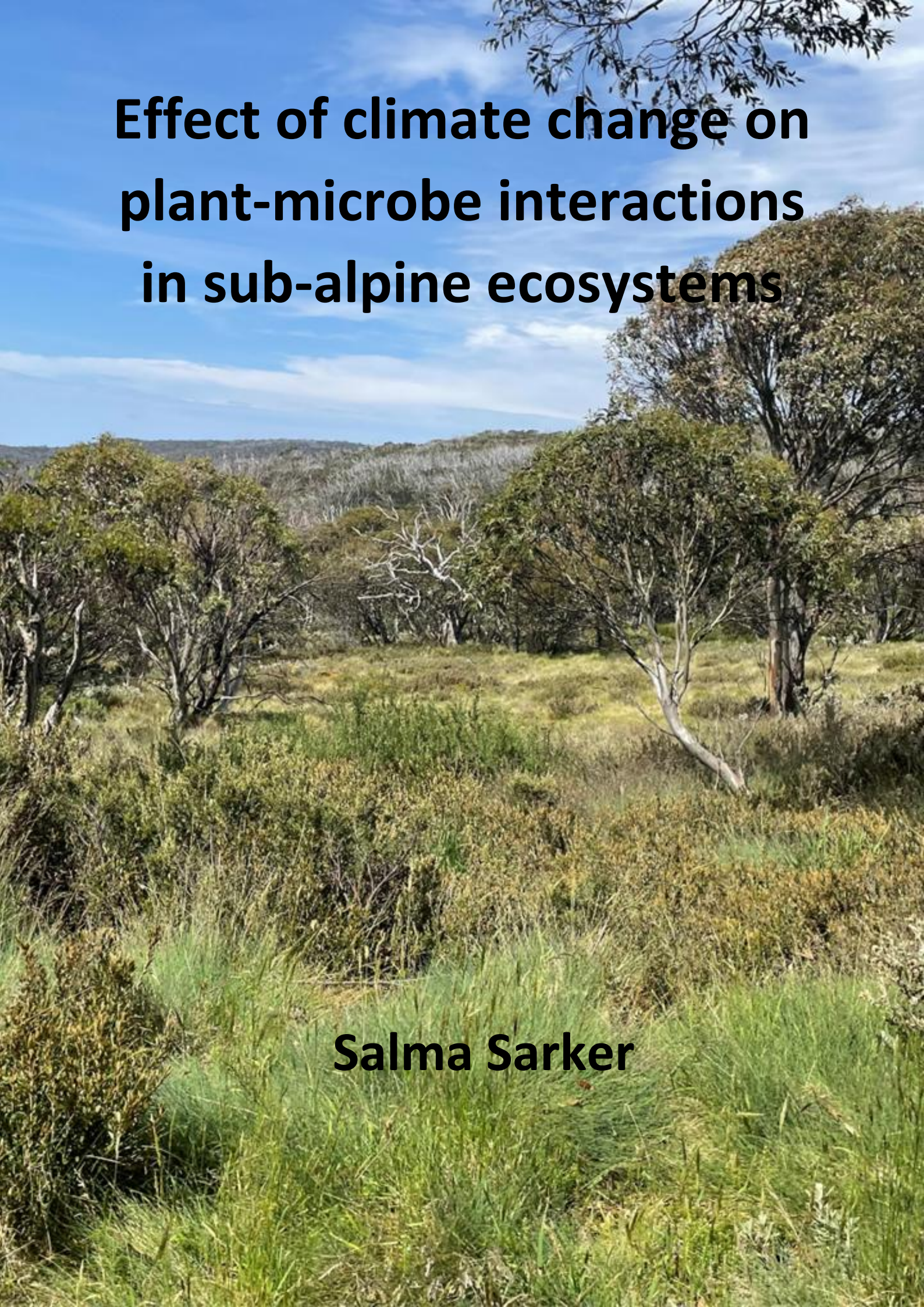
Effect of climate change on plant-microbe interactions in sub-alpine ecosystems



Salma Sarker



Australian National University

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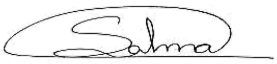
October 2026

A thesis submitted for the degree of Doctor of Philosophy of
The Australian National University

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Candidate's declaration

I declare that this thesis is my own original work, completed during my candidature at the Australian National University. It has not been submitted, in whole or in part, for the award of any other degree or diploma at any institution. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due acknowledgment has been made. All results presented in this thesis are derived from this research.



(Salma Sarker)

09 October 2026

Statement of Contribution

This thesis follows a traditional PhD structure. It begins with an introductory chapter that outlines the background, research objectives, and overall thesis structure. Each subsequent chapter contains its own materials and methods section, detailing the experimental design, laboratory procedures, sequencing workflows, bioinformatics processing, and statistical analyses relevant to that chapter. Methods are presented concisely within each chapter to minimise repetition while maintaining clarity. The thesis concludes with a combined discussion and conclusion chapter that synthesises the key findings, evaluates their significance, and outlines directions for future research.

Formatting, terminology, and units have been applied consistently across all chapters, and the thesis is written in Australian English throughout.

I was directly involved in all stages of the research, including literature review, development of research questions and hypotheses, experimental design, laboratory work, data processing, statistical analysis, and interpretation of results. Throughout the research process, I received guidance and support from my supervisors: Dr Celeste C. Linde (CCS), Dr Adrienne B. Nicotra (ABN), and Dr Justin O. Borevitz (JOB).

Acknowledgements

All praise and thanks are due to Almighty Allah, whose mercy, guidance, and blessings made this journey possible. During moments of uncertainty, He granted patience; during challenges, He provided strength; and during successes, He reminded me to remain grateful and humble.

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I acknowledge Research School of Biology for providing a welcoming and stimulating environment in which this research could flourish. My thanks extend to the academic, technical, and professional staff whose assistance, expertise, and support contributed to the successful completion of this work. In particular, I am deeply grateful to Niccy Aitken and Monica Ruibal for their assistance and expertise in the molecular laboratory, and to Elizabeth Broady from the Ecogenomics and Bioinformatics Lab (EBL) for her support and assistance. I am especially thankful to Niccy Aitken, who was always willing to offer practical solutions and guidance whenever challenges arose. Her support played an important role in the successful completion of this research. I am grateful to Teresa Neeman, Jonathan Rocco, and Jiajia Lee from the Biological Data Science Institute for sharing their knowledge and helping me develop my understanding of statistical analyses. I also thank the Plant Services team for their support with the greenhouse experiments, Penelope Burrell for her assistance with plant scanning and soil chemical analyses, Prof. Jeremy Bruhl for his advice on the germination of Carex seeds, and Dr Felipe Albornoz for sharing his expertise in microbial data analysis.

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long-term warming and drought experimental platform in the Australian sub-alpine ecosystem formed the foundation of several chapters of this thesis and enabled research that would not otherwise have been possible. I sincerely acknowledge the Australian Plant Phenomics Facility for awarding me the 2022 Postgraduate Internship Award and for providing access to their exceptional research facilities. The controlled growth capsules and 3D plant scanning platform played an important role in the greenhouse component of this research, enabling the collection of high-quality phenotypic data. I am grateful to the Ecological Society of Australia and the Holsworth Wildlife Research Endowment for awarding me a 2024 Holsworth Wildlife Research Grant. This funding partially supported the microbiome analyses associated with the transplant experiment conducted as part of this thesis. I also thank the Biomolecular Resource Facility (BRF), ANU, for sequencing support and the Australian National Botanic Gardens for providing seeds used in the greenhouse and transplant experiments.

To my lab mates, fellow students, and friends, thank you for making the journey far less lonely than it could have been. I am especially grateful to Sadia Ayyub for her willingness to share her knowledge and experience, to Dr Ryan O'Donnell for his thoughtful feedback on my thesis, and to Hosna Nisha for her companionship throughout this journey. A very special thanks goes to Dr Sharmin Akter for her unwavering support throughout this journey. Over the years, she became far more than a friend; she became a mentor, a sister, and a source of strength. I would also like to thank my mentor, Dr Pieter Arnold, for his guidance, support, and advice during the early stages of my PhD, which helped me navigate the challenges of settling into a new country and academic environment.

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My deepest appreciation belongs to my family. To my parents, thank you for the sacrifices you made, the values you instilled in me, and the unwavering belief you placed in my abilities long before I believed in them myself. Your prayers, encouragement, and love have been the foundation upon which every achievement in my life has been built. To my siblings, thank you for your constant support, understanding, and encouragement.

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A very special place in my heart belongs to my beloved child, Rifaya, who passed away at the age of six during my PhD journey. Losing her was the greatest sorrow of my life. Although she was with us for such a short time, her presence filled our lives with love and happiness and changed me forever. Carrying her memory gave me strength through some of the hardest moments of this journey. I pray that Almighty Allah grants her eternal peace and the highest place in Jannah. She will always be loved, always be remembered, and forever remain in my heart.

During this journey, I was also blessed with the arrival of my daughter, Numaira. Her presence brought happiness, hope, and light back into our lives. Her laughter, innocence, and love have been a source of comfort and strength, reminding me to keep moving forward even during difficult times.

This thesis may bear my name, but it is also a reflection of the many people whose generosity, support, and belief in me helped make its completion possible. I will always carry those contributions with gratitude and remember this journey not only for what I learned, but also for the remarkable people who shared it with me.

Abstract

Climate change is rapidly altering high-elevation ecosystems, where increases in temperature and shifts in rainfall patterns are expected to strongly influence both plant communities and the soil environments that support them. Microbial communities play a central role in these systems by regulating nutrient cycling, supporting plant growth, and maintaining ecosystem stability. However, how climate stressors especially multiple climate stressors interact to reshape these microbial communities and how such changes influence plant-microbe interactions remain poorly understood in this high-elevation ecosystem. My thesis investigates how warming and drought influence soil- and root-associated microbial communities in Australian sub-alpine grasslands, and how these changes affect plant performance and root colonisation. A set of field-based manipulations and controlled greenhouse experiments were used to examine microbial diversity, community structure, functional potential and plant-microbe interactions.

Across the thesis, combined warming and drought consistently imposed the strongest impacts on both soil- and root-associated microbial communities and plant performance, indicating that interacting climatic stressors amplify ecological disruption in sub-alpine ecosystems. Among individual stressors, warming generally exerted broader and more consistent negative effects than drought alone, particularly for fungal and arbuscular mycorrhizal fungal (AMF) communities. In contrast, drought effects were often more variable and appeared especially important for bacterial community restructuring. Moreover, prolonged climatic stress generally reduced symbiotic fungal groups while favouring saprotrophic, opportunistic, and stress-associated taxa across both soil- and root-associated communities. These shifts suggest a potential weakening of beneficial plant-microbe associations under climatic stress, which may increase plant vulnerability to environmental stress and disease pressure under future climate change.

Collectively, climate stress led to consistent but group-specific changes in soil microbial communities, with combined warming and drought producing the strongest effects. These changes extended beyond reductions in diversity to altered microbial interactions and functional potential, indicating that climate stress reshapes not only the composition of

microbial communities but also their ecological roles. However, plant responses to warming and drought in control growth chambers were highly species-dependent and links between root fungal community changes and plant performance were often weak, suggesting that above and belowground responses do not always change in parallel. Finally, a field-based transplant experiment demonstrated that climate stress and host species reshapes root-associated fungal communities during plant establishment by altering which taxa successfully colonise plant roots.

Overall, this thesis demonstrates that climate change reshapes soil- and root- microbiomes in group-specific ways, influencing not only microbial diversity and function but also the assembly of root-associated communities. These findings provide new insights into ecosystem responses to climate change and have important implications for predicting ecosystem resilience and informing restoration strategies in sub-alpine systems.

List of abbreviations

%IncMSE	Percent Increase in Mean Square Error
16s rRNA	16S ribosomal ribonucleic acid
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of Variance
ASV	Amplicon Sequence Variants
CI	Confidence Interval
CPM	Counts per million
DADA2	Divisive Amplicon Denoising Algorithm 2
dbRDA	Distance-Based Redundancy Analysis
DNA	Deoxyribonucleic acid
ECs	Enzyme Commission numbers
EMM	Estimated Marginal Mean
FDR	False Discovery Rate
FUNGuild	Functional Guild
gDNA	Genomic DNA
ITS	Internal Transcribed Spacer
LDA	Linear Discriminant Analysis
LEfSe	Linear discriminant analysis Effect Size
LMMs	Linear Mixed-effects Models
MID	Molecular Identifier
NMDS	Non-metric Multidimensional Scaling
NST	Normalized Stochasticity Ratio
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
PICRUSt2	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

SPEC–OCCU	Specialization-occupancy
TBE	Tris-borate-Ethylenediaminetetraacetic acid
TCA cycle	Tricarboxylic Acid Cycle

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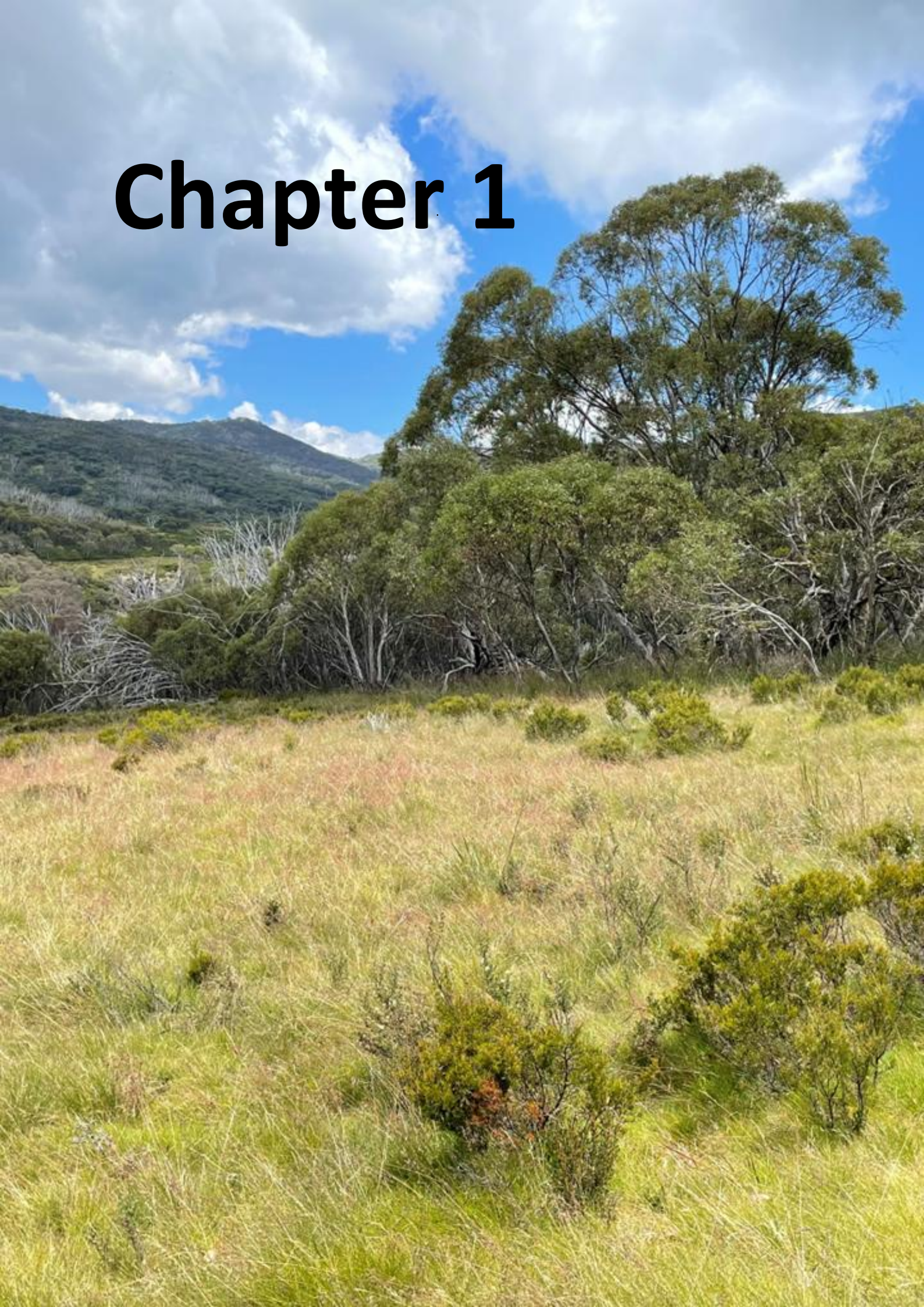
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Chapter 1



Chapter 1: Introduction

Global temperatures are rising, and climate change is already impacting the structure, composition, and functioning of ecosystems worldwide (IPBES, 2019; Pecl et al., 2017). This trend is being driven by greenhouse gas emissions reaching unprecedented levels (Soeder, 2025), resulting in increasing frequency and intensity of record-breaking climate extremes under ongoing climate warming (Fischer et al., 2025; Perkins-Kirkpatrick & Lewis, 2020; Trancoso et al., 2020). With limited progress in reducing emissions (Lamb et al., 2022; Minx et al., 2021), climate change is expected to worsen, leading to increasingly severe impacts on ecosystems (Jha & Dev, 2024).

Changes in the frequency and intensity of extreme climate events such as heatwaves and droughts are increasingly recognised as dominant drivers of ecosystem responses under climate change (Gu et al., 2025; Huang et al., 2025). Elevated temperatures can accelerate metabolic processes, increase evapotranspiration, and intensify water limitation in terrestrial ecosystems (Denissen et al., 2024; Zhang et al., 2024). Similarly, drought is a critical stressor affecting ecosystem structure and function, primarily through reductions in soil moisture availability and disruptions to plant productivity and belowground processes (Canarini et al., 2021; Dai, 2013). Drought conditions can alter microbial activity, nutrient cycling, and carbon dynamics, which impacts ecosystem stability and resilience (De Silva et al., 2025; Malik & Bouskill, 2022). Importantly, warming and drought often occur concurrently as compound climate extremes, where their combined effects can exceed the impacts of individual stressors (Dezetter et al., 2022; Krishnan et al., 2025). These compound events are increasing in frequency and intensity and are associated with amplified ecological consequences, including shifts in species composition, altered community interactions, and reduced ecosystem resilience (Allen et al., 2015; Moss et al., 2024; Müller et al., 2026; Zscheischler et al., 2018).

The effects of climatic stressors vary across ecosystems (von Buttlar et al., 2018), with some systems being particularly sensitive to changes in heat and moisture availability (Gritsch et al., 2015; Hu et al., 2023; Liu et al., 2025). Among various ecosystem types, alpine and sub-

alpine regions are widely recognised as especially vulnerable due to the sensitivity of endemic species adapted to cold and harsh environments (Hoegh-Guldberg et al., 2018; Rowland et al., 2026). Moreover, warming at high elevations often exceeds global averages and precipitation regimes are becoming increasingly variable (Adler et al., 2022; Gobiet et al., 2014; Grabherr et al., 2010; Hock et al., 2019; Ji et al., 2022; Seddon et al., 2016; Verrall & Pickering, 2020). Alpine ecosystems are predominantly distributed across the Northern Hemisphere (Testolin et al., 2020) where research efforts have been disproportionately focused (Hou et al., 2024; Verrall & Pickering, 2020).

The Australian Alps

In comparison to the Northern Hemisphere, Southern Hemisphere alpine regions remain relatively underexplored, despite their significant ecological and social value (Körner, 2003). The alpine landscapes of southeastern Australia, collectively known as the Australian Alps, span approximately 12,330 km² across New South Wales, Victoria, and the Australian Capital Territory (Figure 1.1). The Australian Alps represent a distinctive alpine bioregion characterised by relatively low elevations compared to major mountain systems such as the Himalayas and European Alps, with a maximum elevation of 2228 m at Mount Kosciuszko (Britannica Editors, 2025; NSW National Parks and Wildlife Service, 2003). The Australian Alps have a complex geological history spanning approximately 520 million years and represent an ancient, highly weathered mountain system shaped by prolonged erosion and tectonic processes (Australian Alps Liaison Committee, 2013; Williams et al., 2014). In contrast to younger, steep alpine landscapes, Australian alpine mountains are geologically older and more weathered, resulting in comparatively gentle slopes and deep, organic-rich soils (Costin, 1954). Climatically, the region is cool and moist relative to much of the Australian continent, but remains within a temperate zone, experiencing milder temperature extremes, shorter and less consistent snow cover, and relatively warm summers (Huber et al., 2011).

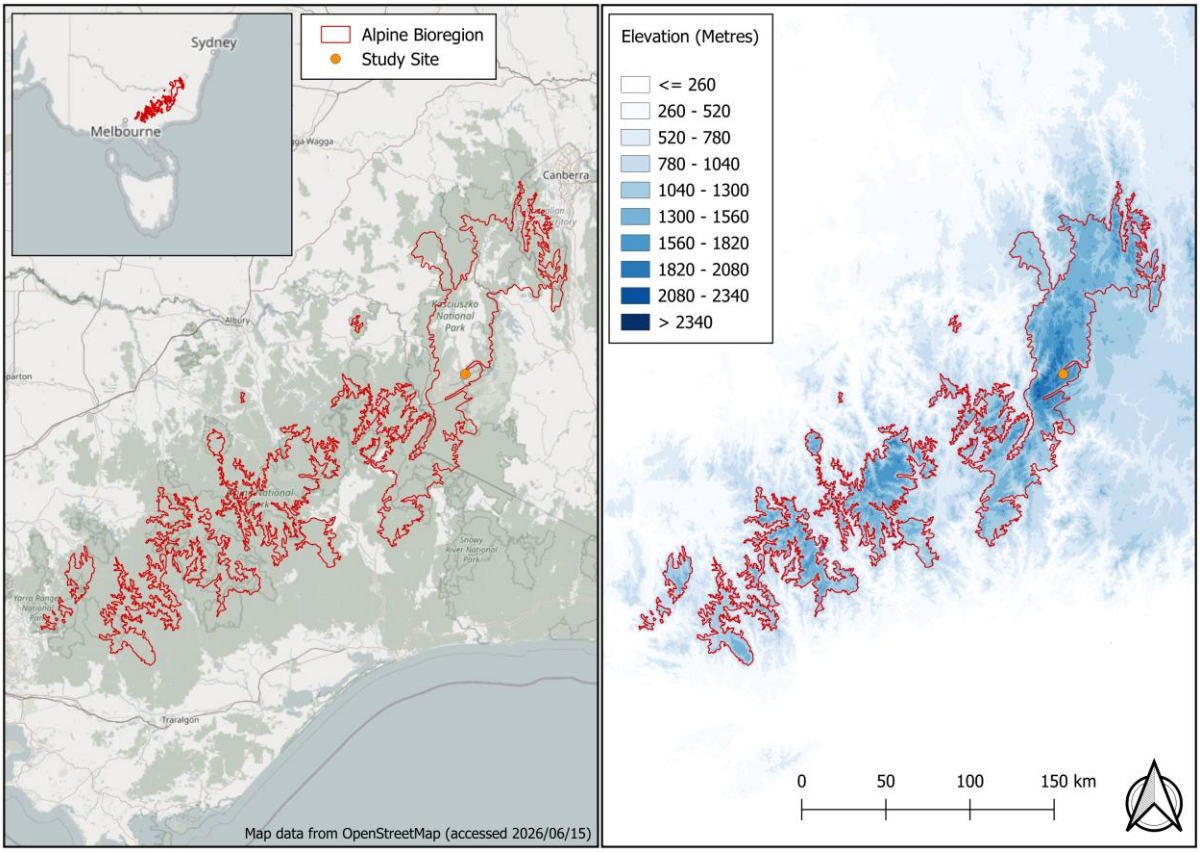


Figure 1.1: Map of Australian Alpine Bioregion and its elevation distribution. The left panel shows the distribution of the Australian Alpine Bioregion (red outlines) across south-eastern Australia. The inset map indicates the location of the Australian Alps within Australia. The right panel shows the elevation distribution of the Australian Alpine Bioregion. The study site location in Kosciuszko National Park, New South Wales indicated by the orange point.

The region is high in biodiversity and supports numerous endemic species, while also contributing to key ecosystem services such as carbon storage, water regulation, and catchment function within the Murray–Darling Basin (Ji et al., 2022; Kirkpatrick, 1994; Slatyer, 2010; Wilson et al., 2022; Worboys et al., 2011). Unlike many high-elevation regions globally, alpine areas in Australia are often vegetated above the treeline, with limited exposure of bare rock (Costin, 1955). Only about 5% of this region lies above the treeline and is classified as alpine, with the majority comprising sub-alpine environments (Figure 1.2) below the treeline (Bilish et al., 2018; Costin et al., 2000).



Figure 1.2: Australian sub-alpine landscape.

Vegetation in Australian alpine environment

The Australian alpine and sub-alpine regions support a diverse range of vegetation communities which, include grasslands, heathlands, bogs and fens, alpine herbfields (tall and short), fjaeldmark, sub-alpine woodlands, and eucalypt forests, with approximately 30% of plant species being endemic to the region (McDougall & Walsh, 2007). These vegetation types form a heterogeneous mosaic of structurally and floristically distinct formations, often occurring as sharply delineated patches across the landscape (Costin, 1954; Costin et al., 1979). Small-scale variation in topography can generate contrasting microhabitats over short distances, which may act as refuges for species under changing climatic conditions (Bliss, 1962; Scherrer & Körner, 2011). Together, these features make this region a valuable system for studying how ecological communities respond to environmental variability (Figure 1.3).



Figure 1.3: Australian sub-alpine grassland.

Climate change projections in the Australian sub-alpine

The Australian Alps are projected to be among the first alpine regions in the Southern Hemisphere to experience the impacts of climate change (Thompson, 2016). Temperatures in the region have already increased at an average rate of approximately 0.2°C per decade and are projected to rise by 4–5°C by 2100 (Harris et al., 2016). Climate projections further indicate declining precipitation and reductions of rainfall during summer and autumn, earlier snowmelt, and a decrease in seasonal snow cover (Green, 2010a; Harris et al., 2016; Hennessy et al., 2003; Hennessy et al., 2008; Pepler et al., 2015). Moreover, warming is predicted to occur more rapidly than in the Australian Alps compared to surrounding areas (Ji et al., 2022).

Consequently, climatic changes are expected to influence alpine biodiversity and key ecosystem functioning (Green, 2010b; Hughes, 2003; Steffen, 2009). Many alpine plant

species are shifting their distributions upslope in response to climate warming (Rixen et al., 2014; Stanisci et al., 2020); however, the limited elevation range of the Australian Alps restricts available habitat, increasing the risk of local extinction for some species (Auld et al., 2022; Rixen et al., 2022). This risk is further compounded by the upward encroachment of sub-alpine vegetation, which increases competition and reduces suitable alpine habitat (Hoffmann et al., 2019). Together, these changes highlight the need to better understand how biological communities respond to warming and changing moisture conditions in this system.

Vegetation changes in the Alpine ecosystem with climate change

Among all the abiotic stresses that plants are exposed to, warming and drought stress are the two most significant stressors that affect plant growth, development and yield quality (Iqbal et al., 2023; Prasad et al., 2008). In alpine and sub-alpine grasslands, drought generally reduces plant growth and can offset or reverse positive effects of moderate warming on productivity (De Boeck et al., 2018; Doležal et al., 2022; Zuo et al., 2023). Warming is also reported as a major stressor that extensively affects plant growth and development (Lee et al., 2020) and can cause shifts in plant phenology (Chen et al., 2017a; Cleland et al., 2012). However, studies also showed that the impact of warming varies among different species irrespective of the ecosystem (Carroll et al., 2021; Partzsch, 2019). For instance, a study in the Rocky Mountains, USA found that warming significantly increased the growth of montane tree species without reducing survivorship but had no effect on the growth of sub-alpine fir, which experienced complete mortality at higher temperatures (Carroll et al., 2021).

While experimental studies have demonstrated that combined warming and precipitation extremes can interactively influence plant responses (Bai et al., 2010; Zhu et al., 2016), broader syntheses indicate that their combined effects are often additive rather than strongly interactive (Song et al., 2019; Wilschut et al., 2022; Wu et al., 2011). Importantly, the magnitude and direction of these responses vary with plant growth form and experimental context, with herbaceous systems generally showing more negative responses than woody systems, and stronger negative effects are observed in greenhouse compared to field

conditions (Wilschut et al., 2022). Evidently, plant responses to climate change are highly context-dependent and remain difficult to predict across different ecosystems and experimental settings. This is particularly relevant in alpine and sub-alpine systems, where species are already near their environmental limits and may respond differently to combined climate stressors. Thus, there is a need to better understand how warming and altered moisture regimes influence plant performance and community dynamics under realistic field conditions.

Soil- and root-associated microbial communities

Soils represent one of the most biodiverse ecosystems on Earth, harbouring diverse microbial communities that play fundamental roles in nutrient cycling, soil formation, plant productivity, carbon dynamics and ecosystem functioning (Sayer et al., 2017; Schimel & Schaeffer, 2012; van der Heijden et al., 2008; Waldrop et al., 2017). While soil provides a large and diverse microbial pool, plants do not interact with this community randomly. Instead, they actively recruit and shape specific subsets of microorganisms in the root zone through processes such as root exudation and rhizodeposition, forming distinct root-associated microbiomes (Dini-Andreote, 2020; Stone et al., 2000). These interactions occur across the rhizosphere, rhizoplane, and endosphere, where microbial communities influence plant nutrient acquisition, growth, immune responses, and tolerance to environmental stress (Chukwuneme & Babalola, 2025; Oleńska et al., 2020; Pantigoso et al., 2022; Philippot et al., 2013).

