

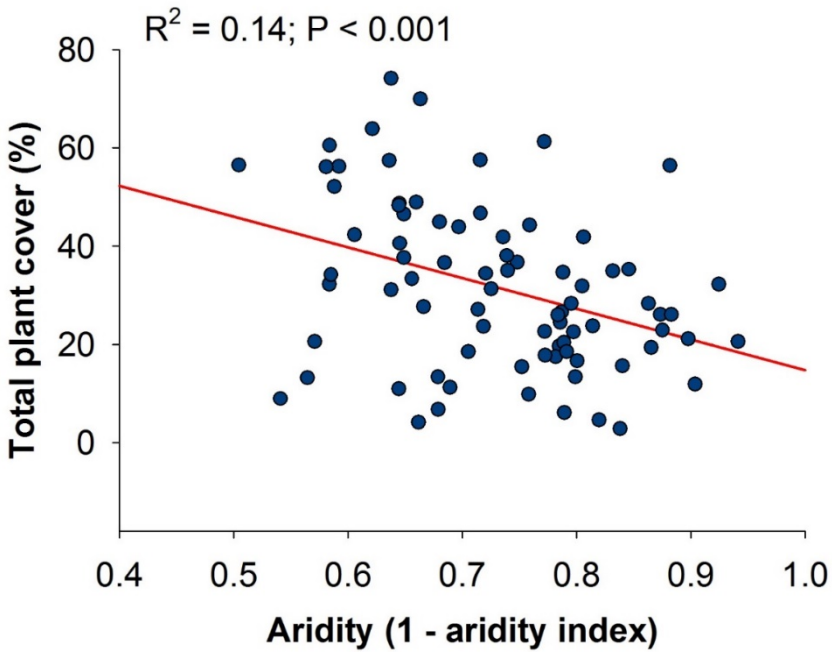
Plant trait	Group	Microbial attributes	F-value	P-value
Growth form	**AOA**	**Abundance**	3.518	0.02
		Richness	6.3	0.001
		Mod0	2.834	0.045
		Mod1	5.383	0.002
		Mod2	1.075	0.366
	AOB	**Abundance**	5.219	0.003
		Richness	2.469	0.07
		Mod3	1.508	0.221
		Mod4	2.051	0.115
			F-value	**P-value**
N fixer	**AOA**	**Abundance**	0.591	0.447
		Richness	0.04	0.843
		Mod0	1.055	0.311
		Mod1	0.034	0.855
		Mod2	0.609	0.44
	AOB	**Abundance**	1.808	0.187
		Richness	4.5	0.041
		Mod3	4.707	0.037
		Mod4	0.45	0.506
			F-value	**P-value**
Root type	**AOA**	**Abundance**	4.507	0.015
		Richness	7.167	0.002
		Mod0	4.092	0.021
		Mod1	5.163	0.008
		Mod2	1.519	0.226
	AOB	**Abundance**	5.832	0.005
		Richness	1.377	0.259
		Mod3	0.854	0.431
		Mod4	3.07	0.053
			Pearson r	**P-value**
Plant richness	**AOA**	**Abundance**	0.505	<0.001
		Richness	0.228	0.06
		Mod0	0.11	0.367
		Mod1	0.184	0.13
		Mod2	0.212	0.08
	AOB	**Abundance**	0.212	0.08
		Richness	-0.05	0.681
		Mod3	0.061	0.617
		Mod4	-0.203	0.094



Supplementary Figure 5.1. Relationship between the human influence index used there and that a similar index for N deposition (Dentener *et al.,* 2006) and N fertilization (Potter *et al.,* 2011).



Supplementary Figure 5.2. A priori SEM model evaluating the role of multiple environmental predictors in regulating the distribution of AOA and AOB across the global drylands.



Supplementary Figure 5.3. Relationship between aridity and plant cover in global drylands.

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