Among soil microbial organisms, fungi and bacteria are major contributors to decomposition, collectively breaking down complex organic materials and contributing to soil carbon dynamics (Vivanco & Martiny, 2025). Moreover, fungi and bacteria also drive nutrient cycling, enhance soil structure, and support plant growth through symbiotic and growth promoting interactions (Bardgett & van der Putten, 2014; Zhang et al., 2022). One notable interaction is the partnership between plants and arbuscular mycorrhizal fungi (AMF). Approximately 80% of terrestrial plants form symbioses with AMF predominantly within the phylum Glomeromycota, facilitating nutrient uptake (particularly phosphorus) in exchange for carbon

(Parniske, 2008; Tedersoo et al., 2014; van der Heijden et al., 2008; van der Heijden et al., 2015).

Additionally, archaea represent a major (albeit historically understudied) component of soil microbial communities (Robinson, 2018; Spang et al., 2017). Archaea exhibit considerable metabolic diversity and play important roles in carbon and nitrogen cycling through processes such as methanogenesis, methane oxidation, and ammonia oxidation, influencing nutrient availability and greenhouse gas emissions (Offre et al., 2013). Among these functional groups, methanogenic archaea are particularly important because they are the only known biological producers of methane and play a key role in carbon transformation under anoxic conditions, thereby contributing to global carbon cycling (Aydin & Gurerk, 2025). Archaea are often regarded as tolerant to environmental stress (Matarredona et al., 2020; Stan-Lotter & Fendrihan, 2015), with some soil-relevant groups including ammonia-oxidizing and methanogenic archaea showing resistance to moisture fluctuations and desiccation (Chen et al., 2017b; Kimberly et al., 2012; Morozova & Wagner, 2007).

Historically, microbial communities were studied primarily using culture-based methods and microscopy (Hirsch et al., 2010). While these approaches provided valuable insights into microbial morphology, physiology, and biomass, they captured only a small fraction of naturally occurring microbial diversity because many microorganisms cannot be readily cultivated under laboratory conditions (Wydro, 2022). Consequently, early studies substantially underestimated the diversity and complexity of soil microbial communities (Lennon & Locey, 2016). The development of culture-independent molecular approaches, based on the analysis of nucleic acids (DNA and RNA) extracted directly from the soil are now used to assess the biodiversity of the soil environment, which speeds up research procedures (Tanveer et al., 2016; Verma et al., 2017). High-throughput sequencing has transformed microbial ecology by enabling the simultaneous characterization of thousands of microbial taxa directly from environmental samples. One widely used approach is amplicon sequencing, in which taxonomically informative marker regions are selectively amplified and sequenced to characterise different microbial groups (Porath-Krause et al., 2022). Commonly targeted markers include the internal transcribed spacer (ITS) region for fungi (Schoch et al., 2012), the

18S rRNA/SSU region for arbuscular mycorrhizal fungi (Hart et al., 2015), and regions of the 16S rRNA gene for bacteria and archaea (Pérez-Cobas et al., 2020). These marker regions differ in evolutionary rate, taxonomic coverage and resolution, and marker selection therefore depends on the microbial group and research objective (Hart et al., 2015; Porath-Krause et al., 2022). Consequently, amplicon-based sequencing has revealed previously hidden diversity and have greatly improved our understanding of the composition, assembly, and ecological responses of soil- and root-associated microbiomes to environmental change (Lennon & Locey, 2016).

Soils and microbial communities in Australian alpine and sub-alpine ecosystems

Soil microbial communities form highly complex networks of interacting microorganisms and represent a major reservoir of genetic and functional diversity (Johansen et al., 2025; Sveen et al., 2025), particularly in mountain ecosystems (Looby & Martin, 2020; Shen et al., 2016). Soil microbial communities are strongly shaped by environmental conditions, with factors such as temperature, moisture, soil pH, and organic matter acting as key filters that regulate community composition, abundance, and activity (Donhauser & Frey, 2018; Fierer & Jackson, 2006; Frindte et al., 2019; Lauber et al., 2008). Given their sensitivity to environmental conditions, soil microbial communities are likely to be highly vulnerable to ongoing climate change. Alterations in temperature and precipitation regimes may disrupt microbial composition and function, with potential consequences for ecosystem processes and plant performance.

Despite the harsh climatic conditions' characteristic of high-elevation environments, mountain soils can harbour considerable microbial diversity (Rime et al., 2015). In contrast to plants and animals, which typically decline in diversity with increasing altitude, microbial communities often exhibit greater resilience and persistence under cold and inhospitable conditions, resulting in distribution patterns that differ from those of macroorganisms (Fierer et al., 2011; Shen et al., 2014). However, soil microbial diversity and function (particularly in

the Australian Alps) remains relatively understudied (Wilson et al., 2022; Wunderlin et al., 2016).

Australian alpine landscapes characterized by distinctive alpine humus soils that differ from those described in many Northern Hemisphere alpine regions (Wood, 1970), which play a critical role in ecosystem functioning (NSW National Parks Wildlife Service, 2014). These humus soils are the most common and widespread type in the Australian Alps, typically found above 1500 m elevation. Unlike many alpine regions globally, much of these Australian alpine soils are relatively deep, reflecting prolonged weathering processes and sustained organic inputs on predominantly gentle slopes of an uplifted land surface (Costin, 1955; Costin et al., 1952). Soil organic content in Australian alpine region generally increases with elevation, resulting in conditions that more closely resemble temperate montane systems than the steep, rocky environments typical of many alpine regions worldwide (Donhauser & Frey, 2018; Wilson et al., 2022). These unique soil and climatic characteristics create distinct belowground environments that are likely to strongly influence soil microbial communities and their responses to climate change.

Plant microbes' interaction under climate change

As climate change can strongly influence plant-microbe interaction (Chakraborty et al., 2024; Classen et al., 2015; Compant et al., 2010), understanding how microorganisms interact with plants is critical for predicting ecosystem responses to climate change. Microbial community assembly is shaped by both host plant traits (Kivlin et al., 2022; Morella et al., 2020) and environmental conditions, particularly temperature and soil moisture (Classen et al., 2015; in 't Zandt et al., 2025), which act as key filters determining which microbial taxa successfully colonise plant roots (Lorberau et al., 2017; Trivedi et al., 2022; van der Putten et al., 2013). Furthermore, as plant responses to temperature shape the quantity and quality of organic inputs to the soil (e.g. litter and root-derived resources), shifts in plant communities can subsequently alter microbial biomass, diversity, and functional activity (Classen et al., 2015).

Microbial responses to temperature are highly variable among taxa due to differences in physiological traits such as temperature tolerance, moisture sensitivity, and growth rates

(Bardgett et al., 2008). Additionally, microbial communities exhibit clear temporal dynamics and seasonal patterns shaped by changes in soil temperature, moisture, nutrient availability, and plant phenology (Baldrian et al., 2013; Bardgett et al., 2005; in ’t Zandt et al., 2025). Seasonal variation is an important driver of soil- and root-associated microbial communities in alpine and sub-alpine ecosystems (Lazzaro et al., 2015; Shigyo et al., 2019; Zinger et al., 2009). Although detailed seasonal studies in the Australian Alps remain limited, these ecosystems experience strong seasonal variation in temperature, moisture, and growing-season length. Thus, seasonality is likely to play an important role in structuring soil- and root-associated microbial communities in this system.

Community ecology seeks to understand how species interactions and environmental conditions shape the structure and diversity of biological communities (Molles & Barker, 1999). In microbial systems, community composition is increasingly recognised to follow non-random biogeographical patterns influenced by environmental gradients and spatial processes (Graham & Knelman, 2023; Nemergut et al., 2013). Traditionally, these patterns have been explained through deterministic processes, such as environmental filtering and species interactions, which are driven by factors including temperature and moisture (Chesson, 2000; Fargione et al., 2003). However, stochastic processes, including dispersal limitation and ecological drift, are also now understood to play an important role in shaping microbial community assembly (Zhou & Ning, 2017). Understanding the balance between deterministic and stochastic processes can help predict which microbial taxa successfully colonise plant roots under changing environmental conditions, particularly under warming and drought.

Research gap

Evidently, there are several factors which impact microbial responses to environmental stimuli. Thus, predicting how microbial community structure and ecosystem processes will respond under changing climatic conditions is necessarily complex. Due to logistical constraints and the technical challenges associated with microbial research, the combined effects of warming and altered moisture on soil- and root microbial colonisation remain

poorly understood, particularly in alpine ecosystems. Moreover, climate manipulation studies in alpine and sub-alpine ecosystems have predominantly focused on plant responses (Alatalo & Little, 2014; Hollister et al., 2023; Hu et al., 2021). In contrast, soil and plant-associated microbial communities have received comparatively less attention (Donhauser & Frey, 2018; Hou et al., 2024). Consequently, our understanding of how soil- and root-associated microbial communities respond to climate warming and other interacting climate drivers in these systems is limited. Addressing these gaps is essential for improving predictions of ecosystem responses to climate change and for understanding the role of microbial communities in supporting plant resilience and ecological restoration in climate-sensitive sub-alpine systems.

Studies have often focussed on plant responses or individual microbial components under single climate drivers, such as warming or drought, often overlooking their combined effects (Ali et al., 2023). In addition, many studies have examined individual plant species, despite evidence that plant responses and associated microbial communities can vary substantially among species (Burns et al., 2015; Choi & Lee, 2024). Methodologically, many studies rely on in situ manipulation of existing field communities, which provide valuable ecological realism but can be influenced by environmental variability and site-specific constraints (Gurevitch & Collins, 1994; Morin, 1998). However, field-based transplant experiments provide a powerful complementary approach by introducing plants into manipulated environments, allowing direct assessment of which microbial taxa successfully colonise plant roots under altered climatic conditions (Nooten & Andrew, 2017). Studies integrating soil- and root-associated microbiome analyses across seasonal variation and multiple climatic stressors remain limited, particularly in sub-alpine ecosystems. To date, no study has examined how soil- and root-associated microbial communities in the Australian Alps respond to seasonal variation and the combined effects of warming and drought.

Focal plant species

In this thesis, I used a set of common and functionally distinct plant species (Figure 1.4) representative of Australian sub-alpine ecosystems, including *Poa costiniana*, *Celmisia pugioniformis*, *Celmisia costiniana*, *Carex capillacea*, and *Podolepis robusta* (McDougall &

Walsh, 2002). These species encompass contrasting growth forms (tussock grass, sedge and rosetted forbs) and ecological strategies, allowing comparison of plant-microbiome interactions across different host types under climate change (Andrew et al., 2021; Jaroszynska et al., 2024). Below is more information about each of these species.

Poa costiniana

Poa costiniana (Poaceae), commonly known as bog snow-grass, is a perennial tussock-forming grass (Figure 1.4a) that is widespread in sub-alpine and alpine grasslands of the Australian Alps (Costin et al., 1979; Wilson, 1993). It is a dominant graminoid that contributes substantially to primary productivity and ground cover in these ecosystems. As a cold-adapted C3 grass, *Poa* species are well known for their tolerance to low temperatures and variable moisture conditions. Owing to its ecological dominance and extensive root system, *P. costiniana* plays an important role in soil stabilisation and belowground carbon inputs, making it a key species for studying plant-soil-microbiome interactions under climate change (Griffin & Hoffmann, 2014; Warren & Taranto, 2011).

Celmisia costiniana

Celmisia costiniana (Asteraceae) is a perennial, rosette-forming alpine herb native to the Australian Alps (Figure 1.4b). It occurs in alpine and higher subalpine environments, where it is commonly found in tall alpine herbfields and often forms extensive carpet-like patches, contributing to community structure (DEECA, 2021). The species has a woody, rhizomatous base with persistent fibrous leaf remains and produces large, daisy-like inflorescences characteristic of the Asteraceae (Gray & Given, 1999).

Carex capillacea

Carex capillacea (Cyperaceae) is a small, perennial graminoid sedge (Figure 1.4c) occurring in montane to alpine environments (Orchard & Thompson, 1999). It is distributed across Australia and New Zealand, where it inhabits montane forest, scrub, and tussock grasslands (DNRET, 2026; Keith et al., 1999). The species forms a low-growing, matted habit with fine, narrow leaves and short rhizomes (NZPCN, 2026; Walsh & Entwisle, 1994). It is typically

associated with damp alpine and sub-alpine habitats, including wetlands and drainage lines and alpine swamps (Barrett, 2013; Harden, 1993). Although species of *Carex* are generally considered non-mycorrhizal or weakly mycorrhizal, Zhang et al., (2019) reported that *C. capillacea* can exhibit limited colonisation by arbuscular mycorrhizal fungi under specific environmental conditions, particularly in alpine settings, where colonisation levels may increase and interactions with AM fungi can influence plant performance. However, the study did not provide microscopic images to independently verify the reported AM fungal structures.

Celmisia pugioniformis

Celmisia pugioniformis (Asteraceae) is a perennial, woody-based rosette herb native to alpine and subalpine regions of southeastern Australia (Figure 1.4d). It occurs across a range of habitats, including herbfields, heaths, sphagnum bogs, and subalpine woodlands, and is characterised by narrow leaves with a dense woolly tomentum on the underside. The species may co-occur with *C. costiniana* and contribute to forb-dominated alpine communities (Gray & Given, 1999).

Podolepis robusta

Podolepis robusta (Asteraceae) is a perennial herbaceous forb (Figure 1.4e) native to alpine and subalpine regions of southeastern Australia (Frood, 2015). It forms an erect growth habit with multiple shoots arising from a basal rosette and a stout taproot (Jeanes, 1999). The species occurs predominantly in subalpine and alpine grasslands and open heathlands, typically above the treeline or in cold-air drainage hollows, at elevations of approximately 1200–1920 m (PlantNET, 2026). As a member of the Asteraceae, it is characterised by composite inflorescences and contributes to forb diversity within alpine plant communities. *Podolepis robusta* (Asteraceae) is very likely to form arbuscular mycorrhizal (AMF) associations based on genus- and family-level patterns, although species-specific confirmation is currently lacking (Brundrett, 2008).

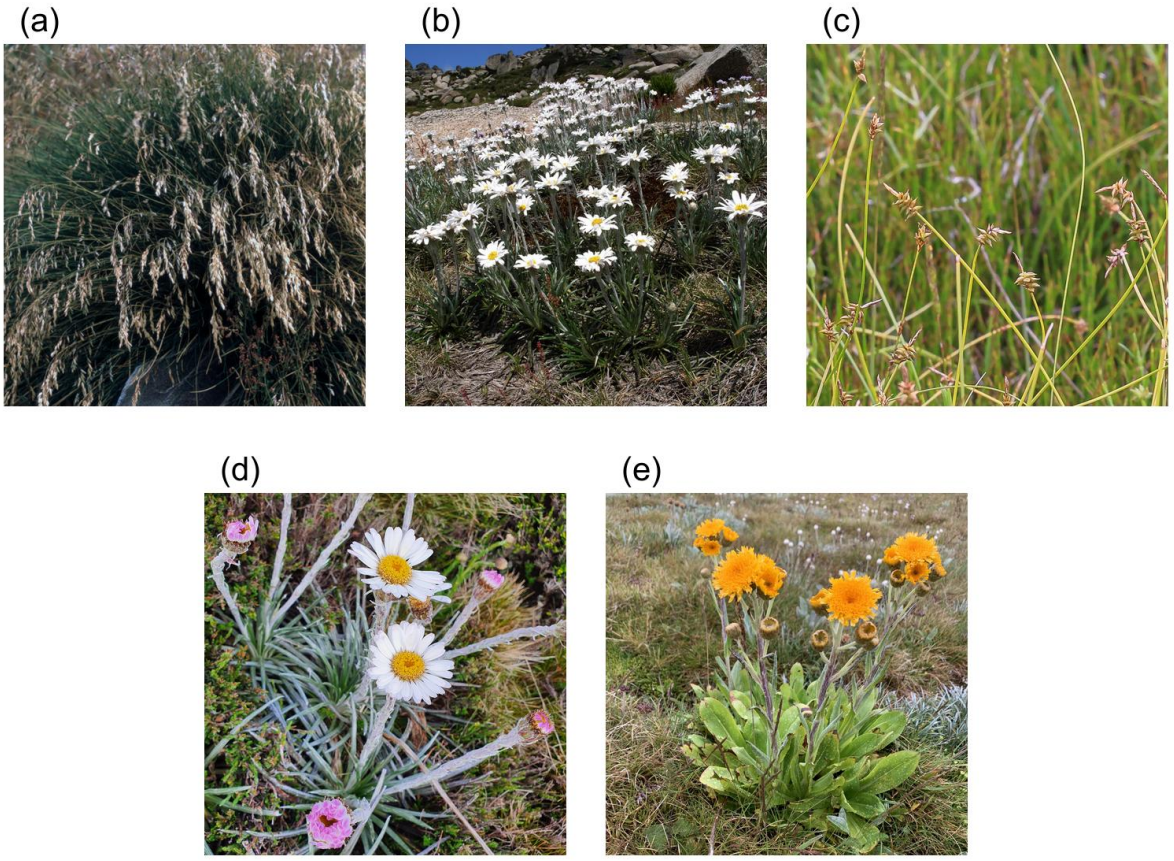


Figure 1.4: Representative images of study species: (a) *Poa costiniana*, (b) *Celmisia costiniana*, (c) *Carex capillacea*, (d) *Celmisia pugioniformis* and (e) *Podolepis robusta*. Images sourced from.

Note. (a) Photograph by Totterdell, C., 2026, APII (https://canbr.gov.au/photo/apii/id/a/11245). CC BY 3.0 AU; (b) Photograph by Fagg, M., 208, APII (https://www.inaturalist.org/photos/345624241). CC BY 4.0; (c) Photograph by Collier, P., 2026, iNaturalist (https://www.inaturalist.org/photos/609860554). CC BY-NC 4.0; (d) Photograph by Mikey, G., 2023, iNaturalist (https://www.inaturalist.org/photos/252840742). CC BY-NC 4.0; (e) Photograph by Medenis, A., 2022, iNaturalist (https://www.inaturalist.org/photos/182553028). CC BY-NC 4.0.

Research approach

This thesis aims to elucidate how climate change, particularly warming and drought, influences soil- and root-associated microbial communities and native sub-alpine plants in

Australian sub-alpine grasslands using an integrated, multifactorial experimental approach. Specifically, I address the following questions:

1) How do warming, drought, seasonality, and their interactions shape the diversity, composition, and functioning of soil microbial communities in Australian sub-alpine ecosystems?; and

2) How do climate stressors alter plant-microbe interactions and the assembly of root-associated microbial communities during plant establishment?

To interrogate these questions, this thesis adopts an integrated framework combining field-based experimental manipulation and controlled greenhouse experiments; to investigate how native sub-alpine plants, soil- and root-associated microbial communities respond to climate change in sub-alpine ecosystems.

In Chapters 2 and **3** I focus on the soil microbiome and I examine how soil microbial communities respond to climate change drivers. **Chapter 2** examines how soil microbial communities vary in diversity and structure under warming and drought across seasonal time scales, providing insights into temporal patterns of microbial community structure. Building on this, **Chapter 3** extends the analysis to a functional and interaction-based perspective, exploring how microbial interactions and potential functional shifts respond to climate treatments, with implications for broader ecosystem processes (Bardgett & van der Putten, 2014; Fierer, 2017).

In **Chapters 4** and **5**, I shift focus towards the root-associated microbiome. **Chapter 4** examines how warming and drought influence plant performance and associated root fungal and arbuscular mycorrhizal fungal (AMF) communities under controlled conditions, providing insight into the mechanisms underlying plant-microbe interactions. **Chapter 5** then extends this to natural field conditions, investigating how root-associated microbial communities assemble under climate change, with a particular focus on microbial colonisation under combined warming and drought. Together, these chapters provide an integrated understanding of microbial responses to climate change, connecting shifts in community structure and function with underlying mechanisms and colonisation processes. By linking

changes in microbial diversity, community structure, and functional interactions with plant performance and microbial colonisation processes, this research provides new insights into the mechanisms underpinning ecosystem responses to climate change. The findings contribute to advancing microbial ecological theory and provide a foundation for improving biodiversity conservation and ecological restoration strategies in climate-sensitive sub-alpine systems.

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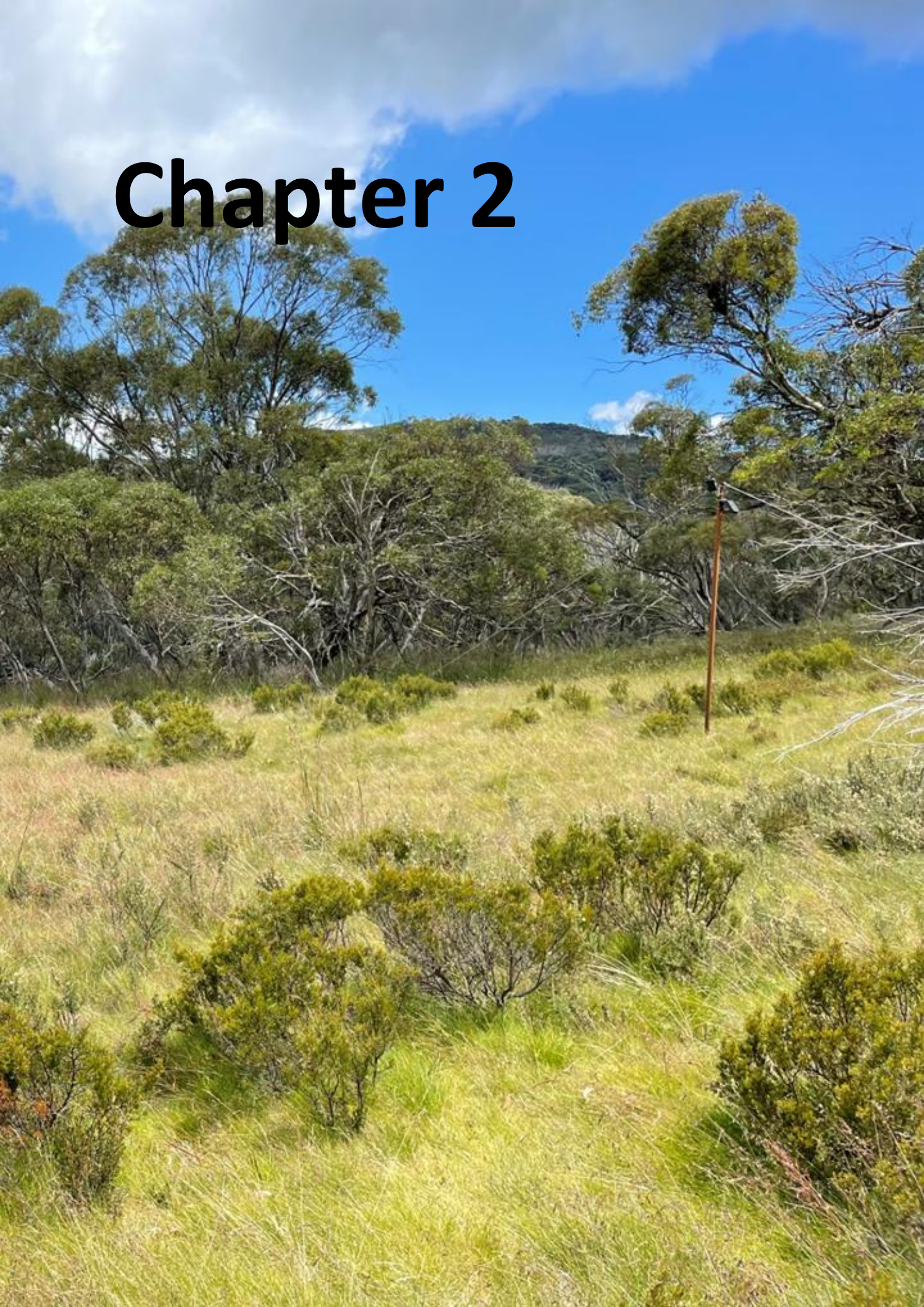
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Chapter 2



Chapter 2: Seasonal and climatic stress on soil microbial community in a sub-alpine grassland

Abstract

Soil microbial communities are central to ecosystem functioning, yet their responses to simultaneous climate drivers and seasonal variation remain poorly understood. This chapter examines how fungal, arbuscular mycorrhizal fungi (AMF), bacterial, and archaeal communities respond to experimental warming and drought across seasons in an Australian sub-alpine grassland. Specifically, we examine the relative impact of warming and drought and assess whether these effects differ among microbial groups. We predicted that microbial diversity and community structure would differ among microbial groups. The field experiment included a continuous +4°C warming above ambient temperature, an 80% rainfall exclusion, and their combined application. We used Illumina high-throughput amplicon sequencing to profile community diversity and structure across treatments and seasons and subsequently assessed changes in alpha and beta diversity to evaluate seasonal dynamics, treatment effects, and key environmental drivers. Warming consistently reduced apparent richness across all microbial groups, underscoring their sensitivity to elevated temperatures. Drought effects were pronounced for bacteria and AMF but weak for fungi and archaea, indicating group-specific differences in moisture sensitivity. AMF exhibited patterns similar to bacteria, perhaps reflecting their dependence on plant carbon under moisture stress. Seasonal patterns strongly modulated these responses: fungal and archaeal richness peaked in summer, whereas bacterial richness increased progressively over time, suggesting acclimation to repeated fluctuations. Random Forest models identified soil temperature and moisture as dominant predictors of microbial diversity, while rainfall events explained comparatively little variation. These findings reveal that seasonality and climate manipulations interact to shape belowground communities in sub-alpine grasslands, with microbial groups differing in both the magnitude and timing of their responses. This group-specific sensitivity has important implications for predicting ecosystem resilience under

future climate change and raises important questions about how differences in microbial selection and evolutionary potential may cascade to affect key soil processes such as carbon cycling, nutrient turnover, and plant-microbe interactions.

Introduction

Climate change is no longer a distant threat but a current global reality. Out of approximately eight million species, nearly one million face extinction due to global warming (Martin, 2019), with profound consequences for biodiversity and ecosystem functioning (Hooper et al., 2012). Among the many facets of biodiversity, soil microbial communities constitute the largest reservoir of microbial diversity on Earth, harbouring more than half of the planet's total biodiversity (Anthony et al., 2023). These microbial communities are essential for key ecosystem processes such as nutrient cycling, decomposition, and mineralisation (Bakker et al., 2019). Microbial community structure is shaped by environmental factors including moisture, temperature, and nutrient availability, and is frequently used as an indicator of soil quality. Since microbial community structure is strongly regulated by environmental conditions such as moisture, temperature, and nutrient availability, climate-induced shifts in these factors can serve as sensitive indicators of soil quality and ecosystem health (Petersen et al., 2002).

Grasslands, which cover about 40–50% of Earth's terrestrial land and harbour high biodiversity (Petermann & Buzhdygan, 2021; Wilsey, 2018), are threatened by climate change, particularly through altered rainfall and temperature regimes (Straffelini et al., 2024). Alpine grasslands are especially vulnerable, where even small climatic shifts can destabilise ecological dynamics (Du et al., 2022; Li et al., 2020). In the Australian Alps, mean annual temperatures are projected to rise by 4–5°C by the end of the century, accompanied by reductions in winter precipitation and increases in summer rainfall (Harris et al., 2016). Such changes are expected to alter microbial diversity and community composition, with potential cascading effects on ecosystem functioning (Donhauser & Frey, 2018).

The impacts of climate change on microbial communities in an experimental setup have been varied. Several short- and long-term manipulations have documented pronounced shifts in microbial community structure under warming or precipitation changes (Horz et al., 2004; Xiong et al., 2014; Zhang et al., 2014). Other experiments showed little to no effect even after years of warming and altered precipitation treatments (Cruz-Martínez et al., 2009; T. Weedon et al., 2012; Williams, 2007). Similarly, studies from temperate ecosystems have reported

contrasting outcomes, where warming combined with natural drought suppressed microbial populations due to reduced soil moisture (Sheik et al., 2011), whereas a global meta-analysis found that warming generally increased microbial abundance, with the strongest responses observed in colder ecosystems such as tundra and histosols (Chen et al., 2015). Precipitation has been proposed as a key factor that can modulate the influence of warming on community composition (Zhang et al., 2016). A meta-analysis of 85 experimental studies showed that interactive effects of warming and precipitation changes were generally weaker than expected from the additive effects of each factor applied individually, suggesting that their effects can be non-additive (Wu et al., 2011). Another meta-analysis with 75 experimental studies showed that warming and drought reduce the abundance and activity of fungi and bacteria (Blankinship et al., 2011). As in other studies indicate that microbial responses depend on ecosystem sensitivity and the duration of experimental manipulation (Allison & Martiny, 2008; DeAngelis et al., 2015; Xue et al., 2016). Besides, soil microbes differ markedly in physiology, ecological roles, and sensitivity to environmental change; thus, treating them as a single group overlooks key functional distinctions. Overall, drought and warming strongly shape soil microbiomes, yet research has focused mainly on aboveground plant responses (Wilson et al., 2022). Greater understanding of how belowground microbial communities respond to altered temperature, rainfall, and seasonal dynamics can improve predictions of ecosystem responses to climate change.

Seasonal variation is a major driver of soil microbial diversity and community structure, especially in alpine and sub-alpine ecosystems where temperature and moisture fluctuate strongly across the year (Ernakovich et al., 2014; Gavazov et al., 2017; Ji et al., 2021; Lazzaro et al., 2015). Snowpack, freeze–thaw cycles, and precipitation shifts shape soil thermal and moisture regimes, plant phenology, and nutrient availability, thereby influencing belowground communities (Groffman et al., 2011; Sharma et al., 2006). Cold-adapted microbes persist beneath the snowpack, and distinct community transitions occur during snow accumulation, melt, and the growing season (Broadbent et al., 2021; Libois et al., 2013; Lipson et al., 2002; Lipson et al., 1999). Furthermore, snowmelt often triggers nutrient flushes that stimulate microbial activity and seasonal turnover (Battin et al., 2004). Therefore, seasonality frequently exceeds other environmental factors in structuring bacterial and

archaeal communities (Rasche et al., 2011), though responses differ among ecosystems: prokaryotes may show stronger seasonal shifts than fungi (Han et al., 2021), while arbuscular mycorrhizal fungi (AMF) communities sometimes remain stable across seasons (Rosendahl & Stukenbrock, 2004; Santos-González et al., 2007). Overall, despite evidence of pronounced seasonal reorganization, the dynamics of microbial communities in sub-alpine grasslands remain poorly characterized.

When temperature and moisture conditions shift, numerous other environmental measures also change, either directly or through microbiome-mediated feedback. These co-varying shifts alter soil properties, vegetation dynamics, and ecosystem boundaries, thereby redefining the plant growth environment and the substrates available to microbes (Bradford et al., 2019; Fei et al., 2017; Maestre et al., 2010). Such climatic fluctuations also reshape plant architecture and species composition (Elliott, 2012; Qiu et al., 2021), influencing biotic interactions, fire regimes, and disease dynamics (Liu & Wimberly, 2016; Raffel et al., 2015). Together, these microenvironmental alterations provide feedback to soil microbial community diversity and structure.

Here, we address this knowledge gap by examining how soil microbial communities in a sub-alpine grassland respond to experimental warming and drought over multiple seasons. We hypothesize that overall, microbial diversity and richness will decrease with the elevation of temperature and decrease in rainfall. Furthermore, we hypothesize that the microbiome community structure will be affected by warming and drought, favouring a subset/different set of microbiomes which possess the ability to use the resources efficiently and survive under stress conditions. If so, then different microbial groups will vary in their sensitivity to warming and drought. We hypothesized that while background seasonal dynamics will exert a strong influence on soil microbial diversity and community structure, these will be distinct from the treatment effects and still show differences among the groups. Specifically, we expected higher microbial diversity and greater structural differentiation among communities during the summer, when temperature and biological activity peak, compared to autumn, when environmental conditions become cooler and less favourable for microbial proliferation. Therefore, our aims are: (i) to quantify seasonal and treatment-driven changes in microbial diversity and community structure; with higher diversity expected during warm, moist periods

(summer) and reduced diversity under warming and drought conditions; (ii) to evaluate whether different microbial groups show different sensitivities to warming and drought; and (iii) to identify which of the measured environmental factors: soil thermal extremes (daily maximum and minimum temperature), soil moisture extremes (daily maximum and minimum volumetric water content, VWC), and rainfall regime (total rainfall and rainfall-event frequency), best explain microbial diversity patterns under induced climate change conditions.

Materials and methods

Study site

An in-field (Brown et al., 2024) altered temperature and moisture study was conducted in a sub-alpine meadow, elevation is 1598–1610 m at Kosciuszko National Park in NSW (-36.372° 148.431°; Figure 1.1) as part of the Australian Mountain Research Facility (AMRF; https://www.amrf.org.au/amrf-sites). The regional climate is characterized by a mean maximum air temperature of 11.0°C and a mean minimum of 1.4°C between 2010 and 2024. Mean annual precipitation of 1429.3 mm for 2020-2024 most of which falls as snow in winter and early spring (WillyWeather, 2025). The growing season normally starts at mid-October and ends at mid-May. The overstory vegetation is characterized by Eucalyptus woodland with open grassy areas containing low-growing shrubs, herbs, and grasses including *Poa*, *Carex*, and *Podolepis*, which form a range of structurally and floristically distinct communities (Johnston & Ryan, 2000; Scherrer & Pickering, 2006). The soils are predominantly acidic organo-mineral alpine humus soils (Costin, 1954), notable for their deep loamy texture, a feature uncommon in alpine regions globally (Scherrer, 2004). The surface horizons of these alpine humus soils are heavily influenced by aeolian dust accumulation, while subsurface horizons derive mainly from natural bedrock weathering (Johnston, 2001).

Experimental design

For warming treatments, soil temperature was increased by installing seven vertical heating rods to a depth of 80 cm which are connected with a circular heating cables 50 cm below the

soil surface at a 1 m radius from the plot center (Figure A. 1a). This setup achieved an approximate 4.0°C increase in soil temperature across the 10–100 cm profile. The +4 °C warming treatment was selected to represent projected late-century warming in the Australian Alps, where climate models predict an approximately 4 °C increase in mean temperature by the end of the 21st century (Harris et al., 2016; Farkas et al., 2025). Transparent polycarbonate fencing enclosed each warmed plot to help retain the elevated temperatures. Ambient (control) plots were enclosed with iron mesh fencing similar to the drought plots to maintain consistent structural conditions and protect against wildlife intrusion. To assess microclimatic variation that is soil temperature (°C) and volumetric water content (VWC; m^3/m^3) across experimental treatments, soil probes (CS655 Campbell Scientific) and temperature probes (CS107; Campbell Scientific) were installed at a depth of 10 cm and 30 cm respectively. Environmental data were collected from the FutureClim (Brown et al., 2024) sub-alpine field site in Kosciuszko National Park, NSW, Australia, and were recorded at 5 minutes intervals using Onset data loggers (Campbell Scientific, Logan, Utah, USA). The data loggers recorded at 5 minutes increments from November 2022 to June 2024 (CR1000X; Campbell Scientific). Drought conditions were simulated using rainout shelters designed to exclude approximately 80% of incident precipitation. This treatment was intended to simulate severe future drought conditions and reduce soil water availability, rather than represent an 80% projected decline in annual precipitation or soil moisture. Plots assigned to the combined warming and drought treatment were equipped with both heating and rainout systems to apply simultaneous stressors.

Each treatment plot within five replicate blocks was equipped with an individual sensor system, allowing continuous monitoring of local soil conditions over time. The experiment used a randomized complete block design with five spatial blocks. Each block contained four 3 × 3 m plots that were randomly assigned to a full factorial combination of two temperature levels (ambient, elevated) and two moisture regimes (non-droughted, droughted). The four treatments were: (i) ambient + non-droughted (control), (ii) elevated + non-droughted (warming), (iii) ambient + droughted (drought), and (iv) elevated + droughted (warming and drought). This yielded 20 plots in total (4 per block), with randomization within blocks to control for spatial heterogeneity.

To minimize edge effects and ensure consistency across treatments, a 50 cm buffer zone was maintained around each plot, resulting in a usable internal sampling area of 2 × 2 m (Figure A. 1a, b). Each 2 × 2 m plot was further subdivided into two equal parts with one left intact to monitor change and the other designated for soil sampling and transplant experiments. Within the designated area, a 60 × 120 cm rectangular zone near the centre of the 2 × 2 plot positioned was demarcated for consistent soil sample collection across treatments (Figure A. 1a, b). This minimized variability caused by micro-site disturbance within the plot. Treatments were randomly assigned within blocks to account for spatial heterogeneity at the field site, including natural gradients such as microtopography and background plant community composition. This blocking approach helps isolate treatment effects from environmental variability.

Soil sample collection

Soil samples were collected at three time points (spring, summer and autumn) over each of two Austral growing seasons (2022-2023 and 2023-2024). Thus, in each season we sampled in November, February and May where the spring sample in 2022 was a baseline, prior to treatment implementation), Summer 2023 (February 2023) and Autumn 2023 (May 2023) belongs to season 1. Whereas season 2 includes Spring 2023 (November 2023), Summer 2024 (February 2024) and Autumn 2024 (May 2024). At each sampling event, three soil cores (to 15 cm depth) were collected from every experimental plot, yielding a total of 60 samples per time point (5 blocks × 4 treatments × 3 replicates) and 360 soil samples across the six time points. Each core was divided into two parts, the upper 0-3 cm part was retained for chemical analysis, while the 4 -15 cm section was used for microbial analysis. The uppermost layer was excluded from microbial analysis because it contained a higher proportion of surface organic material and litter, whereas the 4–15 cm section provided a more consistent mineral-soil matrix for microbial community characterisation. These two soil sections were collected into two different 50 ml falcon tubes. Following collection, soil samples were transported to the laboratory in a freezer (−5°C) and stored at −80°C until further analysis. Prior to DNA extraction, all samples were freeze-dried and sieved through a 2 mm mesh to remove plant

roots and debris, ensuring consistency for downstream microbial and physicochemical assessments.

DNA extraction and sequencing

Genomic DNA extraction from soil samples was conducted using the Qiagen DNeasy PowerSoil kit (Qiagen, Carlsbad, USA), which was employed per the manufacturer's protocol. Following extraction, DNA quantification was conducted by using a Qubit dsDNA quantification assay kit (Thermo Fisher) and normalisation was performed by maintaining the DNA concentrations at approximately 5 ng per microliter. For each sample, 12-15 ng DNA were used to amplify the ITS1, 16S rRNA and 18S rRNA, and 16S r RNA genomic regions for fungi, bacteria, AMF and archaea, respectively (Table A. 1 for primers used). Forward primers were modified on the 5’ end to contain a unique 8 bp molecular identifier (MID) and the Illumina adapter for compatibility with the i5 Nextera XT indexing primers. Reverse primers were modified on the 5’ end to contain the Illumina overhang adapter for compatibility with the i7 Nextera XT indexing primers. The PCR was performed on 2.5 µL of DNA with a 20 µL reaction volume (Table A. 2). PCR amplification was carried out using a touchdown thermocycling protocol. Reactions were initiated with an initial denaturation at 98°C for 3 minutes, followed by cycles consisting of 98°C for 30 seconds, 98°C for 15 seconds, and annealing at 69°C for 40 seconds, with the annealing temperature reduced by 3°C every second cycle until a final annealing temperature of 49 °C was reached. This was followed by an extension at 72°C for 70 seconds per cycle. A final extension step was performed at 72 °C for 10 minutes, after which samples were held at 10°C. To ensure that all samples successfully amplified, PCR samples were electrophoresed on a 2% TBE (Tris-borate-EDTA) agarose gel. DNA samples of unsuccessfully amplified samples were cleaned using Sera-Mag bead cleanup and re-amplified to obtain successful PCR products. The concentration of all positive amplicons was measured using a fluorescence machine by Fluorometry. Amplicon pooling was done by the Janson robot so that the concentration of each sample is equimolar. After pooling, the positive amplicons were purified using 2% suspension of sera-Mag Speed-beads Carboxyl Magnetic Beads, added in a ratio of 1:1 vol PCR product and resuspended in 40 µL water. For soil microbiome analysis we have pooled all four loci (fungal, bacterial, archaeal

and arbuscular mycorrhizae) together. The pool was diluted to 5 nM and molarity was confirmed using a Qubit High Sensitivity dsDNA assay (Thermo Fisher). Sequencing was carried out with 2 × 300-bp paired-end reads using the v3 chemistry on the Illumina MiSeq platform at the Biomolecular Resource Facility (BRF) in the Australian National University.

Soil chemical properties

Total carbon and total nitrogen were determined by dry combustion of ≈ 300 mg of samples using a TC/TN analyser calibrated against glycine standards (0–80 mg), with calibration curves serving as quality control, following the method of (Nelson & Sommers, 1996). Available phosphorus (Colwell-P) was measured by bicarbonate extraction (0.5 M $NaHCO_3$, pH 8.5; 1:100, 16 h) and quantified colorimetrically using the molybdenum-blue method, following standard methods (Colwell, 1963; Rayment & Lyons, 2010).

Soil chemical properties (total carbon, total nitrogen, and available phosphorus) were measured from pre-treatment (Spring 2022) and post-treatment (Autumn 2024) samples to assess potential background shifts in nutrient pools over the experimental duration. These analyses provided contextual information on the overall trajectory of soil nutrient dynamics during the 18-month experimental period. In general, total carbon showed an increasing trend while total nitrogen tended to decline between these two time points, consistent with gradual changes in soil organic matter turnover under field conditions (Table A. 3, Figure A. 2). The main effects of temperature and moisture were not significant, though some time-by-treatment interactions indicated that moisture manipulation may have contributed to modest increases in total carbon and available phosphorus in drought plots.

Bioinformatics

Amplicon sequences of fungal internal transcribed spacer (ITS), bacterial and archaeal 16S ribosomal RNA (16S rRNA) and arbuscular mycorrhizal fungal 18S ribosomal RNA (18S r RNA) were processed using the DADA2 v1.28.0 (Callahan et al., 2016) in R (Team, 2023). Demultiplexed paired-end reads trimmed using Cutadapt (Martin, 2011) prior to DADA2 processing. Reads containing ambiguous bases (Ns) or with lengths below 100 bp were removed. Reads were dereplicated, denoised, and merged with a minimum overlap of 15 bp

and up to 3 mismatches to allow longer amplicons to have substantial overlap. Amplicon sequence variants (ASVs) were constructed, screened for target length, and filtered to remove chimeras. Final ASVs were assigned unique identifiers and exported in FASTA format. ASVs were subsequently clustered into operational taxonomic units (OTUs) at 97% sequence identity using the VSEARCH de novo clustering algorithm implemented in QIIME2 v2024.2. Taxonomic classification of fungal ITS and bacterial/archaeal 16S OTU representative sequences was performed using QIIME2's VSEARCH consensus taxonomy classifier (classify-consensus-vsearch). Fungal sequences were classified against the UNITE v10.0 reference database (04.04.2024 release Abarenkov et al., 2024; Nilsson et al., 2019), while bacterial and archaeal sequences were classified against SILVA v138.2 (Quast et al., 2013), using a minimum sequence identity threshold of 90%. For AMF, taxonomy was assigned to OTU representative sequences against the MaarjAM 18S rDNA QIIME reference database (2021 release; Öpik et al., 2010) using a Naive Bayes classifier trained with fit-classifier-naive-bayes and applied using QIIME2's classify-sklearn method.

Following taxonomic assignment, non-target sequences, including mitochondrial and chloroplast sequences, were removed from the dataset and not further analysed. OTU tables were rigorously filtered to minimize sequencing noise and increase data reliability. OTUs that were present in only a single sample across the entire dataset were first removed, as these likely represent sample-specific contaminants or spurious detections. As expected, this step also eliminated all singleton OTUs, defined as those observed only once globally—which was subsequently verified. To further reduce the impact of low-confidence features, OTUs that occurred only twice (doubletons) or three times (triplets) across all samples were also removed. The final OTU tables, along with taxonomic annotations, phylogenetic trees, representative sequences, and sample metadata, were incorporated into phyloseq objects using Phyloseq R package v1.52.0 (McMurdie & Holmes, 2013) for downstream ecological, taxonomic, and statistical analysis using R.

Statistical analysis

To account for stochastic variation during subsampling, empirical rarefaction was performed for each amplicon dataset (fungi, AMF, bacteria, and archaea) following Schloss (2024). Each

dataset was repeatedly rarefied 1,000 times using the rarefy_even_depth() function in the phyloseq R package. Rarefaction thresholds were chosen to balance the retention of microbial diversity with adequate biological replication across treatments: 11,695 reads for fungi, 37,000 for AMF, 10,212 for bacteria, and 6,400 for archaea. A fixed random seed ensured reproducibility (Figure A. 3). After rarefaction, 11 fungal, 2 AMF, 22 bacterial, and 17 archaeal samples were excluded due to insufficient sequencing depth. Data from Spring 2022 (November 2022) were excluded from community structure and diversity analyses due to missing soil temperature and moisture data. For each iteration, Bray–Curtis dissimilarities and alpha diversity metrics were calculated using the vegan package (Oksanen et al., 2025). Distance matrices and alpha diversity values from the 1,000 rarefied iterations were averaged for each sample to obtain final estimates. These final estimates were merged with sample metadata for downstream statistical analyses and visualisation.

Microbial community structure for all groups was evaluated using distance-based redundancy analysis (dbRDA). For each group, the averaged distance matrix was modelled with temperature, moisture, and sampling time (season) and their interaction as fixed effects, while controlling for block as a covariate. Statistical significance was assessed using permutation tests with a restricted design, constraining permutations within blocks and plots nested within blocks (Anderson & Braak, 2003). Pairwise dbRDA tests comparing treatments within sampling time points were conducted, and P-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate (FDR) adjustment (Benjamini & Hochberg, 1995). Ordination plots were visualized with 95% confidence ellipses around treatment centroids.

Alpha diversity metrics were analysed, including Observed richness, Shannon diversity, and Simpson diversity. Observed richness was defined as the number of non-zero taxa per sample. Differences in alpha diversity metrics among treatments and seasons were assessed using linear mixed-effects models. The Shannon and Simpson diversity indices were expressed as Hill numbers (Hill, 1973), representing the effective number of common and dominant species, respectively. Diversity values were modelled as the response variable, with temperature, moisture, and sampling time as fixed effects, and block and plot as random intercepts to account for spatial variability. Model residuals were examined using the

resid_panel() diagnostic function to assess the assumptions of normality and homoscedasticity, which were found to be satisfactorily met. The significance of fixed effects was evaluated using Type II analysis of variance, and post hoc pairwise comparisons were conducted with P-values adjusted for multiple testing. Results are reported with 95% confidence intervals. Because the Shannon and Simpson diversity indexes showed similar patterns, we opted to use the Hill Shannon index, hereafter referred to as species diversity.

Sensor recordings of soil temperature and moisture spanned from December 2022 to June 2024, encompassing five major sampling time points (summer 2023- autumn 2024). Raw data were exported, quality-checked, and aggregated into 7 days rolling averages preceding each sampling date to reduce short-term noise and capture sustained environmental conditions relevant to microbial sampling. Maximum and minimum values for each plot and treatment combination were calculated over this window. The final processed dataset was used to evaluate treatment-specific environmental profiles and to explore relationships between soil parameters and fungal diversity patterns across treatments and time points. Daily and monthly rainfall data for the period 1 May 2022 to 31 May 2024 were obtained from the Australian Bureau of Meteorology (BoM) Australian Gridded Climate Data (AGCD) (Jones et al., 2009). To capture short-term precipitation most relevant to soil microclimatic conditions and microbial responses, we quantified rainfall over the 30 days preceding each sampling date. Two rainfall metrics were derived: (i) total rainfall (mm) accumulated over the 30 days period, and (ii) the number of rainfall events ≥ 3 mm, representing the frequency of effective precipitation events. Days with < 3 mm rainfall were excluded from event counts, as such minor precipitation is considered ecologically negligible for soil moisture recharge and microbial activity (Collins et al., 2025; Parker & Gallant, 2022).

To identify abiotic drivers of microbial alpha diversity, we applied Random Forest regression (Breiman, 2001) in the R randomForest package. Response variables included observed richness and shannon diversity. Candidate predictors were rainfall metrics (rainfall events, total rainfall), soil temperature (maximum and minimum), and volumetric water content (maximum and minimum). Baseline samples (Month 0 that is spring 2022) were excluded, and analyses were conducted on complete cases only. Model performance was evaluated using out-of-bag (OOB) error estimates and the percentage of variance explained. Variable

importance was quantified as the OOB % increase in mean squared error (%IncMSE), with predictors ranked accordingly. Importance values were visualised as horizontal bar plots to enable comparison among environmental predictors.

Results

All 360 samples were successfully amplified and metabarcoded for fungal, arbuscular mycorrhizal, bacterial, and archaeal communities and yielded a total of 4114 unique OTUs within the fungal kingdom, 8775 AMF-specific OTUs, 12491 unique bacterial OTUs within bacterial kingdom and 434 unique OTUs were classified from the archaeal kingdom.

Variation in microbial community structure (beta diversity)

Distance-based redundancy analysis (dbRDA) based on Bray–Curtis dissimilarities revealed significant isolated and interactive effects of treatment and season on community structure across fungi, AMF, and bacteria ($P < 0.05$, Table 2.1, Figure 2.1a-c,). Archaeal communities responded significantly to treatment and season ($P < 0.05$, Table 2.1, Figure 2.1d), but there was not a significant interactive effect. Pairwise comparisons of Bray-Curtis dissimilarities between control and treatments for fungi, AMF, and bacteria diverged significantly across seasons ($P_{adj} < 0.05$, Table 2.2), archaeal responses were more limited, with significant differences detected only sporadically (Table 2.2). The first two constrained dbRDA axes captured substantial proportions of the constrained variation across the microbial groups. For fungi, dbRDA1 and dbRDA2 explained 20.3% and 15.7%, respectively. The corresponding values were 17.7% and 14.2% for AMF, 53.2% and 16.6% for bacteria, and 78.3% and 11.8% for archaea. Ordination plots did not always show clear separation between control and treatment communities across all microbial groups; however, the visualisations of the data in dbRDA space illustrate both the variability and the magnitude and seasonal patterns of divergence varied among communities.

Table 2.1: ANOVA results from distance-based redundancy analysis (dbRDA) showing the effects of treatment (control, drought, warming, warming and drought), season (sampling time), and their interaction on microbial community structure based on Bray–Curtis dissimilarities, with significant P-values shown in bold.

Microbiome	Effect	Df	Sum of Squares	F-value	P-value
Fungi	Treatment	3	3.47	5.65	**0.01**
	Season	4	2.20	2.68	**0.01**
	Treatment: Season	12	2.39	0.97	**0.01**
AMF	Treatment	3	4.49	6.67	**0.01**
	Season	4	4.77	5.32	**0.01**
	Treatment: Season	12	3.32	1.23	**0.01**
Bacteria	Treatment	3	3.14	21.72	**0.01**
	Season	4	1.99	10.33	**0.01**
	Treatment: Season	12	1.37	2.36	**0.01**
Archaea	Treatment	3	0.82	8.43	**0.01**
	Season	4	2.46	18.98	**0.01**
	Treatment: Season	12	0.37	0.95	0.35

Post hoc pairwise comparisons revealed that fungal communities consistently diverged between control and stress treatments across all seasons (Table 2.2, Figure 2.1a). These differences were strongest during summer. There were moderate but significant differences in spring, and weaker divergence in autumn (Table 2.2). AMF communities consistently diverged between control and all manipulated treatments across every season (Table 2.2, Figure 2.1b, $P_{adj} = 0.01$). In the ordinated plots (Figure 2.1b), the control communities tended to cluster separately along the negative side of dbRDA1 across all seasons, while stress treatments were generally shifted toward the positive side. Overall, AMF responses were characterized by consistent control–treatment divergence, but the strength varied seasonally with less clear differentiation between temperature and drought effects as reflected in the greater overlap among stress treatments for AMF than seen for fungi (Table 2.1, Figure 2.1a and b). Bacterial communities consistently diverged between control and stress treatments, with drought driving the strongest and most persistent shifts (Table 2.1, Figure 2.1c). In both summers, control separated significantly from all treatments ($P_{adj} \leq 0.020$), with drought clustering furthest away ($F = 17.3$ in Summer 2023; $F = 11.7$ in Summer 2024; Table 2.2). Overall, community patterns reflect strong drought sensitivity and seasonal dependence, with

summer heat and dryness amplifying microbial responses. Archaeal communities were most responsive to climate manipulations (Table 2.2, Figure 2.1d) in summer ($F = 5–7$, $P_{adj} \leq 0.03$; Table 2.2), variable in autumn, and generally least responsive in spring, except under combined stress.

Table 2.2: Pairwise comparison of beta diversity in microbial communities. Columns show the sampling time (represented as season), treatment compared (Control vs Treatment), pseudo-F statistic (F), raw P-values (P), number of samples per comparison (N), and Benjamini–Hochberg adjusted P-values (P_{adj}). Significant differences between treatments were assessed at FDR-corrected $P_{adj(BH)} \leq 0.05$, with significant adjusted P-values shown in bold.

Microbiome		**Fungi**				**AMF**				**Bacteria**				**Archaea**			
Season	**Control vs Treatment**	**F**	**P**	**N**	**P_{adj}**	**F**	**P**	**N**	**P_{adj}**	**F**	**P**	**N**	**P_{adj}**	**F**	**P**	**N**	**P_{adj}**
Summer 2023	Drought	2.12	0.03	29	**0.03**	3.95	0.01	30	**0.01**	17.25	0.01	29	**0.01**	5.75	0.01	30	**0.02**
Summer 2023	Warming	2.08	0.01	30	**0.01**	3.59	0.01	30	**0.01**	2.36	0.01	29	**0.01**	4.26	0.02	29	**0.04**
Summer 2023	Warming and Drought	2.88	0.01	30	**0.01**	3.64	0.01	30	**0.01**	2.80	0.01	28	**0.01**	4.98	0.01	30	**0.02**
Autumn 2023	Drought	1.93	0.02	30	**0.02**	2.65	0.01	30	**0.01**	20.35	0.01	24	**0.01**	1.49	0.25	29	0.47
Autumn 2023	Warming	1.58	0.02	30	**0.02**	2.49	0.01	29	**0.01**	2.61	0.01	24	**0.01**	0.93	0.32	22	0.47
Autumn 2023	Warming and Drought	1.65	0.04	30	**0.04**	3.04	0.01	30	**0.01**	3.31	0.01	23	**0.01**	0.15	0.94	29	0.94
Spring 2023	Drought	1.76	0.02	30	**0.02**	2.56	0.01	30	**0.01**	19.64	0.01	30	**0.01**	2.24	0.06	29	0.10
Spring 2023	Warming	2.11	0.01	30	**0.02**	3.22	0.01	30	**0.01**	2.17	0.01	30	**0.01**	1.26	0.32	27	0.38
Spring 2023	Warming and Drought	2.79	0.01	30	**0.02**	2.68	0.01	30	**0.01**	3.12	0.01	30	**0.01**	3.71	0.04	29	0.10
Summer 2024	Drought	2.40	0.01	30	**0.01**	1.99	0.01	30	**0.01**	11.68	0.01	30	**0.01**	6.99	0.01	30	**0.03**
Summer 2024	Warming	1.76	0.01	28	**0.01**	2.36	0.01	30	**0.01**	1.80	0.02	30	**0.02**	1.97	0.12	29	0.18
Summer 2024	Warming and Drought	2.16	0.01	29	**0.01**	1.87	0.01	30	**0.01**	1.87	0.02	30	**0.02**	3.86	0.01	30	**0.03**
Autumn 2024	Drought	2.78	0.01	27	**0.01**	2.27	0.01	30	**0.01**	3.24	0.01	27	**0.02**	4.81	0.01	28	**0.03**
Autumn 2024	Warming	1.82	0.01	27	**0.01**	2.07	0.01	30	**0.01**	3.47	0.01	28	**0.01**	1.40	0.22	29	0.26
Autumn 2024	Warming and Drought	1.58	0.04	27	**0.04**	2.15	0.01	30	**0.01**	1.92	0.03	26	**0.03**	3.27	0.01	29	**0.03**

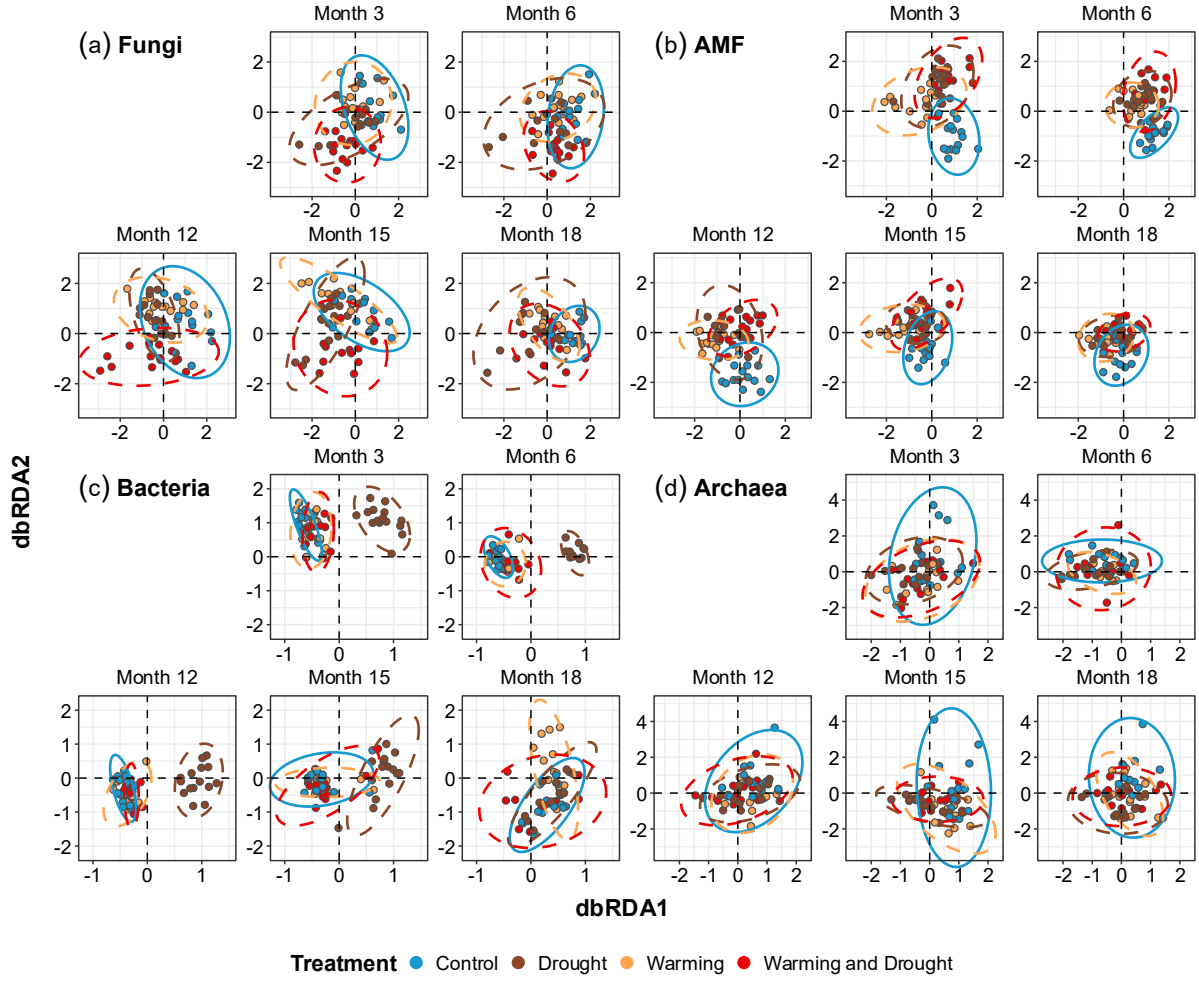


Figure 2.1: Distance-based redundancy analysis (dbRDA) of Bray–Curtis dissimilarities showing community structure of (a) fungi, (b) AMF, (c) bacteria, and (d) archaea across treatments and sampling times. Each point represents a replicate sample, coloured by treatment. Points are displayed by sampling time, with 95% confidence ellipses drawn around treatment groups. Axis percentages indicate the proportion of variation explained by each canonical axis.

Variation in microbial richness and diversity over time reflects seasonal weather patterns

When we examined the effects of temperature and drought treatments on the species richness (observed richness) and diversity (Shannon diversity), one of the strongest effects was sampling time. Not surprisingly, in many cases both metrics were highest in summer and generally lower in spring and autumn (Table 2.3, Figure 2.2).

Table 2.3: ANOVA output of three-way factorial linear mixed-effects models (Temperature × Moisture × Sampling time) for alpha-diversity metrics (Observed richness and Hill-Shannon) of fungi, AMF, bacteria, and archaea. Fixed-effect significance was tested using Type II Wald χ^2 statistics, with significant P-values shown in bold.

Microbiome	Fixed effects	df	Richness		Shannon	
			χ^2	P-value	χ^2	P-value
Fungi	Temperature	1	6.5	**0.01**	0.7	0.41
	Moisture	1	3.7	0.053	0.2	0.65
	Sampling time	4	25.9	**<0.001**	31.4	**<0.001**
	Temperature: Moisture	1	0.1	0.75	0.0	0.93
	Temperature: Sampling time	4	5.2	0.27	1.6	0.82
	Moisture: Sampling time	4	3.1	0.55	3.9	0.43
	Temperature: Moisture: Sampling time	4	3.9	0.42	6.1	0.19
AMF	Temperature	1	5.8	**0.02**	2.0	0.16
	Moisture	1	10.4	**0.0012**	4.4	**0.04**
	Sampling time	4	29.8	**<0.001**	16.3	**0.003**
	Temperature: Moisture	1	9.6	**0.002**	3.1	0.08
	Temperature: Sampling time	4	6.8	0.14	4.6	0.33
	Moisture: Sampling time	4	1.3	0.86	4.4	0.36
	Temperature: Moisture: Sampling time	4	8.0	0.09	2.7	0.61
Bacteria	Temperature	1	6.7	**0.01**	0.5	0.47
	Moisture	1	10.0	**0.002**	11.2	**<0.001**
	Sampling time	4	73.7	**<0.001**	33.4	**<0.001**
	Temperature: Moisture	1	13.3	**<0.001**	2.3	0.13
	Temperature: Sampling time	4	12.1	**0.02**	2.6	0.63
	Moisture: Sampling time	4	4.9	0.30	14.4	0.01
	Temperature: Moisture: Sampling time	4	2.3	0.69	4.5	0.34
Archaea	Temperature	1	4.2	**0.04**	0.04	0.84
	Moisture	1	0.4	0.55	4.9	0.03
	Sampling time	4	109.9	**<0.001**	167.0	**<0.001**
	Temperature: Moisture	1	1.7	0.19	0.01	0.93
	Temperature: Sampling time	4	7.6	0.11	3.7	0.44
	Moisture: Sampling time	4	1.3	0.87	2.1	0.72
	Temperature: Moisture: Sampling time	4	1.1	0.89	1.5	0.83

Fungi showed the clearest seasonal signal: both species richness and diversity consistently peaked in summer and declined in autumn (Figure 2.2a and e), being significantly higher in summer than autumn in each of the years (Table A. 4).

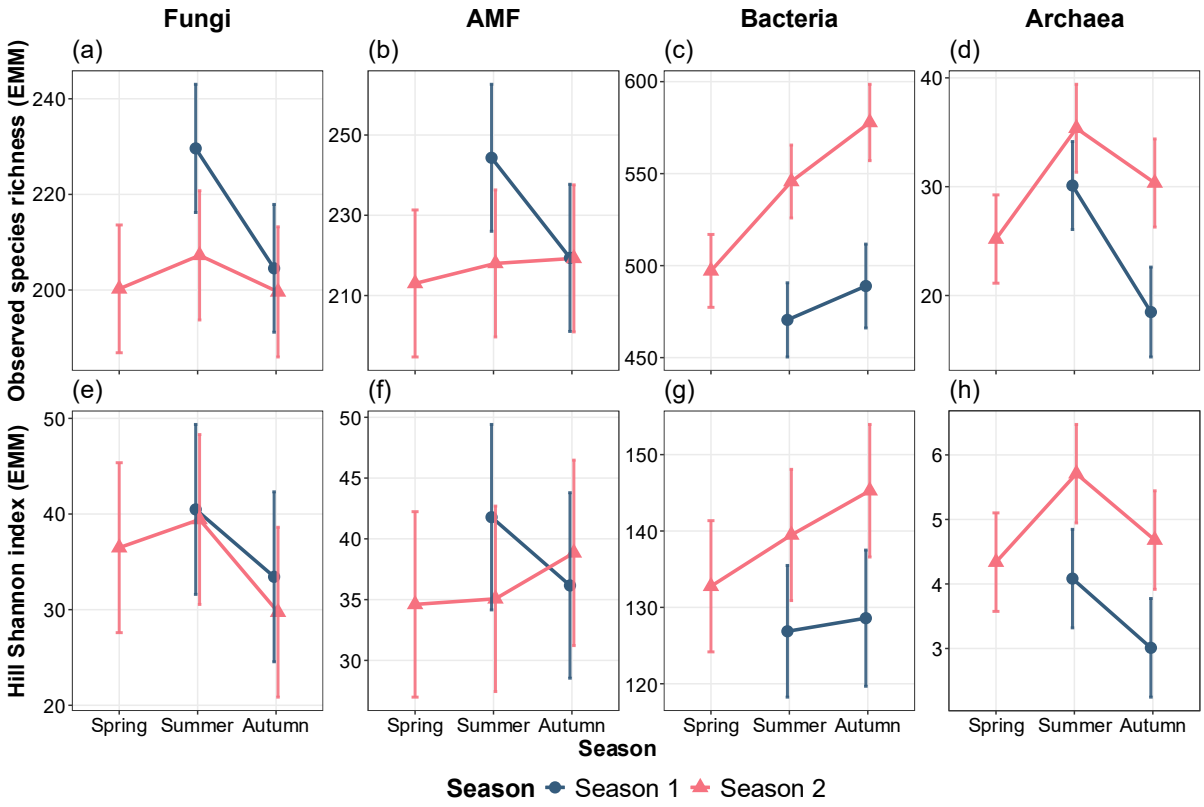


Figure 2.2: Seasonal variation in estimated marginal means (EMMs ± SE) of richness and species diversity for (a) fungal, (b) AMF, (c) bacterial, and (d) archaeal communities. Values were derived from three-way factorial linear mixed-effects models (Temperature × Moisture × Sampling time), with significance assessed using Type II Wald χ^2 tests.

AMF richness also had a distinct maximum in the summer of the first year (Figure 2.2b and f) significantly exceeding all subsequent seasons ($P < 0.01$ to $P < 0.001$; Table A. 4) without as clear a seasonal pattern, while AMF species diversity did not show clear treatment effects (Table A. 4). Bacteria also did not follow a seasonal cycle, instead showing a progressive significant temporal increase (Figure 2.2c and g) in both metrics ($P < 0.01$ to $P < 0.001$; Table A. 4), culminating in the highest levels in the second autumn. Finally, Archaea displayed summer peaks in both richness and species diversity, with the highest levels in summer followed by declines into autumn in both years (Figure 2.2d and h, $P < 0.001$; Table A. 4). Collectively, these results indicate that fungi and archaea exhibit strong and explicable seasonal structuring with summer peaks, whereas bacteria did not exhibit seasonal cycles.

Effects of temperature and moisture on species richness and diversity vary among microbial groups

Experimental warming and drought significantly affected microbial species richness (Figure 2.3a–d; Table A. 5). A consistent signal of temperature sensitivity emerged across all four groups: species richness was reduced under elevated temperature, although the strength of these responses varied.

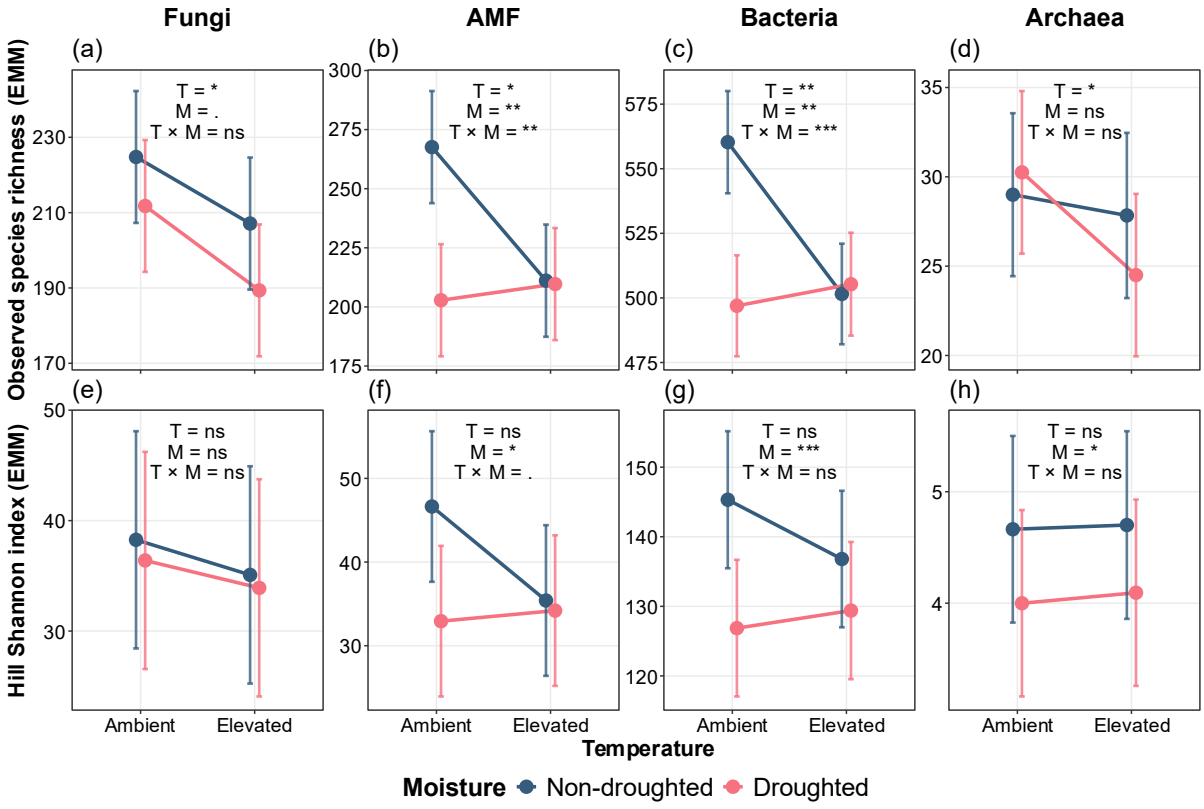


Figure 2.3: Responses of microbial richness and Shannon diversity to experimentally altered temperature and moisture: (a, e) fungal, (b, f) AMF, (c, g) bacterial, and (d, h) archaeal communities. Estimated marginal means (EMMs ± SE) were obtained from three-way factorial linear mixed-effects models (Temperature × Moisture × Month), tested using Type II Wald χ^2 statistics. (T = temperature; M = moisture; significance levels: · P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001; ns = not significant; post-hoc pairwise comparisons of EMMs with adjusted P-values).

Species richness was generally reduced under drought, but the soil moisture effects were more group-specific, with significant impacts on AMF and bacteria and weaker or nonsignificant effects on fungi and archaea. Warming and drought effects on species diversity (Hill Shannon) were similar, but less pronounced (Figure 2.3e–h; Table A. 5). Species diversity (Hill Shannon) of fungal and archaeal communities were largely resilient to experimental warming and drought, while AMF and especially bacteria showed greater sensitivity to temperature and drought.

Edaphic drivers of microbial diversity

Finally, we used Random-forest analyses to examine the extent to which measured edaphic factors such as rainfall, soil temperature and volumetric water content in the plots, drove the observed changes in species richness and diversity across the four groups. Soil temperature and moisture extremes were dominant predictors of microbial species richness across all four groups, whereas background rainfall measures contributed little explanatory power (Figure 2.4). Model performance (percent variance explained) varied by domain: fungi 9.51% (richness) and 42.16% (Shannon); AMF 37.61% and 35.11%; bacteria 29.76% and 28.40%; archaea 31.98% and 48.98% respectively. Overall, Shannon diversity tended to be more predictable than richness, with the highest predictability observed for archaeal Shannon diversity. The archaea were an exception where rainfall events were concerned: species richness was influenced by rainfall events as well as maximum soil temperature, while archaeal species diversity was primarily explained by soil moisture extremes.

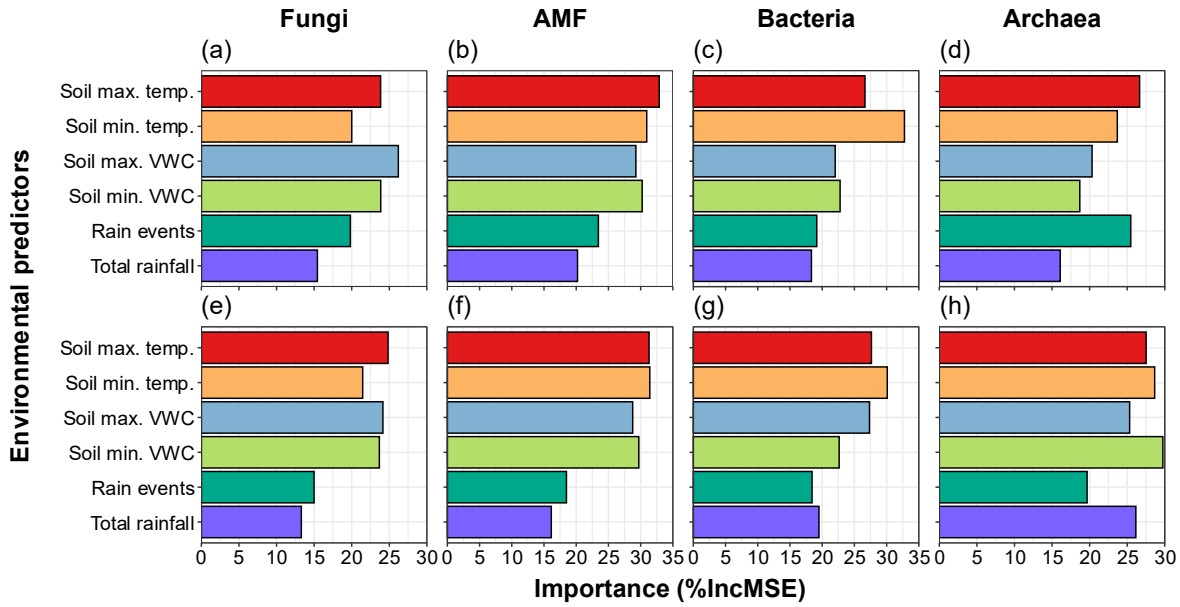


Figure 2.4: Random Forest variable importance (%IncMSE) ranking abiotic predictors of (a, e) fungal, (b, f) AMF, (c, g) bacterial, and (d, h) archaeal richness and shannon diversity across seasons; bars share a fixed colour map so the same variable has the same colour across panels. Observed richness variables are shown in the top panel, whereas Shannon diversity is shown in the bottom panel.

Discussion

We examined the effect of simulated climate change on soil microbial communities in the highly seasonal sub-alpine conditions to assess the extent to which drought, warming and their interaction may modify ecosystem properties and processes. Our study examined changes in microbial community structure over two growing seasons using a factorial climate change experiment in the sub-alpine grasslands of Kosciuszko National Park, NSW, Australia. By integrating alpha and beta diversity analyses and Random Forest models, three key insights emerged. First, community structure diverged consistently between control and manipulated treatments, but the seasonal variation in those effects varied across microbial groups. Additionally, microbial groups differed markedly in their temporal strategies, with fungi and AMF showing higher diversity in the first season than the second, while bacteria did not show such seasonal effects. Second, a consistent signal of temperature sensitivity emerged across all groups: fungal, AMF, bacterial, and archaeal richness were all reduced under elevated temperature (Blankinship et al., 2011), although the strength and nature of these responses

varied. Third, soil temperature and moisture extremes emerged as the dominant predictors of microbial diversity across groups, whereas rainfall events and totals explained comparatively little variation. Together, these findings highlight that microbial communities are both seasonally dynamic and group-specific in their responses to climate change, with implications for predicting ecosystem resilience in sub-alpine environments.

Effect of abiotic stress on microbial community diversity and community structure

We have found warming, drought, and their interaction significantly shaped all microbial groups, though responses varied in magnitude and mechanism. Fungal richness declined mainly under elevated temperature but was less sensitive to drought, indicating temperature-driven filtering in sub-alpine systems (Baldrian et al., 2023; de Oliveira et al., 2020; Tu et al., 2020) which aligns with findings that fungi are generally more tolerant of water stress than bacteria (Bapiri et al., 2010; de Vries et al., 2018; Tu et al., 2020). Despite reduced richness, fungal communities diverged strongly from controls, particularly in summer, reflecting climatic sensitivity (Hawkes et al., 2011; Joergensen & Wichern, 2008). AMF diversity and structure were also reduced under both single and combined stresses (Xie et al., 2024). Reduced water availability typically lowers AMF occurrence and diversity (Frew, 2023; Fu et al., 2022) and soil moisture strongly structures AMF communities (Deveautour et al., 2020). Bacterial diversity declined sharply under both stressors, with drought driving the strongest shifts (Bouskill et al., 2013; Ruan et al., 2024; Wan et al., 2023; Xiao et al., 2025; Yang et al., 2021). Their sensitivity stems from dependence on water-mediated substrate diffusion and limited desiccation tolerance (Austin et al., 2004; Clark et al., 2009). Warming effects became more pronounced over time as labile carbon was depleted, reinforcing bacteria as the most responsive group to combined stress (Purcell et al., 2022; Seaton et al., 2022). Archaeal responses were comparatively weak, with richness declining under warming, especially in droughted soils (Zhang et al., 2023). Reduced substrate availability and competition with other microbes likely constrained archaeal activity, though many taxa persisted via dormancy (Rui et al., 2023; Tian et al., 2015; Tian et al., 2012).

Seasonal dynamics of microbial diversity and community structure

Our results support the hypothesis that microbial communities exhibit strong seasonal dynamics in sub-alpine grasslands. However, seasonal effects on microbial diversity and community structure were group specific. We have found significant seasonal effects were detected for both alpha and beta diversity, with fungi and archaea showing the strongest variation, bacteria the weakest, and AMF an intermediate response; fungal and archaeal diversity peaked in summer, bacterial diversity increased gradually over time, and AMF diversity was highest in the first summer but did not recover to that level thereafter. These contrasting patterns likely reflect fundamental ecological differences among microbial groups. Seasonal peaks in fungal biomass and activity have been documented across alpine, sub-alpine and temperate ecosystems (Schadt et al., 2003; Shigyo et al., 2019; Voříšková et al., 2014; Xie & Yin, 2022), with fungi depending heavily on seasonal pulses of plant litter and root exudates, and their diversity and activity closely track plant phenology and soil resource availability (Lipson et al., 2002; Shigyo et al., 2019; Žifčáková et al., 2016). Root exudates help sustain resident fungal populations through chemical signalling and carbon inputs (Broeckling et al., 2008). Our results of seasonal AMF dynamics are consistent with numerous studies showing that AMF communities fluctuate seasonally in response to plant phenology and environmental conditions, with richness typically peaking in summer and declining during autumn and spring (Ji et al., 2021; Rosendahl & Stukenbrock, 2004; Santos-González et al., 2007). These seasonal patterns reflect shifts in root colonization, spore production, and nutrient demand, where favourable moisture and temperature in summer enhance AMF activity, while cooler, resource-limited periods reduce symbiotic interactions (Bennett et al., 2013; Dumbrell et al., 2011; Escudero & Mendoza, 2005; Lugo & Cabello, 2002; Pringle & Bever, 2002).

Bacterial communities tend to maintain activity across seasons due to their physiological flexibility and ability to use diverse carbon sources (Goldfarb et al., 2011; Steinberger et al., 2024). Repeated inputs of labile carbon from rhizodeposition and snowmelt promote sustained or gradually increasing bacterial diversity rather than distinct seasonal peaks

(Broadbent et al., 2021; Liu et al., 2010; Mau et al., 2015). High summer diversity of archaea coincides with peak plant productivity and ammonium turnover, consistent with reports of elevated archaeal activity during periods of optimal temperature and substrate availability in alpine and sub-alpine ecosystems (Lauber et al., 2013; Rasche et al., 2011; Zhang et al., 2019; Zhang et al., 2023). Collectively, these results highlight that seasonality exerts a strong structuring force on sub-alpine soil microbiomes, modulated by the distinct life-history traits and resource dependencies of different microbial groups.

Conclusions and future directions

This study indicates a rapid (1-2 year) response of microbial communities on simulated climate change effects. How long these persist or what the accumulated effects on those microbial communities will be, remains to be seen. These patterns may be quite stable, might accumulate, or could be transient patterns. The results also raise interesting questions about the taxonomic drivers, ecological guilds, functional potentials, or interaction networks that may underlie these patterns. Future work should integrate such analyses to better link community shifts with ecological processes. While the manipulations realistically simulated warming and drought, they represent fixed conditions and may not capture the full spectrum of natural climatic variability, including extreme or irregular events that are projected to become more frequent under climate change.

This study shows that soil microbial communities in sub-alpine grasslands respond in group-specific ways to experimental warming, drought, and seasonal fluctuations. Warming consistently reduced richness across all microbial groups, highlighting the sensitivity of sub-alpine soil biota to elevated temperatures. Drought effects were strong for bacteria and AMF but weak for fungi and archaea, reflecting differences in moisture sensitivity among groups. Seasonal patterns further shaped these responses: fungal and archaeal richness exhibited clear summer peaks and declined in autumn and spring, whereas bacterial richness increased gradually over time, suggesting a rapid acclimation to repeated environmental fluctuations. Soil temperature and moisture emerged as the main predictors of microbial diversity, while rainfall events played a minor role. These results demonstrate that seasonality interacts with climate treatments to shape belowground communities, with distinct diversity trajectories

among microbial groups. Seasonal changes in microbial diversity have important implications for understanding the global significance and biogeochemical functioning of seasonally snow-covered environments. The contrasting diversity patterns among fungi, AMF, bacteria, and archaea suggest that cold, snow-covered soils may represent an underappreciated reservoir of biological diversity. Incorporating these differential responses into ecosystem models will be crucial for predicting the resilience and functioning of alpine ecosystems under future climatic scenarios.

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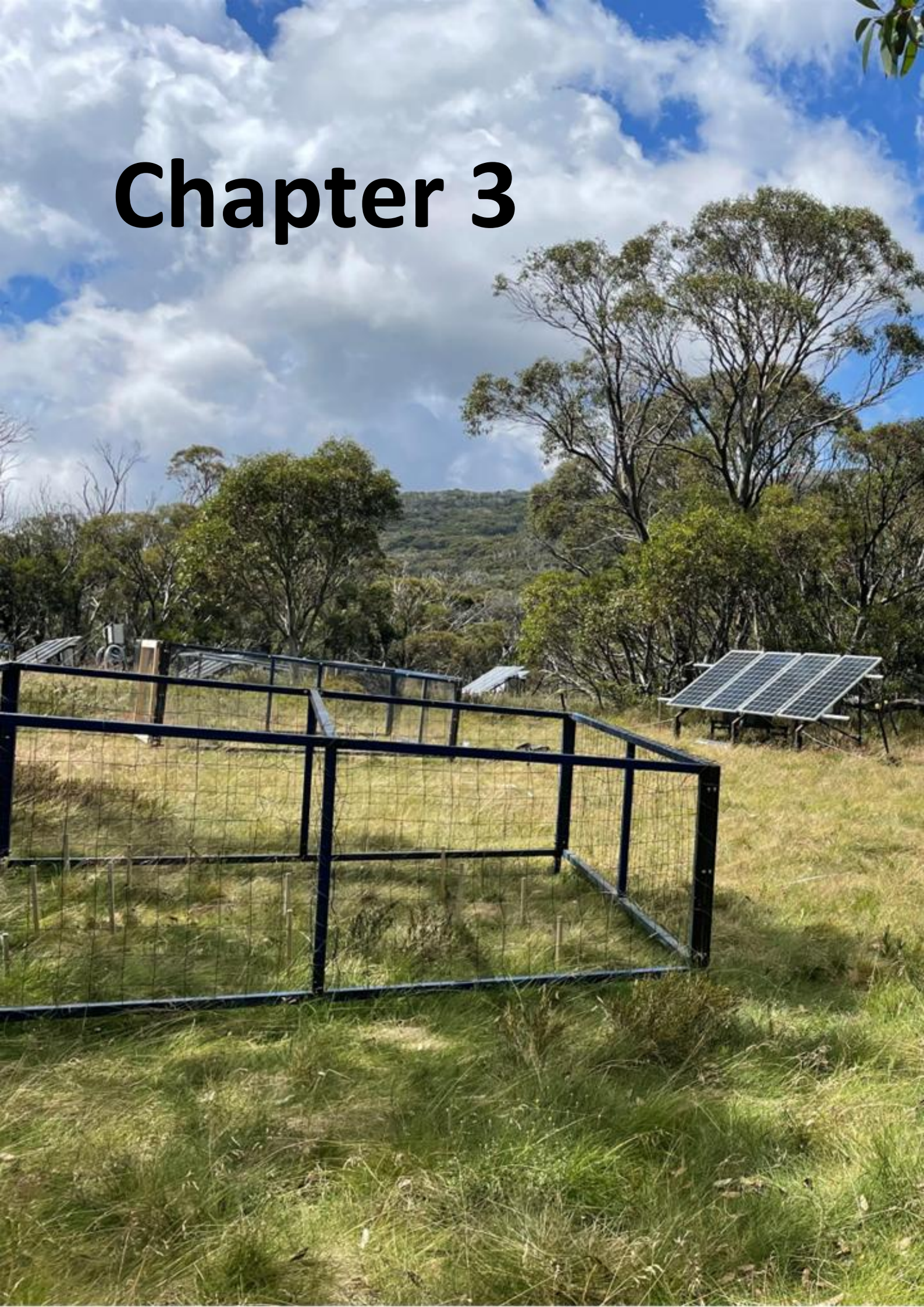
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Chapter 3



Chapter 3: Climate stress alters microbial functional potential and co-occurrence networks in sub-alpine soils

Abstract

Climate change may have profound influence on alpine environments, yet the responses of their belowground microbial communities remain poorly understood. Here we use Illumina metabarcoding to assess the single and combined effects of drought and a 4°C increase in temperature on community composition, co-occurrence networks and predicted functional potential of four major soil microbial groups i.e. fungi, arbuscular mycorrhizal fungi (AMF), bacteria and archaea, across two summers in an Australian sub-alpine field experiment. Consistent with our expectation of group-specific sensitivity, LEfSe analyses revealed that fungi exhibited the strongest early compositional turnover under combined warming + drought, whereas bacteria showed the most persistent and pronounced taxonomic shifts under drought alone. AMF and archaea were dominated by widespread lineage suppression under all stresses, indicating broad climatic sensitivity. Fungal trophic analysis further showed that prolonged drought and combined stress significantly reduced symbiotrophs and produced a gradual increase in pathotrophs, suggesting weakened beneficial fungal associations and a subtle shift towards opportunistic fungi under moisture and temperature stress. Network analysis further demonstrated that microbial interactions reorganised in a group-specific manner: fungal and AMF networks consistently showed reduced network complexity, i.e. they lost nodes, edges, and overall connectivity under combined stress, whereas bacterial networks showed no significant structural change and even increased connectivity under warming. Based on the functional prediction of the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) we found parallel shifts across loci, with fungi and AMF transitioning from early reductions in amino-acid and nucleotide pathways to later suppression of central carbon and energy metabolism. Bacterial functional responses were dominated by drought-driven losses in carbon and aromatic compound degradation pathways, while archaea showed strong suppression under

prolonged combined stress of methanogenesis and coenzyme-linked energy metabolism across stresses. These patterns suggest that climate stresses reorganize soil microbiomes in group-specific ways that may alter long-term ecosystem functioning. Together, these results demonstrate that belowground microbial responses to climatic stress are not uniform. Combined warming and drought most strongly alter fungal and AMF composition, trophic structure, and network connectivity whereas drought exerts the greatest compositional and metabolic pressure on bacteria, while methanogenic archaea remain highly vulnerable across stressors. These group-specific responses highlight differential resilience within the soil microbiome and underscore the potential for climate change to alter ecosystem functioning through changes in microbial composition, trophic roles, connectivity, and metabolic capacity. These findings highlight that warming and moisture decline reshape not only microbial taxonomic structure but also the interaction architecture and the predicted functional potentials of soil communities. Together, the results underscore the vulnerability of sub-alpine soils to compounded climate stress and demonstrate that differentially abundant taxa, microbial networks, and functional pathways act as sensitive indicators of ecosystem stability under ongoing global change. However, our ability to predict global change effects on sub-alpine soil microbiome is still insufficient and requires further experimental work and long-term observations.

Introduction

Despite growing evidence of climate-induced biological changes in alpine and sub-alpine ecosystems, the magnitude and trajectory of these cascading effects remain poorly resolved (Chen & Gao, 2022; Dainese et al., 2024; Rowland et al., 2025). Climate models project an average 4°C increase in mean temperature and a 5–20% decline in precipitation across the Australian Alps by 2100, together with greater likelihood of extreme climatic events such as drought (Harris et al., 2016). Consequently, substantial changes in snow regimes across the Australian Alps are projected, reducing seasonal snow accumulation and increasingly exposing sub-alpine soils to drought and greater temperature variability (Di Luca et al., 2018; Whetton et al., 1996). These changes are expected to challenge the resilience of high-elevation ecosystems (Pauli & Halloy, 2019). Understanding how these climatic shifts translate into ecological and biological responses remains a critical challenge for predicting the future of alpine ecosystems.

Soil ecosystems underpin landscapes by regulating nutrient, carbon and nitrogen cycling and hosting diverse microbial communities that drive organic matter decomposition and maintain soil functionality, thereby supporting ecosystem stability, resilience, and post-disturbance recovery in alpine grassland systems (Allison & Martiny, 2008; Balser & Firestone, 2005; Du et al., 2025; Wang et al., 2016). Moreover, microbial communities often exhibit functional redundancy, where different taxa can perform similar ecological functions, thus enabling the maintenance of essential ecosystem processes despite significant shifts in community composition (Chen et al., 2022). This redundancy enhances ecosystem resilience, providing a buffer against environmental fluctuations and disturbances (Louca et al., 2018). However, this functional overlap does not imply uniformity in response to stressors, distinct microbial groups deploy varying strategies to cope with drought and warming, such as producing extracellular polymeric substances, forming resistant spores, or altering metabolic pathways (Bardgett & Caruso, 2020; Evans & Wallenstein, 2014). Consequently, the degree of sensitivity among microbial taxa can differ markedly, leading to shifts in community structure and functional capacity under climate change scenarios (Zhou et al., 2019). As warmer and drier conditions become more prevalent, particularly during critical seasonal windows such as the

summer growing season, such climate extremes may disproportionately affect sensitive taxa, with cascading effects on decomposition rates, nutrient cycling, and overall ecosystem stability (Kotlarski et al., 2023).

Emerging evidence suggests that the stability of ecosystem functioning under climate stress relies not only on microbial redundancy but also on the adaptive strategies and plasticity of microbial communities (Bardgett & Caruso, 2020; Chen et al., 2022). Climate-driven disturbances can reorganise microbial associations, reshaping network complexity and stability across ecosystems. For instance, soil warming and cooling have altered bacterial and fungal network structures in Tibetan alpine soils (Fu et al., 2024), whereas drought destabilised bacterial but not fungal networks in temperate grasslands (de Vries et al., 2018). In Chapter 2 we found that altered temperature and moisture, as well as seasonal variability affected microbial diversity and community structure in sub-alpine grasslands. Specifically, warming continuously reduced microbial richness, drought impacts were group-specific, and strong seasonal patterns occurred in most of the microbial groups. Yet, in the Australian Alps, the effects of warming and drought on microbial differentially abundant taxa shifts, network stability and functional potential remain largely unknown.

While responses of macro-organism communities to novel conditions are often reported, it has been more difficult to study these responses in microbial communities due to their extreme diversity, rapid turnover and the technical challenges that come with observing them. Microbial activity and diversity in alpine soils depend strongly on temperature and moisture, both of which change under climate warming and drying, affecting nutrient cycling and ecosystem function (Donhauser & Frey, 2018). In relation to the Australian Alps, Wilson et al. (2022) highlighted that the paucity of research on microbial populations in sub-alpine soils and their responses to climate threats limits our ability to predict the consequences of future climate change. Although Birnbaum et al. (2023) provided valuable insights into prokaryotic and fungal communities within degraded and intact peatlands of the Australian Alps, their work focused on land-use induced degradation rather than climatic stressors. Chapter 2 showed that the soil microbiome diversity and community structure respond rapidly (1-2 seasons) to induced warming and drought in the Australian Alps. Given the sensitivity of alpine soil ecosystems to shifts in temperature and moisture, understanding

their responses to ongoing climatic changes is a prerequisite for developing informed conservation and policy frameworks (Praeg et al., 2025). Therefore, here we will focus on functional analyses of the microbial communities to provide insight into below-ground ecological responses to climate change in the Australian Alps.

In this study, we investigate how isolated and combined drought and warming treatments influence soil microbial community structure and function (including fungi, bacteria, arbuscular mycorrhizal fungi (AMF), and archaea) in the Australian sub-alpine ecosystem. Given our previous work demonstrated that richness and diversity respond to altered drought and warming, and that these patterns differ among major taxonomic groups, we hypothesized that the different microbial groups would respond differentially to single and combined climatic stresses in the Australian sub-alpine ecosystem. For example, each stress treatment will select a unique group of microorganisms that act as differentially abundant taxa of that environmental change. We anticipated a shift of ecological guild composition towards stress tolerance and resource exploitation under warming and drought. In addition, Wagg et al. (2019) reported that network complexity is positively linked to ecosystem function and stability, so we hypothesised that experimental drought and warming would decrease the complexity of microbial co-occurrence networks and thereby limit resources availability (Canarini et al., 2024). We further anticipated that these reductions in network complexity would not be uniform across microbial groups. Accordingly, we expected that the combined warming and drought treatment would affect the network structure most, reflecting reduced microbial diversity, weakened interaction strength and diminished ecological connectivity under combined stress. Building on this, we hypothesised that these compositional and co-occurrence shifts would be reflected in functional potential. Specifically, we expected drought and warming individually and in combination to differentially reshape key metabolic pathways, with combined stress exerting the strongest constraint on overall functional capacity.

Therefore, the specific objectives of this study were to (1) identify microbial taxa that are differentially responsive to climate stress in sub-alpine soils, (2) assess the effects of warming and drought on microbial co-occurrence networks to evaluate community stability, and (3) predict potential functional shifts in microbial communities under stress conditions. Microbial

communities in Australian sub-alpine soils are extremely understudied, thus, our findings will provide crucial baseline data on belowground microbial dynamics and their implications for carbon cycling, are crucial to informing climate-adaptation and restoration strategies for these sensitive ecosystems.

Materials and methods

Experimental design and sequencing

This chapter used the same study site, experimental setup, soil sampling design, and amplicon sequencing datasets described in Chapter 2. Briefly, soil samples were collected from the FutureClim (Brown et al., 2024) factorial drought and warming experiment in the Australian sub-alpine grassland (Kosciuszko National Park), comprising four treatments that are control, drought, warming and a combination of warming and drought, across five replicate blocks. Soil collection, DNA extraction, PCR and Illumina amplicon sequencing of fungi, AMF, bacteria and archaea are outlined in Chapter 2. Because Chapter 2 showed substantial seasonal differences in microbial richness and diversity, with the highest values occurring during the summer growing season, we therefore focus the present analysis to the two summer datasets (3 and 15 months into the treatment period).

Bioinformatics and statistical analyses

Sequence processing and phyloseq object generation were outlined previously (Chapter 2). Briefly, samples with insufficient sequence depth have been removed to ensure sufficient sequence depth and adequate biological replication across treatments. Samples having reads below 11,695 for fungi, 37,000 for AMF, 10,212 for bacteria, and 6,400 for archaea, have been removed. Unlike chapter 2, where empirical rarefaction was applied to standardize sequencing depth, no rarefaction was performed here because each analytical framework used its own internal normalization or filtering approach. All analyses, including differential abundance (LEfSe) and co-occurrence network construction were performed in R (v4.5.1) using phyloseq-derived objects and the packages phyloseq v1.52.0, microbiomeMarker v1.13.2, Hmisc v5.2.3, igraph v2.1.4, vegan v2.7.1, and tidyverse v2.0.0 utilities.

Functional predictions were generated using PICRUSt2 v2.6.2 through the command-line interface. Fungal trophic modes were assigned using FUNGuild v1.1 Beta (Nguyen et al., 2016).

Potential differentially abundant microbial taxa were identified by the linear discriminate analysis (LDA) effect size (LEfSe) method with CPM normalization (Segata et al., 2011) at highest feature (OTU) level. Taxonomic labels were truncated to the highest reliable rank, and for AMF this was limited to the genus level to avoid species-level noise. Briefly, the non-factorial parametric Kruskal Wallis (KW) sum-rank test was used to identify differences in the abundance of microbiome groups. Pairwise comparisons were performed between the control and each treatment group (drought, warming and warming and drought) to identify taxa differentially enriched after stress treatments. LEfSe reports a LDA score on a $\log_{10}$ scale that reflects discriminative effect size. Significant taxa were identified using an LDA score > 4.0 for fungi and bacteria, combined with a Benjamini–Hochberg adjusted P-value (P_{adj}) < 0.05 to ensure both biological and statistical relevance. For AMF and archaea, we applied the default LEfSe threshold of LDA > 2.0, as using the higher cutoff yielded very few discriminant taxa. LDA scores were centred such that positive values indicate enrichment in the treatment group and negative values indicate enrichment in the control.

Trophic level classifications from FUNGuild were curated by retaining only “highly probable” and “probable” assignments. Fungal taxa were classified into three broad trophic modes: saprotrophs, which obtain nutrients from dead organic material; symbiotrophs, which obtain nutrients through beneficial associations with living hosts; and pathotrophs, which obtain nutrients by harming host cells or causing disease. Where taxa were associated with multiple trophic modes, abundances were proportionally divided among the assigned categories. The communities that could not be identified were omitted from the analysis. Relative abundances were summarized per treatment. Beta regressions tested treatment effects for each trophic mode (control = reference); contrasts and 95% CIs were obtained using emmeans, with FDR correction (Benjamini–Hochberg).

Co-occurrence networks were inferred at the OTU level from the phyloseq object to explore potential microbial associations across treatments. To ensure robust network inference, taxa

were filtered within each treatment and sampling time subset before correlation analysis. Taxa with <30 % prevalence across samples were excluded to remove rare features, and taxa with zero variance in relative abundance were discarded to avoid undefined or inflated correlation estimates. To enhance computational efficiency and focus on ecologically dominant community members, each subset was further limited to the 200 most abundant OTUs following filtering. Pairwise Spearman correlations (ρ) were calculated using relative-abundance data, and false discovery rates were controlled using the Benjamini–Hochberg procedure. Significant associations were defined as $|\rho| \geq 0.60$ with FDR-adjusted P value (P_{adj}) ≤ 0.05. Significant correlations were then assembled into undirected networks, in which nodes represented OTUs and edges represented significant correlations. Network topology metrics including average degree (mean number of connections per node), graph density, network diameter, modularity, path length, and transitivity were then computed in R using the igraph package v2.1.4, as these metrics collectively describe overall network connectivity, cohesiveness, and structural complexity. Node size was scaled by degree centrality (number of significant co-occurrence connections per OTU). Because correlations derived from compositional data represent co-variation rather than direct ecological interactions, networks were interpreted comparatively across treatments under uniform analytical thresholds. To statistically test whether drought, warming, or combined stress significantly altered network structure relative to the control, we used a permutation test on sample labels. For each Treatment and each summer comparison, we randomly permuted treatment labels 999 times within each month and calculated the metric differences to generate a null distribution. Two-sided P-values were obtained as the proportion of permuted differences more extreme than the observed value. Significant increases or decreases were defined as $P < 0.05$.

Functional predictions for each microbial group were generated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) pipeline (Douglas et al., 2020). Fungal communities (ITS) and AMF (18S) were analysed with their respective eukaryotic pipelines, which predict Enzyme Commission (EC) numbers only; these were likewise integrated into pathway abundances using MetaCyc. Bacterial and archaeal communities were analysed with the 16S (prokaryotic) pipeline, which estimates 16S rRNA gene copy numbers, EC numbers, and KEGG Orthologs (KOs), followed by pathway inference

with the MetaCyc database (Caspi et al., 2016). For fungi, AMF and archaea, the minimum alignment threshold (min_align) was set to 0.5, while default parameters were used for bacteria. To improve visuality, for bacteria and archaea, only pathways with $|\log_2 FC| > 2$ were visualized in the main text, while all significant pathways (FDR < 0.05) are provided in Appendix B in Figure B. 1 and Figure B. 2.

Results

Climate stress consistently reshapes differentially abundant taxa

We applied LEfSe to identify differentially abundant microbial taxa between climate treatments during the first and second summer. This analysis indicates which lineages were enriched or suppressed by each stressor. In line with our hypothesis, treatments produced distinct sets of differentially abundant taxa, with LEfSe showing clear group-specific responses. Fungi showed the strongest response under the combined warming and drought treatment, with 23 lineages suppressed and 16 enhanced over the two summer sampling periods, indicating substantial restructuring of the fungal community under multiple simultaneous stressors. For this treatment, the largest response was noted in the first summer. Drought alone and warming alone had more moderate effects on fungi (13 and 11 suppressed lineages, with only 2 and 1 enhanced, respectively). In contrast, AMF exhibited similar numbers of affected taxa across all treatments, although the majority were suppressed rather than enriched and most in second summer, suggesting a general sensitivity rather than treatment-specific shifts. For bacteria, responses were consistent and dominated by the drought treatment across both summers, which resulted in more than three times the number of affected bacterial lineages compared with the other treatments (21 suppressed, 14 enhanced). Finally, archaeal taxa were predominantly suppressed under warming and combined stresses, indicating that archaeal groups are also differentially impacted by climatic extremes. Collectively, these results show that microbial groups differ in their sensitivity to climate stress, with fungi responding most strongly to combined warming and drought, while bacteria respond most strongly to drought alone (Figure 3.1).

Effects of treatments on major lineages varied among groups. Among fungi, all stress treatments consistently suppressed (with LDA scores > |4.0|) three macro-Basidiomycete lineages Rossbeevera, Cuphophyllus, and Boletales revealing a shared core of stress-sensitive taxa despite their different trophic roles. Beyond this common pattern, each stressor also produced distinct shifts: combined stress caused the strongest overall declines in the first summer, drought consistently enriched Agaricomycetes lineages in both summer and warming favoured an ectomycorrhizal lineage (*Tomentella*). In contrast, AMF showed widespread declines across key lineages (Mucoromycota, Diversisporales, Archaeosporales, Claroideoglomeraceae), reflecting strong sensitivity to climate stress, while Paraglomeromycetes and Gigasporaceae (*Scutellospora*) showed more selective treatment-specific tolerance. Interestingly, *Diversispora* increased only under combined warming × drought in the first summer despite overall suppression of Diversisporales. (Figure 3.1a, b).

Bacteria showed a persistent drought-driven restructuring of the bacterial community across both summers. Drought consistently enriched several taxa associated with resource-limited and stress-prone environments, including *Ktedonobacteria*, *Acidobacteriota* (Subgroup-2 and *Terriglobales*), *Gemmataceae*, and several Planctomycetes lineages. The enrichment of *Acidobacteriota* is consistent with an oligotrophic ecological strategy, as members of this group are commonly associated with low resource availability (Fierer et al., 2007). In contrast, drought suppressed several moisture- and resource-responsive taxa, including *Proteobacteria*, *Actinomycetota*, *Bradyrhizobium*, and *Candidatus Udaeobacter*. Taken together, drought exerted the strongest bacterial response of all stressors, shifting communities towards slow-growing, stress-adapted lineages (Figure 3.1c). Archaeal communities showed consistent suppression of methanogenic lineages across all stressors, while drought and combined stress selectively enriched nitrifying *Thermoproteota*/*Nitrososphaeria*, indicating broad stress sensitivity among methanogens and moisture-driven responses in ammonia-oxidizing archaea (Figure 3.1d).



Figure 3.1: Differentially abundant microbial taxa between treatment and control in first summer and second summer as identified with LEfSe analyses. After counts-per-million (CPM) normalization, only taxa with LDA > |4.0| for fungi and bacteria and with LDA > |2.0| for AMF and archaea, along with false discovery rate (FDR)-adjusted P < 0.05 were retained.

Effect of drought and warming on fungal trophic modes

FunGuild was used to estimate the potential fungal trophic modes of fungal communities in order to investigate the microecological fungi activities in response to drought and warming stress in alpine soils (Figure 3.2). Beta-regression analysis confirmed no significant differences in trophic mode composition among treatments during the first summer. By the second summer, however, clear trends began to emerge. Symbiotrophs declined significantly under combined warming and drought (P_{adj} < 0.05, Figure 3.2b) and showed a marginal decline under drought alone, indicating that prolonged moisture stress reduced beneficial fungal associations. Saprotrophs and pathotrophs did not change significantly, although a small increase in pathotroph abundance under combined stress suggested a gradual shift towards more opportunistic taxa. This shift suggests that prolonged warming and drought may weaken beneficial fungal associations and slightly favor opportunistic or disease-causing groups.

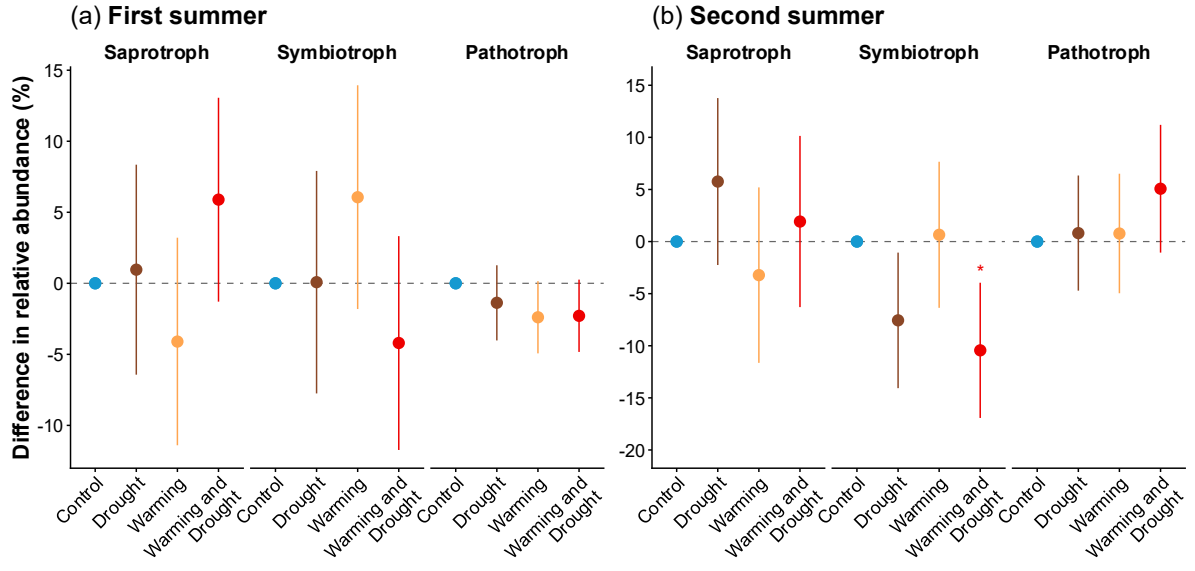


Figure 3.2: Beta-regression analysis of fungal trophic modes across treatments during the first and second summers. Values represent mean percentage-point differences (Δ) in relative abundance compared with the control (± 95 % CI). Significance is based on FDR-adjusted P-values. *, ** and *** indicate P < 0.05, P < 0.01 and P < 0.001 respectively.

Effect of drought and warming on co-occurrence networks of soil microbial communities

To determine whether climate stresses reshaped microbial interactions beyond taxonomic shifts, we constructed treatment-specific co-occurrence networks for each summer. Consistent with our expectation, the network responses were strongly group-specific. Stress, particularly the combination of warming and drought, reduced network complexity, reflecting destabilisation of microbial interaction structure under stress. Fungal networks showed strong and consistent reductions in structural complexity under climate stress across both summers, with drought, warming and combined warming and drought producing significant losses in nodes, edges, network diameter and average degree ($P_{adj} < 0.05–0.001$) (Figure 3.3a and Table B. 1). For AMF, in the first summer, combined warming and drought produced a significantly simpler network, with significant declines in nodes, edges, average degree, and network diameter ($P_{adj} < 0.05–0.001$) (Figure 3.3b and Table B. 1). In contrast, bacterial co-occurrence networks showed no statistically significant changes under any stressor ($P_{adj} > 0.05$), despite clear numerical shifts in several network topology metrics (Table B. 1). Drought sharply reduced connectivity in the first summer but became more complex than the control by the second, warming consistently increased network connectivity across years, and only the combined warming + drought treatment consistently produced the simplest and most fragmented networks, although these reductions were not statistically significant (Fig 3c and Table B. 1). Similarly, archaeal networks were generally resilient to single stressors, maintaining or even strengthening connectivity under drought and warming, but combined warming and drought caused significant network collapse, especially in the second summer ($P_{adj} < 0.05$) (Figure 3.3d and Table B. 1). Overall, microbial networks reorganised under stress, but each group exhibited its own characteristic level of resilience or vulnerability (Figure 3.3 and Table B. 1).

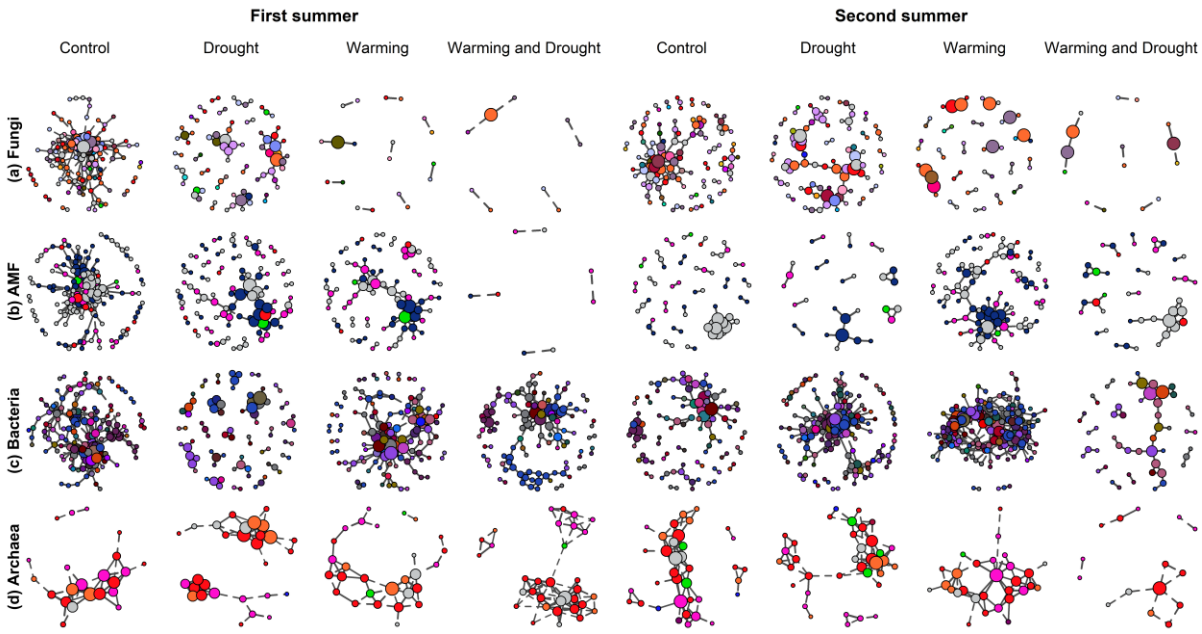


Figure 3.3: Microbial OTU-level co-occurrence networks coloured by order across treatments at first summer (Month 3) and second summer (Month 15). Each node represents a microbial OTU, coloured by its taxonomic order. Edges indicate significant pairwise co-occurrence relationships (Spearman $|\rho| \geq 0.60$, Benjamini–Hochberg $q \leq 0.05$). Node size corresponds to degree centrality.

Drought and warming effects on predicted functional pathway analysis

To assess whether the compositional and network shifts translated into changes in microbial functional potential, we analysed predicted metabolic pathways across treatments for each summer. We expected that warming and drought individually and in combination would differentially shape functional profiles. Consistent with expectation, functional predictions revealed clear treatment-dependent and group- specific shifts in metabolic capacity. Across both fungi and AMF, functional responses to climate stress showed a common trajectory: early disruption of biosynthetic pathways followed by reduced carbon- and energy-generating metabolism over time, with gluconeogenesis consistently suppressed, highlighting a shared energetic constraint across soil fungal symbionts under prolonged warming and drought. Bacteria showed consistent and strong stress-driven, particularly drought-driven, losses in nitrogen metabolism, carbon degradation, and aromatic compound breakdown,

indicating a central vulnerability in bacterial metabolic capacity and stress tolerance. Methanogenesis and its key supporting pathways Coenzyme M/B biosynthesis, flavin biosynthesis, and the archaeal TCA cycle were consistently suppressed across all stressors, revealing a universal climate-sensitive collapse in archaeal energy metabolism (Figure 3.4).

Fungal functional responses showed a clear progression from early disruption of amino-acid and nitrogen metabolism ($P_{adj} \leq 0.05$) to later, widespread suppression of carbon- and energy-producing pathways. Combined warming and drought caused the broadest early functional losses ($P_{adj} \leq 0.05$), and all stress treatments ultimately converged on significantly reduced gluconeogenesis I ($P_{adj} \leq 0.05$), revealing a shared energetic limitation under prolonged climatic stress (Figure 3.4a). Similarly, AMF functional responses showed a clear shift from early disruption of biosynthetic and nucleotide-salvage pathways to later suppression of central carbon and energy metabolism. During the first summer, all stressors significantly reduced ($P_{adj} < 0.001$) pathways linked to nucleotide salvage, amino-acid biosynthesis, and lipid/phospholipid metabolism, indicating strong early impacts on cellular maintenance. By the second summer, these effects converged on core carbon pathways, most consistently gluconeogenesis I ($P_{adj} < 0.001$), revealing sustained energetic limitation under prolonged stress. As in fungi, AMF ultimately showed convergence on reduced gluconeogenesis under all prolonged stresses, highlighting a shared energetic bottleneck across loci (Figure 3.4b).

Bacterial functional responses were dominated by drought-driven suppression of core metabolic pathways, with pronounced reductions in nitrogen-linked amino acid metabolism, carbon metabolism, carbohydrate degradation, and aromatic compound breakdown ($P_{adj} \leq 0.05$). Across all stressors and both summers, nitrogen-associated stress-response functions (e.g., super pathway of ornithine degradation, creatinine degradation etc) showed strongest and consistent declines ($P_{adj} < 0.001$), emerging as the most climate-sensitive metabolic bottleneck, linking disruptions in nitrogen recycling, reduced osmoprotection, and diminished energy balance. Warming and combined warming and drought also reduced multiple carbon- and aromatic-degrading pathways ($P_{adj} < 0.001$). Together, these patterns indicate that bacterial metabolism is broadly and consistently impaired under climate stress, with nitrogen and carbon-linked processes particularly vulnerable (Figure 3.4c). Across all stressors, archaeal functional profiles were marked by strong suppression of core energy and cofactor

pathways, most consistently methanogenesis, TCA cycle VI, and coenzyme M/B biosynthesis ($P_{adj} \leq 0.001$). Drought caused early reductions in amino-acid, nucleotide, and coenzyme metabolism ($P_{adj} \leq 0.001$), with targeted inhibition of methionine biosynthesis ($P_{adj} \leq 0.01$) by the second summer. Warming produced persistent and intensifying suppression of energy-yielding pathways including coenzyme M/B, TCA cycle VI, flavin biosynthesis, and L-glutamate/glutamine biosynthesis across both summers ($P_{adj} \leq 0.001$). Combined warming and drought imposed the strongest constraint, with widespread reductions in coenzyme, amino-acid, nucleotide, and central carbon metabolism during both summers ($P_{adj} \leq 0.001$), indicating severe and cumulative functional impairment under multi-stressor conditions (Figure 3.4d).

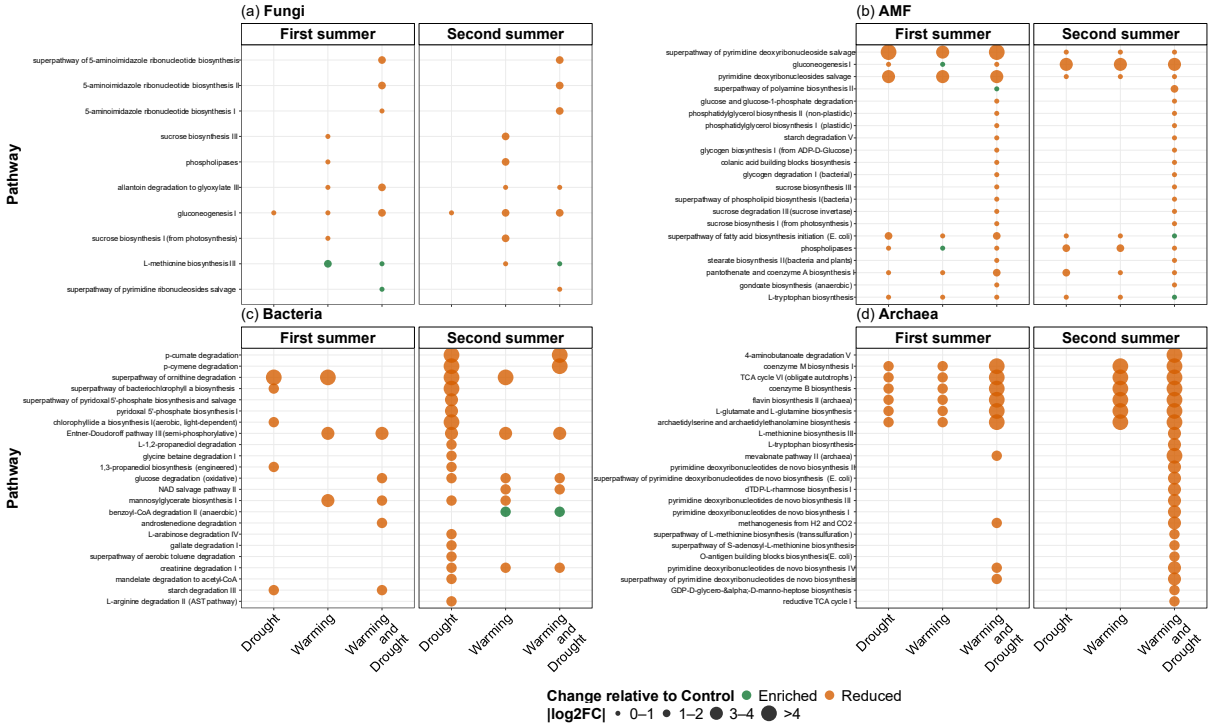


Figure 3.4: Differentially abundant functional pathways (PICRUSt2) for fungi, AMF, bacteria, and archaea to drought, warming, and combined warming-drought across two summers. Bubble colour indicates enrichment or reduction relative to the control, and bubble size reflects $|\log_2 FC|$. For bacteria and archaea, only pathways with $|\log_2 FC| > 2$ are shown to reduce visual complexity.

Discussion

Our results broadly support the hypothesis that soil microbial communities respond differentially to single and combined climatic stresses in the Australian sub-alpine ecosystem. Each group exhibited distinct compositional, network and functional shifts under warming, drought, and their combination, confirming that specific microbial lineages act as differentially abundant taxa of climatic perturbation. According to LEfSe analysis, responses differed among microbial groups; fungi showed their largest compositional shifts under combined stress during the first summer, whereas bacteria displayed the most persistent and pronounced turnover under drought. These contrasting patterns highlight the group-specific ways in which soil microbiomes reorganise under climate stress. Bacteria and archaea demonstrated greater compositional shift from copiotroph (thrive in environments with high nutrient concentrations, exhibiting rapid growth rates and feast-and-famine life strategies) to oligotroph (adapted to nutrient-poor environments, characterized by slow, efficient metabolism and the ability to grow at very low nutrient levels). Complementing these taxonomic patterns, fungal trophic mode responses indicated that prolonged climate stress weakened beneficial symbiotic associations while slightly favouring more opportunistic fungal groups.

Network analysis reinforced these compositional differences by showing that climate stresses reorganised microbial interactions in a group-specific manner. Revealing stress-induced reduction in connectivity and network complexity, most notably under combine stress. For fungi and AMF, combined stress consistently produced the simplest and least connected networks, with significant losses of nodes, edges, and average degree indicating weakened ecological connectivity under co-occurring stress.

In contrast, bacterial networks showed no significant reductions in connectivity under stress and even became more complex under warming, highlighting strong resilience and domain-specific differences in how microbial interactions respond to climatic change. Our analysis reveals a similar trend when viewed through a functional lens. We found clear stress-specific metabolic constraints: fungi and AMF consistently converged on suppressed carbon- and energy-producing pathways under prolonged stress, bacteria showed strong drought-driven

reductions in core carbon, nitrogen, and aromatic-compound metabolism, and Archaea exhibited repeated suppression of methanogenesis and central energy pathways together indicating that climate stress broadly constrains belowground metabolic capacity. Collectively, the way that the combined stresses exacerbated suppression of multiple fungal and archaeal lineages likely reflects the compounded physiological limitations imposed by reduced soil moisture and elevated temperature on growth, enzyme activity, and resource acquisition.

Below we consider the drivers of differential sensitivity among the groups, the effects on community networks and the functional implications of these changes. We place these results in the context of effects of extended climate change on microbial community composition, fungal trophic mode response, co-occurrence network and function.

Differential sensitivity of microbial groups to climate stress

Analysis using LEfSe revealed that fungal communities were most responsive to simulated climate change, with extensive loss of ectomycorrhizal and macro-Basidiomycete lineages and concurrent enrichment of saprotrophs. This pattern suggests a shift from mutualistic and resource-sharing interactions toward stress-tolerant, fast-growing decomposers, potentially weakening plant-fungal symbioses essential for nutrient turnover (Lozano et al., 2021; Maisnam et al., 2023). Among AMF, different Diversisporales lineage suppressed under all stress in both summers can be explain as its high sensitivity towards moisture stress (Weber et al., 2019) and which also results its low root infection (Zhang et al., 2018). Though *Diversispora* increased only under combined warming and drought in the first summer and showed suppression to all three stress treatments by the second summer, suggesting an opportunistic early response that was not sustained under prolonged climatic stress. However, another study documented that in temperate managed grassland *Diversispora* enhanced by overruling the drought sensitivity (Albracht et al., 2023). In contrast, *Paraglomus* lineages consistently enhanced under warming and combined warming and drought treatment, suggesting its tolerance towards stress also consistent to literature (Albracht et al., 2023). This genus is rhizophilic (high allocation to intraradical hyphae, with limited extraradical hyphae) with high abundance in grass roots (Weber et al., 2019) and was

previously reported as having a positive influence on drought resistance of plants (Tian et al., 2013; Zhang et al., 2018). The decline of Glomerales under prolonged stress, align with previous studies with similar stress conditions (Alguacil et al., 2022). This suggests that only a few AMF groups can tolerate concurrent warming and moisture limitation stressors. However, these results reflect relative rather than absolute abundance, and the observed patterns may also arise from the persistence of these genera while others decline.

For bacteria, a strong and persistent effect of drought and a shift from copiotrophic (carbon-demanding, fast growing) to oligotrophic (stress-tolerant, slow growing) dominance is indicated, which aligns with theoretical expectations and previous observations that environmental stressors such as warming and drought suppress fast-growing, carbon-demanding copiotrophs while favouring slow-growing, stress-tolerant oligotrophs (Barnard et al., 2013; Fuchslueger et al., 2014). Furthermore, drought reduces plant productivity and nitrogen fixation (Ma et al., 2020), thereby decreasing the supply of soil total nitrogen (TN) and limiting the growth and multiplication of bacteria. Under drought stress, soil moisture mobility is poor, affecting soil nutrient mobility (Moyano et al., 2013), thus bacteria perish or are downregulated because of a lack of sufficient energy sources. Such transitions reflect microbial community reorganisation towards more resource-efficient, resilient assemblages under conditions of carbon and water limitation in sub-alpine soils. In case of archaea, methanogenic groups declined under drought, warming and combined warming and drought stress, whereas ammonia-oxidizing archaea, such as *Nitrososphaeria*, increased under stress conditions. This contrasting response indicates a functional shift in the archaeal community from methane production to nitrification. The decline of methanogenic groups is consistent with previous studies showing that drier soils reduce anaerobic conditions and led to a substantial decline in methanogenic archaea and associated methane emissions (Tian et al., 2012). Similarly, the increase of abundance of ammonia-oxidizing archaea under drought and warming agrees with previous findings Sheik et al. (2011). This pattern is further supported by evidence that drought can enhance ammonia oxidation and nitrification processes in soils (Yan et al., 2023). This suggests increased nitrate (NO_3^-) production, a key form of nitrogen available for plant uptake. However, this does not necessarily improve plant nutrition, as drought limits nutrient movement and root uptake (He & Dijkstra, 2014). Moreover, nitrate

is highly mobile and prone to loss via leaching or gaseous pathways (Wang et al., 2015). The enrichment of ammonia-oxidizing archaea therefore reflects a microbial adaptation to more oxygenated and resource-limited environments, highlighting the role of archaea in maintaining nitrogen cycling under environmental stress rather than indicating a direct benefit to plant performance.

Collectively, these changes indicate combined warming and drought caused the disappearance of certain microbial taxa, reorganized soil microbial communities toward stress-resilient, low-activity assemblages with reduced functional redundancy. These changes would potentially slow carbon turnover and altering nitrogen cycling pathways in alpine soils, though these losses are unlikely to be rapidly compensated by the colonization of adapted microbes (Álvarez-Garrido et al., 2019).

Effect of drought and warming on fungal trophic modes

Patterns in fungal trophic modes reflected subtle but ecologically meaningful shifts under prolonged climate stress. Although no significant differences emerged during the first summer, the second summer showed a significant decline in symbiotrophs under combined warming and drought, with a marginal decline under drought alone. At the same time, the upward trend in pathotrophs suggests that plant-mutualistic guilds are among the most sensitive to warming and moisture loss, whereas plant pathogens and certain saprotrophic decomposers are better able to cope or even increase under sustained stress (Baldrian et al., 2022; Lozano et al., 2021; Wan et al., 2023). A likely mechanism, supported by previous work, moisture stress reduces plant productivity and increases mortality of plants and soil organisms, resulting in greater inputs of dead organic material and subsequent enrichment of soil organic matter (SOM) (de Vries et al., 2018). This SOM pulse favours saprotrophic and pathogenic guilds while suppressing symbiotic fungi, driving a functional shift from symbiotroph-dominated roles toward saprotroph- and pathotroph-associated strategies (Liu & He, 2021; Sokol et al., 2022; Wan et al., 2023). This aligns with evidence that mycorrhizal and other mutualistic fungi are often sensitive to sustained moisture limitation and elevated temperatures, frequently showing reduced activity, impaired carbon exchange, or diminished colonisation under prolonged stress (Compant et al., 2010; Sendek et al., 2019; Wilson et al.,

2016), resulting in impaired plant growth and disease resistance (Umer et al., 2025; Wahab et al., 2023).

Effect of drought and warming on soil microbial community networks

We hypothesized that experimental drought and warming would reduce the complexity of microbial associations, as greater interconnectivity among taxa can promote ecosystem stability and functional efficiency (Högberg et al., 2007; Wagg et al., 2019). Consistently, our co-occurrence network analysis revealed that climate perturbations restructure microbial co-occurrence architectures in ways that are strongly group-specific, with fungi being the most consistently vulnerable, archaea showing intermediate resilience, and bacteria responding in more dynamic but statistically weaker ways. Under combined stress, all microbial groups showed simpler networks, meaning their interactions were disrupted and the overall community became less stable. This might be because sub-alpine microbial communities are inherently adapted to cooler, wetter environments (Bardgett & Caruso, 2020; Donhauser & Frey, 2018), so combined warming and drought-imposed conditions outside their niche, leading to a marked reduction in network complexity (de Vries et al., 2018; Fu et al., 2024). Previous work suggests that fungi generally exhibit greater resistance to drought than bacteria, owing to their hyphal structure and capacity to access microscale water pockets (Bapiri et al., 2010; de Vries et al., 2018; de Vries & Shade, 2013). In contrast, bacteria typically show low resistance but high resilience, collapsing under moisture limitation but recovering rapidly upon rewetting (Barnard et al., 2013). Our bacterial networks followed this pattern: drought caused an initial loss of connectivity during the first summer, but networks expanded substantially by the second summer, indicating strong resilience. However, our sub-alpine fungal networks did not match the expected drought resistance. Instead, fungi showed pronounced reductions in network complexity under all stress, particularly under warming and combined warming and drought suggesting that in alpine soils, temperature stress may override the moisture-buffering capacity often attributed to fungal communities. In addition, in alpine grasslands, moisture stress sharply limits plant productivity and belowground carbon allocation (Moravcová et al., 2023), hitting mycorrhizal and root-associated fungi that rely on

fresh plant carbon (Qi et al., 2021). As a result, the hyphal traits that often make fungi drought resistant elsewhere cannot fully offset this carbon shortage, so mutualistic fungal networks collapse even though some saprotrophic, stress tolerant taxa persist, which also align with our finding for AMF (Hernandez et al., 2021; Luo et al., 2023). Exposure to warmer and drier conditions likely imposed strong stress and drove compositional turnover, so that communities became increasingly dominated by thermotolerant taxa while more sensitive taxa declined (Bardgett & Caruso, 2020). While these fungi may successfully colonize and survive, their ecological relationships became simplified, with fewer and weaker connections among taxa, resulting in lower overall network cohesion. Such simplification reflects a loss of functional redundancy and may reduce the resilience of fungal-mediated processes such as decomposition and nutrient mobilization. In contrast, bacterial interactions likely stem from their high metabolic flexibility, rapid turnover, and capacity to exploit resource fluctuations under stress, leading to strengthened co-occurrence patterns and tighter regulatory linkages. These divergent trajectories suggest a gradual reorganization of soil microbial networks under climate stress, with bacteria becoming the dominant regulators of belowground interactions as fungi lose structural complexity, potentially altering ecosystem multifunctionality and soil carbon stability in alpine environments.

Previous studies have shown that network complexity is positively correlated with ecosystem functioning (Li et al., 2022; Wagg et al., 2019). An increase in network complexity improves the efficiency of energy flow and material cycling in ecosystems, which positively affects the functioning of an ecosystem (Högberg et al., 2007). In this study, we found that climate stresses increased bacterial network complexity but decreased fungal network complexity. It appears as if fungi are not well equipped to survive under warming and combined stresses, leading to survival and colonization of a subset of fungi that are stress adapted or tolerant, and a loss of structural complexity in fungal networks. In contrast, under stresses especially warming and combined warming and drought stresses bacterial network showed increased complexity. After warming, the warm and rich environments promote bacterial growth and colonisation, and the interactions and regulation among bacterial communities are enhanced, subsequently increasing the complexity of bacterial networks. This result is consistent with the findings of Yuan et al. (2021), who found that warming increases the complexity of

bacterial networks in their warming experiments on grassland soils. This process may have implications for ecosystem soil multifunctionality and plant community stability (Chen et al., 2023; in ‘t Zandt et al., 2023; Wang et al., 2024) and requires further research and understanding.

Predicted functional potential of microbial communities under climatic stress

Predicted fungal and AMF functions showed early disruption of protein synthesis and nitrogen metabolism, followed by strong downregulation of carbohydrate-energy pathways (gluconeogenesis and sucrose biosynthesis) as stress persisted. These patterns are consistent with established microbial stress mechanisms such as altered N-processing enzymes (Munene et al., 2025), reduced plant belowground carbon allocation (Wang et al., 2021), shifts toward stress-tolerant fungal taxa with lower investment of carbohydrate degradation (Morrison et al., 2019), and depletion of labile carbon substrate (Pold et al., 2016). These mechanisms collectively explain the progressive downregulation of fungal and AMF pathways under environmental stress.

Bacteria showed the strongest and broadest functional suppression, with drought, rapidly reducing dozens of carbohydrate-degradation pathways, far exceeding the magnitude of change observed in other groups. The reason maybe that due to a high susceptibility of bacteria to drought (Kaisermann et al., 2017; Zhang et al., 2016), indicating that the stability and interaction of bacterial communities were severely impaired by drought stress. The strong suppression of bacterial carbon-degradation pathways in our system spanning starch, sucrose, glucose, glycogen, fucose, rhamnose, sugar acids, and aromatic compounds is consistent with established drought mechanisms. Water limitation severely restricts substrate diffusion and reduces the efficiency of extracellular carbon-degrading enzymes (Sardans & Peñuelas, 2010), leading to immediate downregulation of functional pathways involved in labile C breakdown. Bacterial communities are known to respond within hours to changes in water availability (Blazewicz et al., 2014; Nielsen & Ball, 2015), which aligns with the rapid functional decline observed in first summer and consisted in the 2nd summer.

Similar reductions in carbon- and nitrogen-cycling genes have been reported under drought in alpine peatland ecosystems (Yan et al., 2023), suggesting that moisture and temperature stress jointly constrain bacterial metabolic potential. By the second summer, suppression extended to pathways linked to sugar acids, sulphur cycling, and aromatic compound degradation, indicating a sustained decline in bacterial contributions to soil carbon turnover. Together, these mechanisms support the view that drought imposes the strongest functional constraint on bacterial communities, driving widespread inhibition of carbon-processing pathways across seasonal timescales (Wan et al., 2023).

Archaea showed strong functional sensitivity to warming and drought, with consistent suppression of core energy, cofactor, and amino acid/nucleotide biosynthesis pathways. These impacts intensified over time, shifting from broad metabolic suppression in the first summer to deeper, targeted inhibition of central metabolism especially methanogenesis, TCA cycle activity, and coenzyme M/B biosynthesis in the second summer. Combined warming and drought produced the most severe declines, indicating an escalating collapse of archaeal metabolic capacity under dual stress. These responses align with established archaeal metabolic constraints: archaea evolved under chronic energy limitation and rely on costly, highly specialized cofactors, making their metabolic networks particularly vulnerable to environmental stress (Thor et al., 2017). Valentine (2007) similarly demonstrated that archaeal metabolism is organised around fragile, low-energy yield pathways, particularly methanogenesis and cofactor-dependent energy conservation, making the strong suppression of methanogenesis, coenzyme M/B biosynthesis, and TCA cycle activity observed here an expected consequence of energetic disruption under warming and drought.

Conclusion and future directions

The microbial communities in Australian sub-alpine ecosystems are extremely understudied, and we lack a baseline knowledge on the microbial composition and function in these soils. However, it is important to better understand the soil microbial communities in this ecosystem if we seek to understand the effect of climate change on soil microbial composition and function. Both are likely to respond to environmental changes and impacts the productivity and C storage ability of this ecosystem. Our study has demonstrated that isolated

and combined climatic stresses distinctly reshape the composition, interactions, and functional potential of soil microbial communities in the Australian sub-alpine ecosystem. Combined warming and drought imposed the strongest and most pervasive impacts, driving the enhance of stress-tolerant microbial taxa. Results of LEfSe analysis identified group-specific differentially abundant taxa that reflect variance adaptive strategies, climate stress shifts the prokaryotic communities from copiotroph to oligotroph and shifted the relative abundance of fungal guilds towards more saprotrophic and opportunistic communities. Network analyses revealed a loss of connectivity and stability under concurrent stress, suggesting weakened ecological cooperation. These changes in composition and connectivity were further illustrated by the functional predictions. We found evidence of consistent reductions in metabolic diversity and biosynthetic capacity, particularly under prolonged warming and moisture limitation, signalling a reorganization of belowground ecological roles. Collectively, these findings highlight that climate-induced stress not only alters microbial diversity but also reorganizes community function and interactions, potentially constraining the resilience of sub-alpine soils to future warming and drying. Thus, current and future changes of climate conditions in this ecosystem are likely to lead to altered microbial communities, and associated network and functions, which may in turn have implications for broader ecosystem function changes in this sub-alpine ecosystem.

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Chapter 4



Chapter 4: Prolonged drought and warming cause differential changes in the root microbiome and growth of sub-alpine plants

Abstract

Climate change is reshaping the structure and function of high-elevation ecosystems, affecting plant performance, root microbial communities, and the ecological context of plant-microbe interactions. Integrating long-term observations with manipulative multi-stress experiments provides powerful yet underutilized, approach for assessing the sensitivity of such ecosystems to climatic change. We conducted growth chamber experiments to isolate the microbial-mediated effect on plant performance and to evaluate the impacts of drought, warming and their combination on plant performance and symbiosis. Warming and drought effects on biomass accumulation rate interacted and were strongly plant species-dependent. Under drought, root fungal trophic mode richness increased relative to well-watered plants, irrespective of the temperature. Our results suggest that microbial legacy effects and mutualist partners can facilitate plants to grow faster and partially mediate plant responses to climate stress. However, we detected only weak coordination between plant performance and microbial community attributes, suggesting a partial decoupling of above- and belowground responses. Overall, these results demonstrate that drought and warming differentially affect plant performance and root fungal diversity, while community assembly and interaction structure remain buffered against short-term climate perturbations. This decoupling highlights the resilience and functional redundancy of root fungal communities in sub-alpine plants and suggests that microbial restructuring under climate stress does not necessarily translate into proportional effects on host growth.

Introduction

In the previous two chapters, we demonstrated that soil microbial communities in Australian sub-alpine systems are highly sensitive to climatic stressors such as drought and warming, exhibiting shifts in microbial diversity, community structure, and functional potential, consistent with findings from other ecosystems (Allison & Treseder, 2008; Feng et al., 2022; Melillo et al., 2017). However, although plants recruit their root-associated microbiome from the surrounding soil microbial community (Badri & Vivanco, 2009), it remains unclear whether host-associated microbiomes respond to drought and/or warming in parallel with broader soil microbial communities. Changes in soil microbial composition may translate into altered root-associated communities and modified functional outcomes for host plants (Fitzpatrick et al., 2019; Rubin et al., 2018). In particular, root-associated fungi, including symbiotic taxa, can enhance plant access to limiting nutrients and water, confer protection against pathogens, and influence plant stress tolerance (Ali et al., 2022; Allison & Treseder, 2008; Berendsen et al., 2012; Koide, 1991; Quiroga et al., 2017). Consequently, root-associated fungi are considered a critical component for forecasting the impacts of future climate change on terrestrial ecosystems (Su et al., 2020; Xie & Yin, 2022; Zhang et al., 2018) and their response to climate stressors should be investigated to predict their resilience in future climate change scenarios.

The capacity of root-associated fungi to buffer plants against abiotic stress such as drought and warming is not universal and may depend on host identity and environmental context (Lorberau et al., 2017; Wei et al., 2022). Climatic stressors may alter microbial interactions along a continuum from mutualism to parasitism, particularly under stressful environmental conditions (Davis et al., 2015; Rudgers et al., 2020). Consequently, microbial buffering effects can weaken or break down entirely under extreme stress (Santos-Medellín et al., 2017; Zhang et al., 2024). Despite this, the combined effects of multiple climate stressors on root-associated fungal communities and their consequences for plant growth remain relatively understudied (Kivlin et al., 2013; Lozano et al., 2021), even though multiple climatic drivers are shifting simultaneously (Thuiller, 2007). Moreover, while large-scale studies have examined mycorrhizal fungi in relation to global plant diversity patterns (Fei et al., 2022;

Zhong et al., 2021), less is known how root-associated fungal assemblages respond to controlled experimental manipulations of temperature and moisture within defined host systems.

Despite growing recognition of the importance of plant-fungal-climate interactions, several key gaps remain in the experimental literature. Many studies continue to examine single stressors, such as warming or drought, in isolation (Dobson & Zarnetske, 2025; Karlowsky et al., 2018; Li et al., 2021; Wahren et al., 2013), even though multiple climatic drivers are shifting simultaneously in natural systems (Kivlin et al., 2013; Thuiller, 2007). Moreover, most studies focus almost exclusively on aboveground phenotypic responses (De Boeck et al., 2016; Eziz et al., 2017; Ma et al., 2021; Möhl et al., 2022; Wei et al., 2024a; Wilschut et al., 2022), or alternatively on belowground microbial dynamics (Chen et al., 2025; Lagueux et al., 2021; Wei et al., 2024b), with relatively few studies integrating both components within a unified experimental framework (Melillo et al., 2017; Pec et al., 2021). As a result, interactive effects among stressors, particularly non-additive or threshold responses, remain poorly understood.

Furthermore, even when microbial processes are considered, their effects are often inferred indirectly rather than tested through explicit manipulation of soil inocula. Plant performance is also frequently assessed using static end-point metrics, such as final biomass or survival, which obscure temporal growth dynamics. This limits mechanistic insight into how stress effects accumulate over time and whether plants exhibit early sensitivity, delayed decline, or compensatory growth responses (Classen et al., 2015). Consequently, there is a clear need for integrative experiments that simultaneously manipulate multiple climate stressors, incorporate genotypic resolution, directly manipulate microbial communities, and quantify plant performance using temporally resolved growth measurements.

Climate change is projected to have a significant impact on the Australian sub-alpine ecosystem (Whetton & Chiew, 2021), with ongoing alterations in alpine climates expected to intensify in the coming century (Bhend et al., 2012). It is predicted that every 1°C increase in air temperature will increase soil temperature by 1.5°C in southeastern Australia (Ooi et al., 2012). These changes are anticipated to lead to drier and warmer conditions, and consequently more frequent occurrences of heatwaves, storms, droughts, and extreme fire

conditions (Hennessy et al., 2005). As a result, alteration in the habitat suitability may occur, potentially influencing the competitive dynamics between native and introduced species within the Australian alpine ecosystem (Petitpierre et al., 2016). Moreover, alpine species would have a greater risk of extinction due to global warming (Guisan & Theurillat, 2000).

Australian alpine environments have relatively low maximum elevations (Costin et al., 2000) and a restricted geographic extent, opportunities for upward range shifts under warming are limited, increasing the likelihood that species may 'top-out' as temperatures rise (Staude et al., 2020). This structural constraint positions the Australian Alps as an early indicator of climate-driven change in alpine ecosystems globally. Because opportunities for upward range shifts are constrained in the Australian Alps (Auld et al., 2022; Sritharan et al., 2021) and low dispersal capacity of alpine species (Morgan & Venn, 2017), long-term persistence will depend not only on physiological tolerance but also on ecological interactions that mediate stress responses, including plant-microbe relationships. Experimental manipulation of warming and drought in this system therefore provides a mechanistic framework for assessing both immediate phenotypic responses and potential limits to persistence under future climate change.

The aim of this chapter is to experimentally disentangle the direct effects of warming and drought from microbially mediated effects on plant performance, and to determine whether these responses are species-specific and reflected in root fungal community dynamics under controlled greenhouse conditions. This was tested using a multi-factorial greenhouse experiment manipulating temperature, water availability, and soil inoculum (live vs. sterile), allowing the direct effects of climate stress to be separated from microbially mediated effects. By testing three alpine plant species with contrasting mycorrhizal dependencies under controlled conditions, this design provides a robust framework to disentangle plant and microbial responses to warming and drought.

We hypothesised that warming and drought would reduce plant growth, particularly under combined stress, and that responses would differ among species. We further predicted that native soil microbes would partially buffer plant performance under stress. At the microbial level, we expected climate stress to restructure root fungal communities, with plant identity

contributing to community assembly. Finally, we predicted that shifts in root fungal communities would be associated with variation in plant performance. This study provides mechanistic insight into how warming and drought interact with soil microbial communities to influence plant performance and root fungal dynamics, improving our understanding of plant-microbe responses to climate change in alpine ecosystems.

Materials and methods

We designed a multi-factorial greenhouse experiment to test the direct and microbially mediated effects of warming and drought on plant performance and root fungal communities by manipulating temperature, water availability, and soil inoculum (live vs. sterile). In Kosciuszko National Park (NSW), the subalpine zone is largely characterised by snow gum (*Eucalyptus pauciflora*) woodland interspersed with treeless grassy herbfields, sedgelands and alpine plains occupying frost hollows and exposed high-elevation surfaces (Costin et al., 2000; McDougall & Walsh, 2007). These treeless communities are among the most widespread alpine vegetation types and are structured by tussock grasses such as *Poa costiniana* (prickly snow grass), perennial forbs including *Celmisia pugioniformis* (snow daisy), and sedges such as *Carex capillacea*, alongside other herbaceous and low-shrub species (e.g., *Oxylobium ellipticum*, *Podolepis robusta*, *Plantago muelleri*, and *Podocarpus lawrencei*). Together, these taxa represent the principal growth forms shaping alpine grassy herbfields and contribute substantially to ecosystem productivity and belowground processes. Species within these communities differ in growth form, rooting depth and symbiotic strategy, particularly in their dependence on arbuscular mycorrhizal fungi (Brundrett, 1991; Moyle & Deslippe, 2024). By selecting co-occurring representatives of Poaceae (*Poa*), Asteraceae (*Celmisia*) and Cyperaceae (*Carex*), this study captures functional contrasts in growth form and microbial dependency within a shared alpine community, allowing explicit tests of whether warming and drought responses are conserved across lineages or vary in a species-specific manner.

Experimental design overview

The experiment comprised two sequential phases: (i) a greenhouse establishment phase designed to isolate the effects of soil microbial inoculation in the absence of abiotic stress, and (ii) a growth capsule phase employing a fully factorial design to test the interactive effects of temperature, water availability, and soil inoculum under controlled climatic conditions (Figure 4.1). A total of 324 plants were used in the experiment, representing combinations of three water regimes (well-watered, 50% drought, 70% drought), two temperature regimes (ambient and elevated) and two soil inoculum treatments (live and sterile) with nine replicate plants per treatment combination.

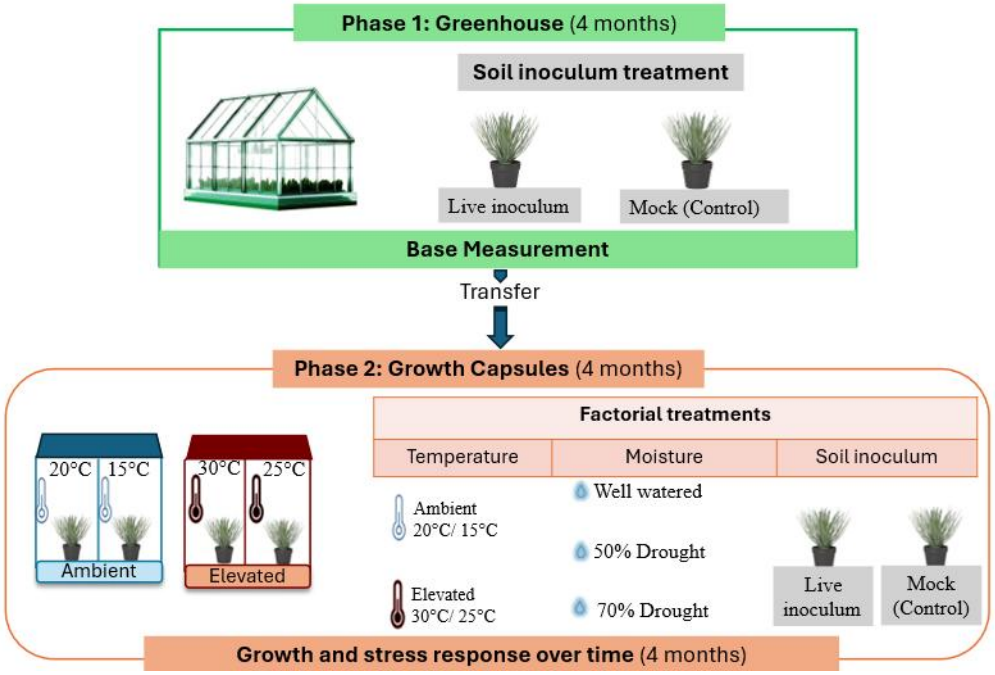


Figure 4.1: Experimental design illustrating the two-phase approach: plants were first grown under live or sterile soil inoculation in a greenhouse to assess baseline microbial effects, then transferred to growth capsules where temperature and moisture treatments were applied in a fully factorial design.

Plant species selection

To examine species-specific responses to warming, drought, and soil microbial inoculum, three widespread alpine plant species characteristics of treeless grassy herbfields in

Kosciuszko National Park were selected: *Poa costiniana*, a perennial tussock grass; *Celmisia pugioniformis*, a perennial rosette forb; and *Carex capillacea*, a perennial sedge. These species co-occur across alpine and subalpine plains and represent dominant functional groups within these systems, capturing structural and ecological diversity among grasses, forbs, and sedges. *Poa costiniana* contributes substantially to aboveground biomass and grassland structure, *Celmisia pugioniformis* represents broad-leaved herbaceous forb that contribute to floristic diversity, and *Carex capillacea* is typical of moist microsites where sedges contribute strongly to belowground biomass and soil stabilisation. Importantly, these species differ in their potential mycorrhizal associations and belowground ecological strategies. Grasses and many Asteraceae are commonly associated with arbuscular mycorrhizal fungi (AMF), whereas sedges frequently exhibit reduced, variable, or facultative mycorrhizal colonisation. These contrasts in phylogenetic diversity, growth form, and likely microbial association provide a suitable framework for testing whether plant responses to climate stress and soil microbial inoculum are consistent across the three study species or are species-specific. Seeds were sourced from the Australian National Botanical Garden Seed Bank and surface sterilised in 5 ml of a 1% NaOCl solution for 5 mins, washed with Milli-Q water and air dried prior to germination. To confirm seed viability, representative seeds were germinated on 1% water ager media at 20°C during the 14 h light period and 15°C during the 10 h dark period (data not shown).

Soil collection and preparation

A pilot experiment was conducted to determine an appropriate potting mix that supported consistent germination and adequate early growth across all three plant species. The mixture selected consisted of 50% coarse sand, 20% potting mix and 30% sub-alpine soil. The sand facilitated harvest of the roots for the microbial study, whereas the alpine soil provided the physiochemical and microbial component of our field experiment site (Kosciuszko National Park), and the potting mix provided some nutrients, soil drainage and aeration. The alpine soil was collected from Kosciusko National Park (-36.374, 148.429) in November 2022. After collection, soil was stored at -18°C until the experiment started in June 2023. All soil components were sieved through a 3.36 mm sieve to remove stones, coarse material and

roots. The soil mixture was homogenised using a cement mixer to ensure thorough and uniform mixing. A total of 184 kg of this soil mixture was sterilized by gamma radiation a dose of 50 kGy (Steritech Pty. Ltd, Wetherill Park, NSW, Australia), leaving aliquots of unsterilized sub-alpine soil for each pot for subsequent microbial inoculation. Gamma irradiation was used because, unlike heat-based sterilization methods such as autoclaving, it is not expected to substantially increase soil nutrient availability through thermal breakdown of soil organic matter. Sterilizing the soil enabled assessment of the role of field biotic factors (soil communities) by comparing plants grown in inoculated soils vs plants grown in uninoculated soils (sterile soil).

Plant propagation

The experiment was conducted using a pot size 0.46L with drainage holes covered with a layer of fly screen (Bunnings Australia) and a piece of paper towel to minimize soil leaching. Each pot was filled with 460g sterilized soil. Five seeds were sown into each pot in June 2023 and then thinned to one healthy individual per pot after a month. To control saprophytes/soil mold developing on the soil mixture surface, the fungicide Banrot 400 WP (active constituents 250g/kg Thiophanate-Methyl and 150g/kg Etridiazole) @ 400g/L was applied once to all pots 3 days after seed sowing. Fungicide application was necessary to prevent mould development from compromising plant establishment and experimental viability; because all pots received the same treatment, any direct effects of the fungicide were applied consistently across experimental treatments.

To assess the effect of soil microbes on plant growth and their ability to alleviate environmental stress, 50% of pots received a live soil inoculum added as a microbial wash, while the remaining pots received a sterile soil inoculum. The inoculum was prepared from field soil collected from the same source used to generate the irradiated potting substrate, with live soil representing 10% of the soil volume. The live soil was mixed with water to create a soil–water slurry and applied two weeks after fungicide treatment. Inoculation was repeated monthly until harvest to maintain microbial assemblages in the live-treated pots, while sterile pots received a mock inoculation using a sterile soil microbial wash. To prepare the microbial wash, 1 kg of live soil was mixed with 5 L of water to form a slurry, and 20 mL

of the suspension was added to each pot once every month starting two weeks after seed sowing.

Plants were fertilised weekly after 10 weeks of seed sowing with a low phosphorus fertilizer, Aquasol @8 g/ 10L (NPK 23: 3.95: 14, including S, Mg, Mn, Cu, Fe, Zn, B) for the next five weeks to ensure establishment of the plants. Each plant received ~40 ml of liquid every time. Fungus gnats were managed using sticky traps throughout the experiment. A single application of a commercial insecticide (Azamax®) was applied along the growth capsule walkway approximately six months after seed sowing.

Greenhouse establishment phase: Baseline effects of soil inoculum

To isolate the effects of soil microbial inoculum in the absence of abiotic stress, plants were first established under controlled greenhouse conditions for sixteen weeks following seed sowing. During this period, all pots were maintained under well-watered conditions. Irrigation was applied twice daily using overhead sprinklers, supplemented by capillary irrigation twice per week, whereby trays were filled with water, and pots were allowed to absorb water from below for approximately three hours.

The greenhouse environment was maintained at 20°C during the 14 h light period and 15°C during the 10 h dark period. Pots were regularly randomised to minimise positional effects. To prevent cross-contamination between microbial treatments, plants receiving live and sterile soil inoculum were maintained on separate benches within the same greenhouse.

At week 16, before applying temperature and drought treatments, digital biomass was quantified to assess baseline growth differences attributable solely to soil inoculum. Plant growth was monitored non-destructively using a 3D multispectral canopy scanning system (Phenospex PlantEye F500 Dualscan, Phenospex, Heerlen, The Netherlands). High-resolution 3D point clouds were used to derive digital biomass, which served as the primary growth metric analysed in this chapter. Phenotyping commenced at week 16 and continued weekly until week 32, spanning both the greenhouse baseline phase and the subsequent climate

treatment phase. All phenotyping was supported by the Australian Plant Phenomics Facility (APPF).

Capsule phase: Factorial temperature and water treatments

Following the greenhouse establishment phase, plants were transferred in October 2023 to controlled growth chambers at the Australian Plant Phenomics Facility (APPF), Australian National University. To examine the interactive effects of climate stress and soil microbial inoculum, plants were subjected to a fully factorial combination of temperature (ambient vs elevated), water availability (well-watered, 50% drought, 70% drought), and soil inoculum (live vs sterile).

Temperature treatments were applied using separate growth chambers. Ambient conditions were maintained at 20°C/15°C (day/night), while elevated temperature conditions were set at 30°C/25°C (day/night). In both chambers, maximum light intensity was 700 µmol m^{-2} s^{-1}, the photoperiod was 12 h light/8 h dark, and relative humidity was maintained at 50%. Day–night temperature transitions were ramped over one-hour intervals at 2°C increments until target temperatures were reached. The 10°C difference between ambient and elevated treatments represents an approximate +5°C increase in air temperature under projected future climate scenarios, plus an additional +5°C increase at the soil level, consistent with predicted alpine soil warming beyond 2050 (Hoyle et al., 2013). Pots were randomly arranged within each chamber, with live and sterile inoculum treatments maintained separately to prevent cross-contamination. Drought treatments were initiated after transfer to the growth chambers. Soil moisture was monitored as percentage volumetric water content (VWC) by weighing pots using an analytical balance (Digitech Digital Bench Scale, Model QM-7264). For drought treatments, watering was withheld for four days until initial wilting symptoms were observed. This wilting response was used to establish the reference moisture condition for subsequent drought treatments. Thereafter, pots were weighed fortnightly and watering was adjusted to maintain the predefined moderate- and severe-drought moisture levels, corresponding to 50% and 30% of the reference soil water level, respectively. After one month, drought-treated pots were re-watered once to restore soil moisture and reduce the risk of plant mortality. Within each water regime, ambient and elevated temperature

treatments were maintained at comparable WHC levels to isolate the direct effects of temperature and minimise indirect warming effects mediated through soil drying.

Harvesting

Plants were harvested after 36 weeks of growth in the capsules. Shoots were excised at the soil surface, and roots were carefully separated from the soil by gently shaking off excess substrate, followed by rinsing with water to remove remaining soil particles, taking care to minimise loss of fine roots. Approximated 700 mg of roots were added to 80% ethanol for molecular analyses of root microbial colonization and kept at -20°C for further processing. All shoots and roots were oven-dried separately at 60°C for five days to constant weight. Dry shoot and dry root biomass were then recorded using a precision balance. To prevent cross-contamination, all instruments were sterilised with ethanol between samples.

DNA extraction and sequencing

Roots from ethanol were thoroughly washed with deionized water and transferred to a 2 ml tube and kept at -80 ^{o}C until DNA extraction. DNA extraction was performed on approximately 100 mg of fresh root material using the DNeasy plant pro kit (250) (Qiagen) as per manufacturer instruction. DNA extraction and sequencing followed the same procedure as chapter 1. Briefly, the quality of extracted DNA was checked with NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA), the quantity was checked with a Qubit Fluorometer (Thermo Fisher Scientific), and normalized. Root endophytes (fungi) and mycorrhizal associations was determined by amplicon sequencing using the primer sets ITS1F (5’-CTTGGTCATTTAGAGGAAGTAA-3’)/ ITS2R (5’-GCTGCGTTCTTCATCGATGC-3') (Caporaso et al., 2012) and AMV4.5NF (5’-AAGCTCGTAGTTGAATTTCG-3’) and AMDGR (5’-CCCAACTATCCCTATTAATCAT-3’ (Sato et al., 2005) to amplify a portion of the fungal ITS1 region and 18S rRNA gene fragment, respectively. Sequencing was carried out with 2×300-bp paired-end reads using the v3 chemistry on the Illumina MiSeq platform at the Biomolecular Resource Facility (BRF) in the Australian National University.

Statistical analysis

Temporal variation in digital biomass was analysed using linear mixed-effects models to quantify treatment effects on plant growth dynamics over time. Biomass measurements derived from repeated Phenospex 3D scans were log-transformed prior to analysis to stabilise variance and model proportional growth. Fixed effects included temperature (ambient, elevated), water availability (well-watered, 50% drought, 70% drought), species (*Poa*, *Celmisia*, *Carex*), day (number of days since transferring pots to the growth chambers), soil inoculum (sterile, live), and all interactions among these factors, with a random intercept for experimental unit to account for repeated measurements on individual plants. Models were fitted using maximum likelihood estimation, and Type III Wald χ^2 tests were used to assess the significance of fixed effects. Treatment effects on biomass growth rate were quantified using estimated marginal trends (emtrends) with respect to time (day), representing the average rate of change in log-transformed biomass per day. Growth rates were estimated separately for each temperature × water combination within species, over Days 6–90 for *Poa* and *Carex* and up to Day 120 for the slower growing *Celmisia*. Temperature effects were evaluated by contrasting elevated versus ambient temperature within each water × species combination, while water-availability effects were assessed using pairwise contrasts among moisture treatments within each temperature × species combination with Tukey adjustment. Growth-rate estimates and their 95% confidence intervals were visualised to illustrate species-specific interactive effects of temperature and water availability. All analyses were conducted in R v4.5.1 (R Core Team, 2025) using the packages lme4, lmerTest, and emmeans.

Root microbiome analysis

Amplicon sequences of fungal internal transcribed spacer (ITS) and arbuscular mycorrhizal fungal 18S ribosomal RNA (18S r RNA) were processed using the same protocol of Chapter 2 (Figure C. 1). The only exception was during read merging, where a minimum overlap of 12 bp and up to one mismatch were permitted to accommodate longer amplicons while ensuring sufficient overlap. Rarefaction was conducted as described in Chapter 2, with sequencing depths set to 10,000 reads for fungi and 20,000 reads for AMF, resulting in the exclusion of 11 fungal samples and one AMF sample (Figure C. 1). Fungal trophic modes were assigned

using FUNGuild v1.1 Beta (Nguyen et al., 2016), retaining only “Probable” and “Highly Probable” classifications. Multiple trophic assignments were separated into individual trophic categories with abundances evenly partitioned, and analyses focused on Saprotroph, Symbiotroph, and Pathotroph trophic modes. Through this approach, we obtained functional information for 55.9% of the Operational Taxonomic Units (OTUs). That is, saprotrophs (26.4%), symbiotrophs (13.5%), and pathotrophs (16.0%). The low percentage of OTUs assigned to these fungal functional groups, which is common in these types of studies, is evidence of a pressing need to improve FUNGuild content and/or similar database information. Observed fungal trophic mode richness and AMF observed richness were analysed using the same linear mixed-effects modelling framework with Temperature, Moisture, and Inoculum as fixed effects and Replication as a random effect. Treatment effects were evaluated using ANOVA and estimated marginal means with Benjamini–Hochberg–adjusted post-hoc comparisons.

Differences in root fungal (ITS) and arbuscular mycorrhizal fungal (AMF) community structure among plant species were evaluated using principal coordinates analysis (PCoA) based on averaged Bray–Curtis dissimilarities calculated from empirical rarefied ASV tables. Statistical differences among plant species were tested using PERMANOVA (adonis2, 999 permutations). To identify host-associated fungal specialists, we calculated specificity and occupancy values for each fungal ASV and visualised these relationships using specificity-occupancy (SPEC–OCCU) diagrams. Specificity was calculated as the proportion of an ASV’s mean relative abundance within a given host species relative to its total mean relative abundance across all host species. Occupancy was calculated as the proportion of samples within a host species in which an ASV was detected. ASVs with both specificity and occupancy values greater than 0.7 were classified as specialist taxa.

To characterise treatment-specific co-occurrence patterns in root fungal (ITS) and arbuscular mycorrhizal fungal (AMF) communities, co-occurrence network analyses were performed separately for each dataset. For each treatment × soil inoculum combination, OTU tables were transformed to relative abundances and filtered to improve network reliability by retaining taxa with a minimum prevalence of 10% of samples per treatment and a mean relative abundance ≥0.01% for ITS and ≥0.01% for AMF. Co-occurrence networks were constructed

based on pairwise Spearman correlation coefficients calculated within treatments, with significant associations defined as $|\rho| \geq 0.6$ and Benjamini–Hochberg false discovery rate (FDR)–adjusted $q \leq 0.05$. Networks were represented as undirected graphs, where nodes corresponded to taxa and edges represented significant positive or negative correlations, and were visualised using igraph package v2.1.4.

Spearman's rank correlations were used to assess relationships among plant growth traits and fungal trophic mode richness metrics. Analyses were performed separately for each plant species and climatic treatment. Plant traits included growth rate, final day digital biomass, and dry shoot biomass (mg), while fungal variables included AMF richness and saprotrophic, symbiotrophic, and pathotrophic trophic richness. Correlation coefficients and p-values were visualised using heatmaps, with significance indicated at $P < 0.05$.

Results

Effects of climate stress and soil inoculum on plant biomass accumulation

Addition of live soil inoculum significant increased aboveground biomass after four months in the greenhouse in all three species, with the strongest effect observed in *Carex* ($P < 0.001$), followed by *Poa* and *Celmisia* ($P < 0.05$, Figure 4.2).

In the capsule phase with warming and drought treatments applied, accumulation rate of digitally estimated biomass, estimated across the last 16 weeks, varied among species and in response to temperature across moisture treatments (Figure 4.3, Table C. 1). These differences arose from consistent divergence in biomass accumulation trajectories over time, particularly in *Celmisia* under ambient temperature and adequate moisture (Figure C. 2).

In *Poa*, elevated temperature consistently reduced growth rates under well-watered and moderate drought conditions (Well-watered: $P < 0.001$; 50% Drought: $P < 0.001$, Table C. 2). In *Celmisia*, elevated temperature caused a pronounced and consistent decline in growth rate across all moisture levels, including well-watered, moderate, and severe drought treatments

(all P < 0.001, Table C. 2). Under elevated temperature, moisture effects were also evident, with growth rates significantly lower under well-watered conditions than under severe drought (P = 0.006; Table C. 2). In contrast, *Carex* responded positively to elevated temperature under well-watered conditions, exhibiting significantly higher growth rates compared to ambient temperature (P < 0.001, Table C. 2). However, this warming advantage diminished under drought, with growth rates declining under elevated temperature at 50% drought (P = 0.003, Table C. 2) and showing no significant temperature effect under severe drought. Pairwise moisture contrasts under elevated temperature further showed consistently higher growth rates under well-watered conditions relative to both moderate and severe drought (both P < 0.001;Table C. 2).

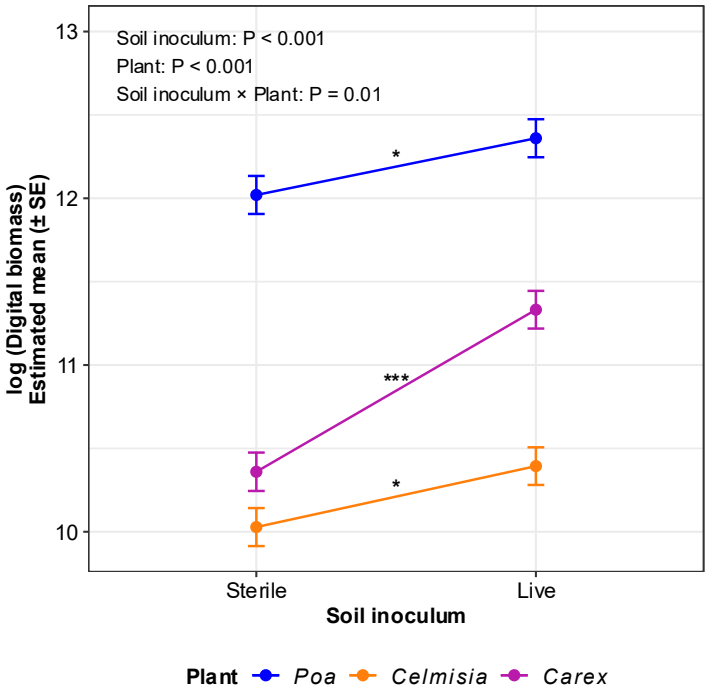


Figure 4.2: Soil inoculum effects on plant growth for three plant species grown in identical benign greenhouse conditions after 16 weeks. Estimated marginal means (± SE) of log-transformed digital biomass after four months growth in the greenhouse are shown for *Poa*, *Celmisia*, and *Carex*, inoculated with sterile or live soil inoculum. Values are estimated marginal means (± SE) derived from a linear model fitted to log-transformed digital biomass, including soil inoculum, species, and their interaction. Asterisks indicate pairwise contrasts between soil inoculum treatments within each species; significance is denoted as P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

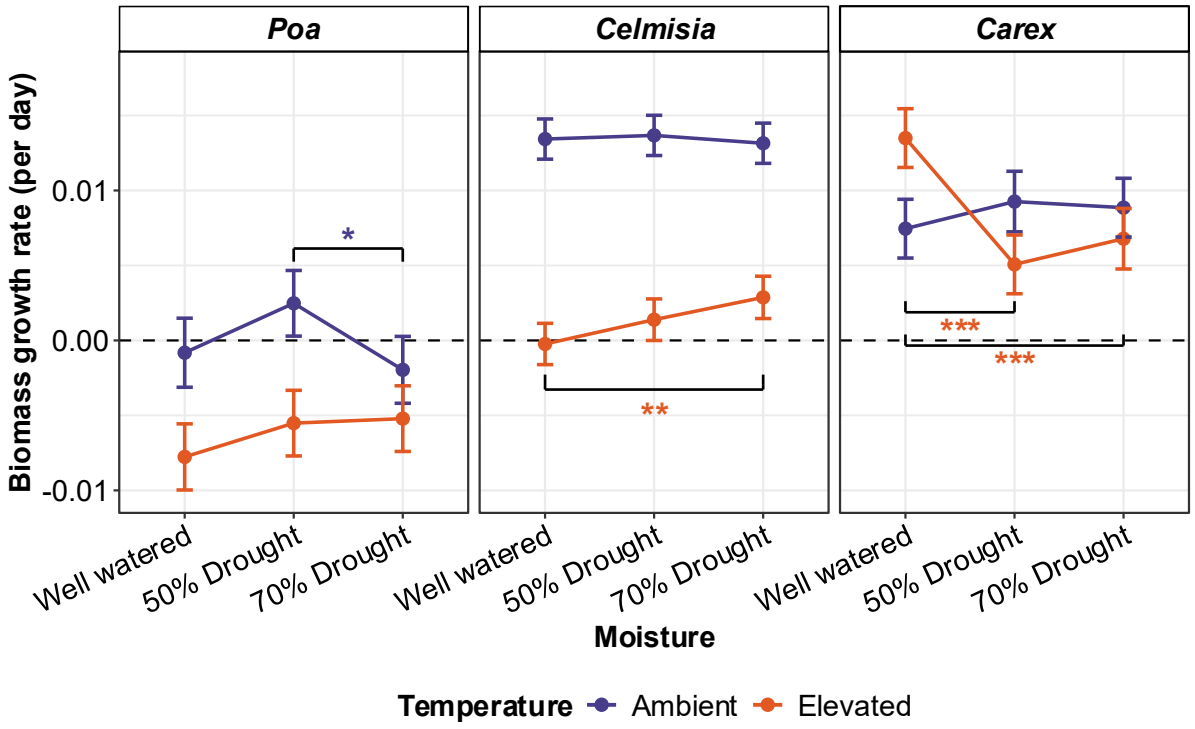


Figure 4.3: Species-specific interactive effects of different temperature and moisture availability on biomass growth rate. Points represent estimated marginal trends (emtrends; slopes per day) of log-transformed digital biomass derived from linear mixed-effects models fitted across the experimental time window, with error bars indicating 95% confidence intervals. The dashed horizontal line indicates zero growth. Asterisks indicate pairwise contrasts among moisture treatments within each species; significance is denoted as $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

At final harvest, dry shoot and root biomass was influenced by temperature, while the effects of drought varied among species (Figure 4.4, Table C. 3). Microbial contributions to dry shoot and root biomass accumulation were limited and not consistent across species or treatments (Figure C. 3). In *Poa*, shoot and root biomass were lower under elevated temperature than under ambient conditions across water treatments, with the highest biomass observed under ambient, well-watered conditions compared with the combined warming and drought treatment (Figure 4.4). In *Celmisia*, elevated temperature significantly reduced shoot and root biomass across moisture regimes (all $P < 0.001$; Figure 4.4) and soil inoculum treatments ($P < 0.05$; Figure C. 3). Similar to *Poa* under ambient temperature, well-watered *Celmisia* plants had greater biomass than drought-treated plants in both sterile and live soils, with the largest

reductions of shoot biomass under 70% drought ($P_{adj} < 0.001$; Figure 4.4). In contrast, *Carex* showed no significant effects of temperature or moisture on shoot biomass across treatments, however showed ($P < 0.05$; Figure 4.4) significant reduction in root biomass under warming combined with extreme drought.

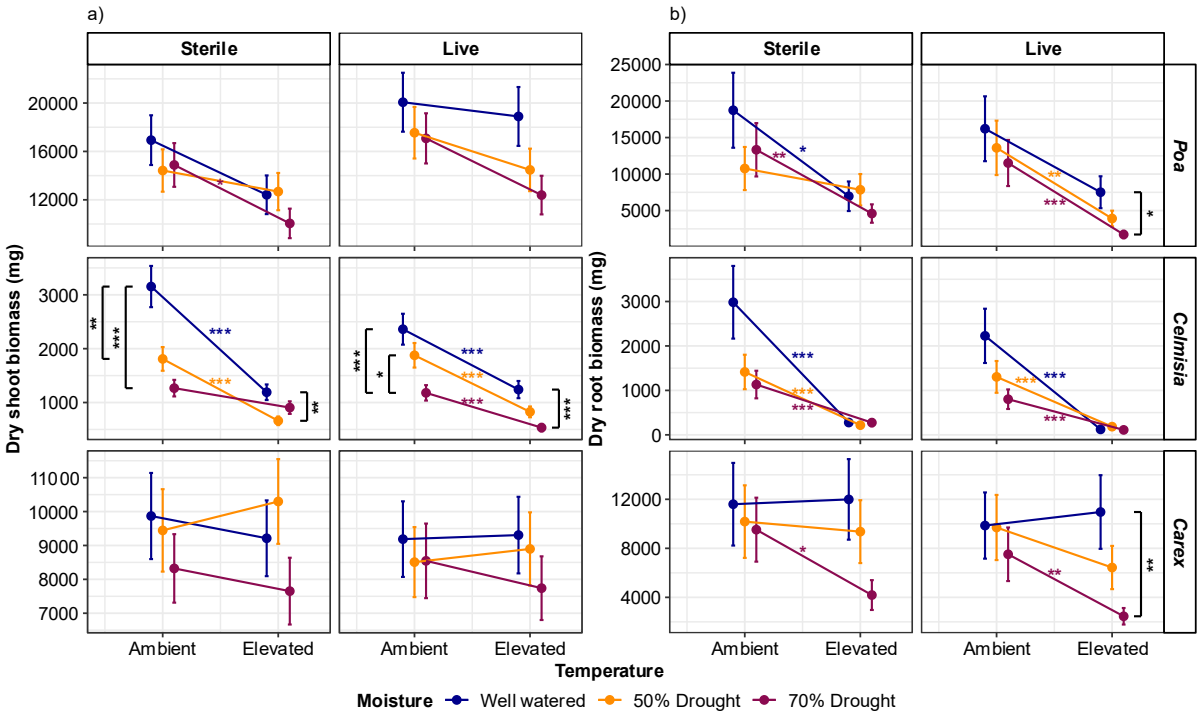


Figure 4.4: Dry biomass at harvest for *Poa*, *Celmisia*, and *Carex* under water and temperature treatments. (a) Dry shoot biomass and (b) dry root biomass. Points represent back-transformed estimated marginal means ± SE from linear models fitted to $\log_{10}$ (dry shoot + 1) and $\log_{10}$ (dry root + 1), respectively. Colours indicate water treatments, and panels distinguish soil inoculum (sterile vs live). Asterisks in bracket denote significant Benjamini–Hochberg–adjusted pairwise contrasts among water treatments (Significance levels: · $P_{adj} < 0.1$, * $P_{adj} < 0.05$, ** $P_{adj} < 0.01$, *** $P_{adj} < 0.001$). Asterisks in colours along with the lines represent significant pairwise contrast between temperature treatments (significance is denoted as $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Climate stress alters root fungal and AMF richness

Root fungal trophic mode richness exhibited contrasting responses to climate treatments, with drought and warming exerting distinct, plant species-specific effects (Table C. 4). Under

ambient temperature, droughted plants generally supported higher richness of saprotrophic, symbiotrophic, and pathotrophic fungi, as well as arbuscular mycorrhizal fungi (AMF), compared with well-watered plants. In contrast, elevated temperature consistently reduced fungal trophic mode richness across moisture treatments (Figure 4.5).

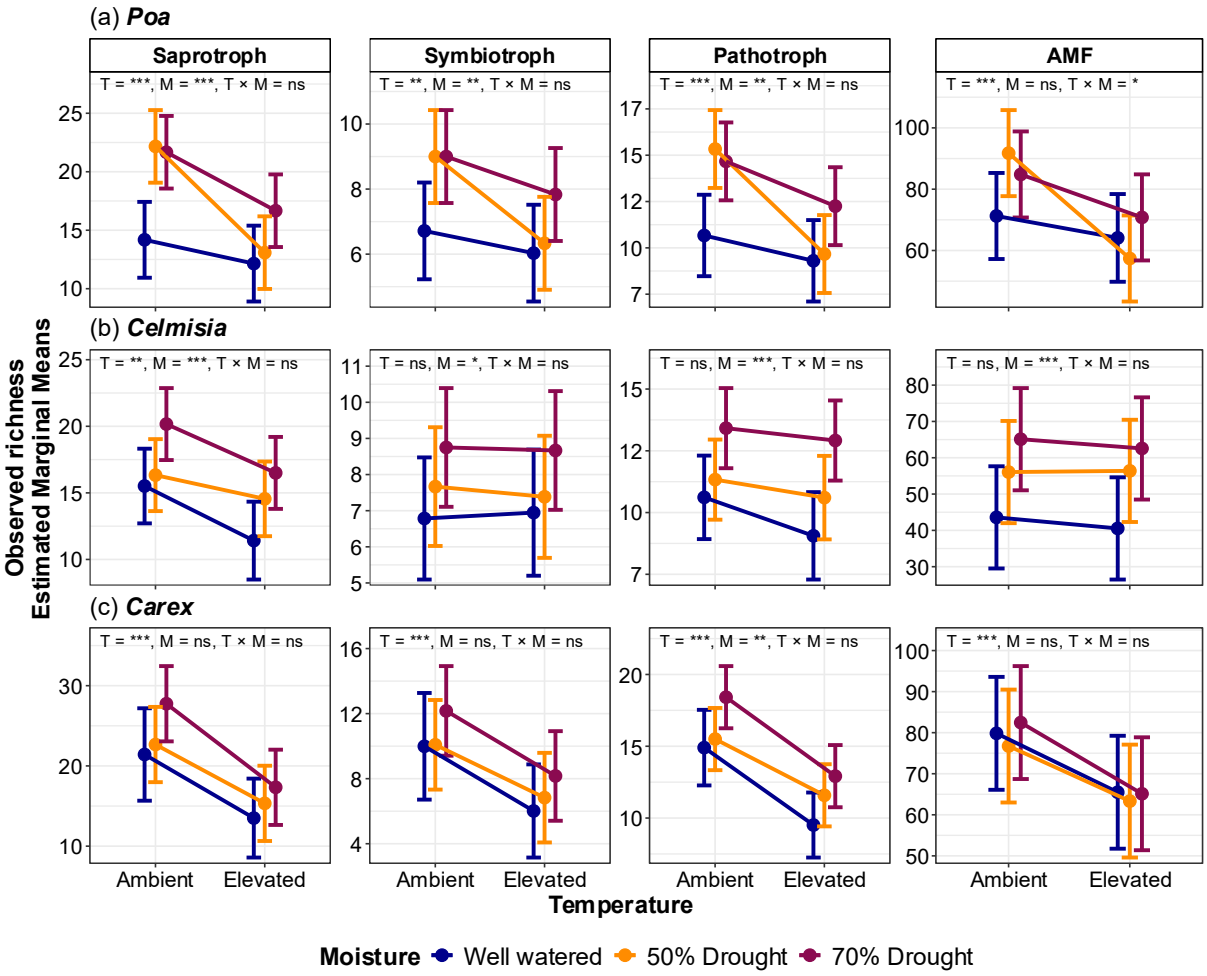


Figure 4.5: Responses of fungal trophic modes and AMF richness to experimentally altered temperature and moisture in *Poa*, *Celmisia* and *Carex*. Estimated marginal means (EMMs ± SE) were obtained from three-way factorial linear mixed-effects models (Temperature × Moisture × soil inoculum), tested using Type II Wald χ^2 statistics. (Significance levels: · P_{adj} < 0.1, * P_{adj} < 0.05, ** P_{adj} < 0.01, *** P_{adj} < 0.001; ns = not significant; post-hoc pairwise comparisons of EMMs with adjusted P-values.) Fungal trophic mode richness calculated from FUNGuild output and AMF richness calculated from metabarcoded AMF output.

In *Poa*, saprotrophic richness increased under drought at ambient temperature (P_{adj} < 0.05), whereas elevated temperature reduced saprotrophic, pathotrophic, and AMF richness, most

strongly under 50% drought ($P_{adj} \leq 0.01$) (Figure 4.5; Table C. 5 – Table C. 6). In *Celmisia*, fungal trophic mode richness showed limited sensitivity to elevated temperature, with moisture exerting a stronger influence than warming across trophic modes (Figure 4.5). In *Carex*, elevated temperature consistently reduced the richness of saprotrophic, symbiotrophic, and pathotrophic fungi, as well as AMF, across moisture treatments (Figure 4.5; Table C. 5 – Table C. 6). Together, these results indicate that drought effects on root fungal richness are most evident under ambient temperature, whereas warming imposes a dominant environmental filter that overrides moisture-driven differences in *Poa* and *Carex* but not in *Celmisia*.

Live soil inoculum consistently supported higher fungal and AMF richness than sterile soil across plant species (Figure C. 4). Saprotrophic and symbiotrophic richness were higher in live soil for *Poa* and *Carex*, whereas *Celmisia* showed a contrasting response with higher pathotrophic richness in sterile soil ($P_{adj} \leq 0.032$; Table C. 7). AMF richness was also significantly higher under live soil inoculum in all three species (*Poa*: $P_{adj} = 0.023$; *Celmisia*: $P_{adj} = 0.001$; *Carex*: $P_{adj} = 0.020$).

Treatment effects on relative abundance of fungal trophic modes

Across all plants, saprotrophic fungi increased progressively from control to drought to warming treatments. However, saprotroph abundance under warming with extreme (70%) drought was lower than under warming with moderate (50%) drought (Figure 4.6). In *Poa*, a significant increase in saprotroph relative abundance was observed under combined warming with 50% drought in live soil ($P < 0.05$, Figure 4.6), whereas in *Celmisia*, only warming resulted in a significant increase ($P < 0.01$, Figure 4.6). In contrast, symbiotrophic fungi remained stable or declined slightly under climatic stress across treatments. Under combined warming and 50% drought, symbiotroph relative abundance in live soil declined significantly ($P < 0.05$, Figure 4.6) in *Poa*, while in *Celmisia* a significant ($P < 0.01$, Figure 4.6) reduction was observed in the sterile treatment under the same conditions. Pathotrophic fungi declined in relative abundance under drought, warming, and combined warming–drought treatments, showing an overall decrease with increasing stress across species. In *Celmisia*, abundance decreased

under warming (P < 0.001, Figure 4.6) and combined warming with 50% drought (P < 0.05, Figure 4.6). In *Poa*, pathotrophs consistently declined across all stress treatments, while exhibited a particularly strong and significant reduction under warming in live soil (P < 0.01, Figure 4.6).

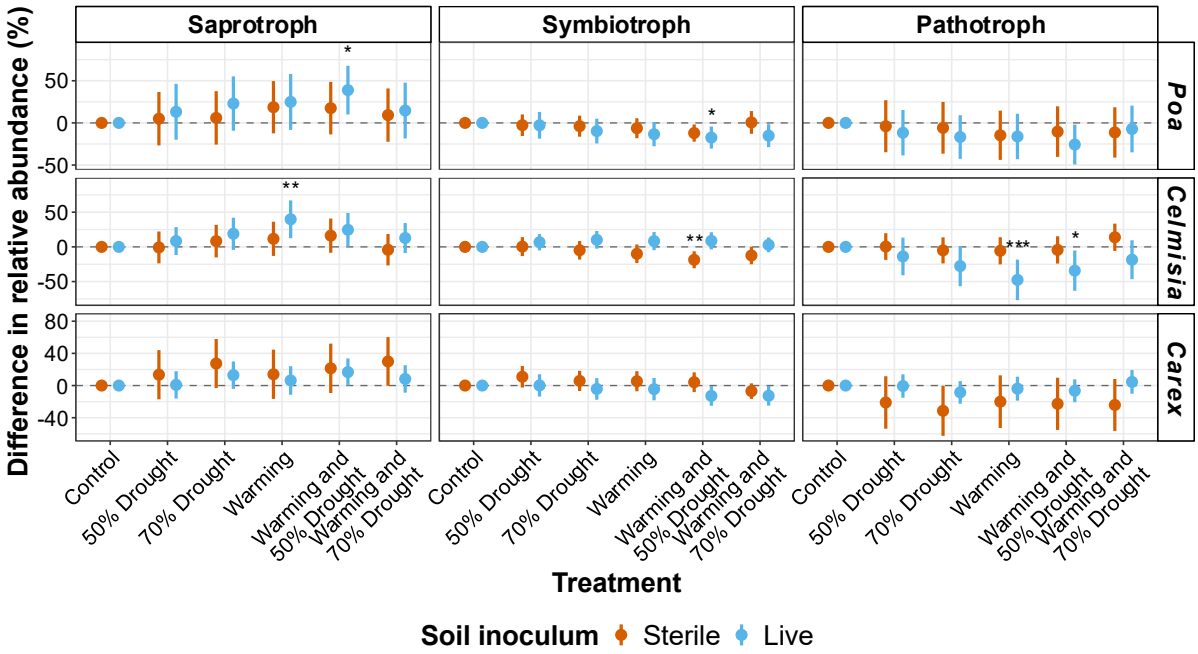


Figure 4.6: Beta-regression analysis of fungal trophic modes across treatments. Values represent mean percentage-point differences (Δ) in relative abundance compared with the control (± 95 % CI). Significance is based on FDR-adjusted p-values. *, ** and *** indicate P < 0.05, P < 0.01 and P < 0.001 respectively.

Root fungal community structure is weakly structured by host identity, while co-occurrence network structure is not affected by drought and warming

Contrary to our expectation that host plant identity would strongly structure root fungal and AMF community composition, we found that plant identity explained a statistically significant but only modest proportion of variation in community structure for both root fungi (PERMANOVA, $R^2 \approx 0.10–0.16$, P= 0.001, Table S8), and AMF ($R^2 \approx 0.05–0.07$, P = 0.001, Figure C. 5, Table C. 8). In contrast, root fungal and arbuscular mycorrhizal fungal (AMF) communities

were largely shared among host plant species (*Poa*, *Celmisia*, and *Carex*) under common soil inoculum conditions (Figure 4.7), with principal coordinates analysis (PCoA) based on averaged Bray–Curtis dissimilarities showed substantial overlap among host species. Low PCoA axes values (9.2-20.4%) explaining total variation, was observed (Figure 4.7).

For co-occurrence networks, contrasting with the expectation and chapter 2, root endophytes (fungi and AMF) showed stability under climate stress, i.e. the network topology remained largely stable across climate treatments. A non-significant increase of the network complexity, irrespective of the type of soil inoculum, was observed under both levels of drought, while the network becomes marginally simplified for live inoculum treatments (Figure C. 6, Table C. 9).

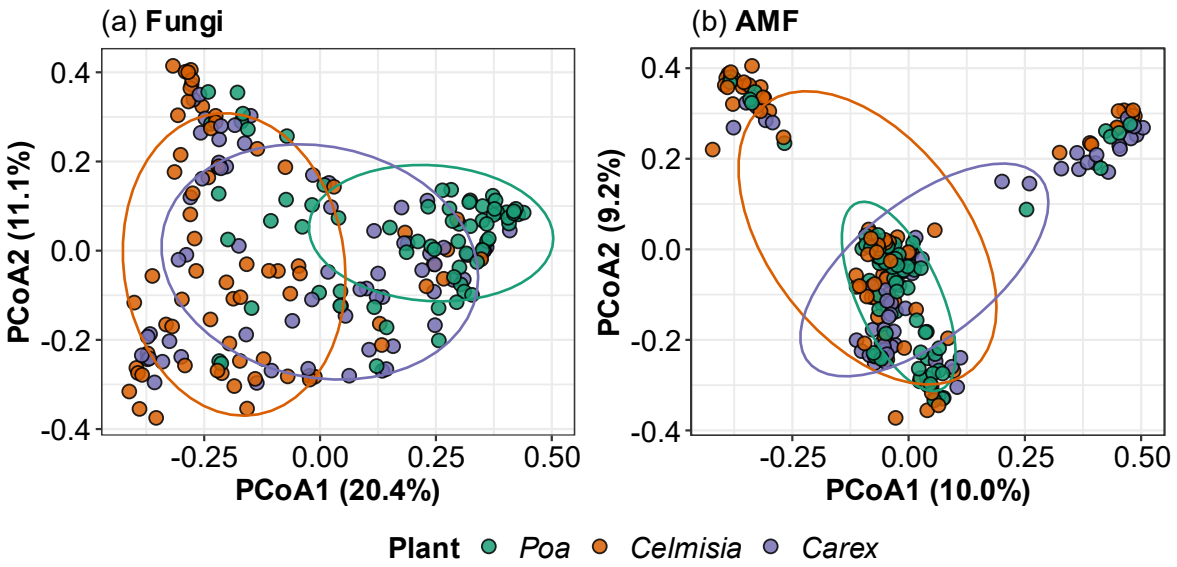


Figure 4.7: Principal coordinates analysis (PCoA) of (a) root fungal and (b) arbuscular mycorrhizal fungal (AMF) communities based on averaged Bray–Curtis dissimilarities showing root-associated fungal and AMF community composition of *Poa*, *Celmisia*, and *Carex* across all climatic treatments. Points represent individual samples, coloured by plant species. Ellipses denote 95% confidence regions around group centroids, illustrating the central tendency and dispersion of communities rather than enclosing all samples.

Weak correlation among plant performance and fungal trophic mode richness

Across all climatic conditions, fungal trophic mode richness metrics were positively correlated across all plant species (Figure 4.8). Although AMF richness was often weakly or neutrally associated with other fungal trophic modes, all significant associations were positive, indicating correlated responses among belowground microbial groups rather than antagonistic trade-offs (Table C. 10). Across all environmental stress conditions, very few microbial–plant trait couplings were significant across plant species, indicating relatively weak correlation between belowground fungal community variation and aboveground plant performance across all plant species.

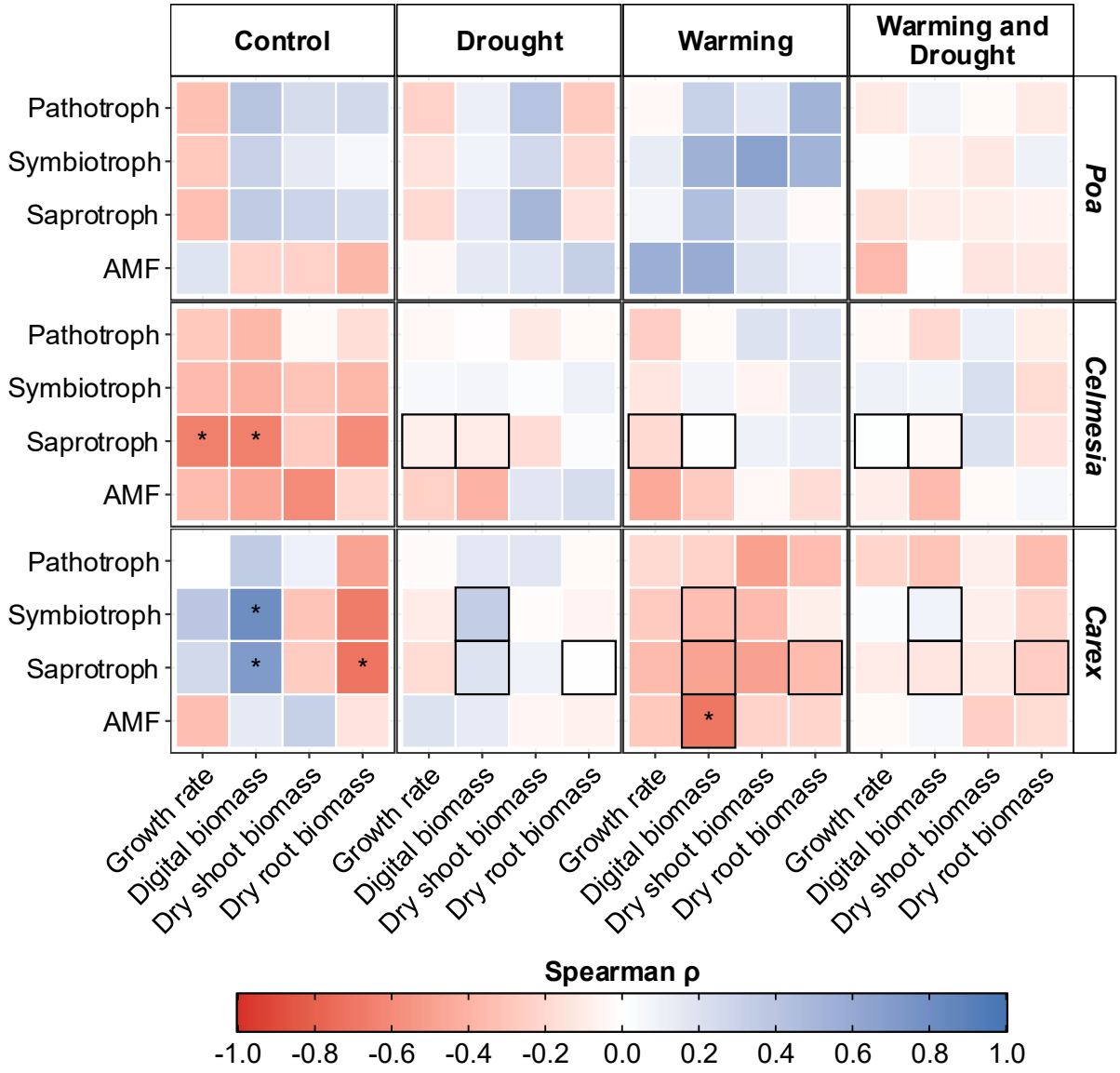


Figure 4.8: Spearman rank correlation heatmaps illustrating relationships between microbial community attributes and plant performance traits for *Poa*, *Celmisia*, and *Carex* under control, drought, warming, and combined warming–drought treatments. Colours represent the strength and direction of strong correlations (|ρ| ≥ 0.6; blue = positive, red = negative), and asterisks indicate statistically significant relationships ($P < 0.05$, $P < 0.01$, $P < 0.001$). Black borders highlight correlations that changed in strength or significance relative to the control treatment.

Discussion

This study assesses the effects of biotic association (microbial inoculum) and abiotic stress on plant performance and root-associated microbiomes using three Australian sub-alpine plants. This multifactorial greenhouse experiment allowed us to test whether plant and root microbial responses to drought and warming were consistent across host species and soil inoculum contexts. Plants which received a live microbial inoculum achieved a significantly higher biomass. After introducing drought and warming stressors, singly and in combination for 4 months, we found that plant growth responses were highly species specific, with warming generally constraining biomass more strongly than drought, whereas combined stress resulted in the largest reductions. Furthermore, *Celmisia* was most sensitive towards all stressors, whereas *Carex* was more resilient. Root fungal communities showed parallel sensitivity to climate stress, exhibiting stress-specific and non-additive shifts in richness, trophic mode composition, and co-occurrence network structure. However, despite pronounced microbial restructuring, plant performance remained only weakly coordinated with microbial community structure, and host identity exerted limited control over microbial assembly under shared soil inoculum. While climate stress clearly influenced both plant phenotypes and root microbiomes, the weak correlation between plant performance and fungal trophic richness likely reflects the influence of multiple interacting drivers rather than a lack of plant-microbe interactions. Soil physicochemical properties (e.g., pH, moisture, temperature, and nutrient availability) are primary regulators of both microbial community structure and plant performance and can therefore obscure direct relationships (Cao et al., 2016; Delgado-Baquerizo et al., 2016; Wang et al., 2014; Xiong et al., 2024). In addition, microbial legacy effects, inoculum composition, and priority effects can shape microbial assembly independently of host responses (Bever et al., 2012; van der Putten et al., 2013). Plant traits and phenological stage further influence root exudation and microbial recruitment (Berg & Smalla, 2009; Trivedi et al., 2020), leading to temporal shifts in community structure that may not directly align with plant performance metrics. Collectively, these processes can decouple plant performance from microbial community patterns, indicating context-dependent assembly rather than weak functional linkage in alpine plant–soil systems.

Microbes assist in plant growth

Applications of microbial washes significantly increased aboveground biomass of sub-alpine grassland plants, indicating that soil microbial communities can facilitate plant growth (Figure 4.2). The addition of microbes may have led to the establishment of microbial populations in the soil which improved soil water-holding and nutrient-retention capacities and also stimulated microbial activities and soil enzymes resulting in better plant growth (Du et al., 2025; Hou et al., 2024; Li et al., 2024). Primary succession and restoration studies of alpine grasslands indicated that *Poa* is strongly influenced, and benefit from, rhizosphere microbial communities, which in turn affect soil enzyme activities and nutrient availability relevant for growth (Du et al., 2025; Tscherko et al., 2004). *Celmisia*, as a sub-alpine perennial with documented mycorrhizal associations, is expected to rely on similar microbially mediated mechanisms (nutrient uptake, stress tolerance, hormone modulation) as other sub-alpine forbs and grasses (Bektaş et al., 2026; Hou et al., 2024). It is interesting that *Carex* attained a high ($P < 0.001$) biomass in live soil compared to sterile soil, which was higher than expected given it is a non-mycorrhizal plant. However, a recent study on alpine sedges showed that non-mycorrhizal *Carex cepillacea* harbours distinct core and keystone bacterial taxa closely linked to soil nutrients and microbiome network structure (Xie et al., 2025). Several of these taxa (e.g. *Sphingomonas*, *Nocardioides*, *Actinoplanes*) are widely associated with plant growth-promoting and stress-tolerance functions in other systems and have also been reported as highly connected taxa in bacterial interaction networks (Luo et al., 2019; Mahmoud et al., 2025; Wang et al., 2022) suggesting potential roles in supporting plant performance in alpine sedges. Furthermore, metagenomic evidence from another alpine sedge (*Carex praeclara*) rhizosphere indicates enrichment of microbial genes involved in nutrient acquisition, as well as nitrogen and phosphorus cycling, suggesting a functional contribution of rhizosphere microbiomes to plant performance (Jian et al., 2022). The microbes from the microbial wash may accelerate the decomposition of organic matter facilitating nutrient availability (Jin et al., 2022; Zhang et al., 2023). In addition, the beneficial microorganisms may inhibit pathogens, decompose cellulose and lignin, and transform them into available nutrients for plants (Berg et al., 2017; Elnahal et al., 2022; Liu et al., 2022),

explaining improved plant growth in live soil compared to sterile soil during the greenhouse phase.

Effects of climate stress on plant biomass accumulation is species -specific

Consistent with global syntheses showing that plant biomass responses to warming and drought are highly context and species-dependent (Eziz et al., 2017; Wilschut et al., 2022), functional-group identity has emerged as an important source of variation, with grasses and sedges often exhibiting more positive or resilient warming responses than forbs (Dobson & Zarnetske, 2025; Hoover et al., 2014). Such differences are thought to reflect variation in thermal tolerance thresholds and physiological capacity to sustain growth under elevated temperatures (Wei et al., 2024a). Moreover, warming effects are strongly ecosystem-specific: while positive shifts in community structure and productivity have been observed in alpine meadows, contrasting negative responses have been reported in alpine steppe systems (Ganjurjav et al., 2016). In line with this broader pattern of context dependence, we found that *Carex* showed enhanced biomass growth under warming when water was sufficient, whereas *Celmisia* exhibited strong growth constraints under elevated temperature even in the absence of moisture limitation.

In the greenhouse phase of the experiment, *Poa* completed most of its vegetative growth within the first four months, such that subsequent warming and drought treatments acted primarily on established plants. This pattern is consistent with alpine grass growth phenology, where graminoids typically achieve rapid early biomass accumulation following snowmelt before entering a maintenance phase later in the season (Körner, 2003). Experimental extension of the alpine growing season further demonstrates that dominant graminoids do not prolong growth even when season length is substantially increased, with senescence initiating within a fixed period after growth onset (Möhl et al., 2022). Studies in alpine systems further show that *Poa* species reach peak phytomass early relative to forbs, supporting the interpretation that later declines in relative biomass reflect stress-induced losses rather than suppressed growth (Venn & Morgan, 2007).

Beyond functional-group contrasts, the effects of elevated temperature on biomass accumulation varied markedly among alpine species and across moisture regimes (De Boeck et al., 2016; De Boeck et al., 2018). *Celmisia* biomass accumulation (dry shoot and root biomass) was markedly reduced under combined stress, whereas *Poa* and *Carex* also showed declined growth. Higher atmospheric dryness under warming can constrain stomatal conductance and reduce transpirational cooling, intensifying heat stress, particularly under drought, resulting in elevated leaf temperatures and reduced carbon gain (Buchner et al., 2017). These interacting stresses are known to substantially reduce aboveground biomass production during hot and drought events in alpine grasslands (De Boeck et al., 2016). The strong sensitivity of *Celmisia* to warming across all moisture treatments aligns with warming and drought studies where forbs declined immediately (~20–35% biomass loss), whereas graminoids resisted via stomatal control and re-sprouting (De Boeck et al., 2018). It also aligns with broader evidence that shallow-rooted alpine forbs are particularly vulnerable to elevated temperatures, likely because they operate near their thermal optima and experience increased respiratory costs or reduced carbon gain under warming (Wahren et al., 2013). In line with alpine snowbed studies showing that drought reduces above-ground biomass through constraints on ecosystem carbon fluxes, the strong reductions in *Celmisia* dry shoot biomass under both drought and elevated temperature suggest that single and combined moisture and thermal stress limit net carbon gain in this species (Johnson et al., 2011). In addition, drought frequently increases root-to-shoot ratios as plants allocate relatively more biomass belowground to enhance water uptake (Zhou et al., 2018). Experimental warming can also promote belowground allocation, particularly in water-limited environments (Zhou et al., 2022). When these stressors occur together, warming may amplify drought effects on plant physiology and biomass allocation (Gargallo-Garriga et al., 2015), although the magnitude of these interactions varies among ecosystems (Zhou et al., 2020).

The response of *Carex* exemplifies patterns observed by Wahren et al. (2013) in Australian alpine systems, where warming can stimulate growth under sufficient water supply but drought rapidly overrides these benefits, emphasizing the central role of moisture availability in shaping plant responses to climate change. In addition, the positive response of *Carex* to warming under well-watered conditions aligns with evidence that grasses and sedges can

exploit warmer conditions through enhanced metabolic activity and growth plasticity when moisture is not limiting (Li et al., 2021). However, the loss of this warming advantage under drought supports evidence that warming frequently amplifies water stress, leading to biomass declines when elevated temperature and drought co-occur (De Boeck et al., 2016). Together, these findings emphasise that alpine plant growth responses to climate change are governed by interacting stressors and species-specific traits, rather than by warming alone.

Climate stress shapes the root-associated fungal community

Consistent with soil microbiome responses described in Chapters 2 and 3, our findings indicate that climate-driven shifts in soil microbial communities extend into the root-associated fungal communities. However, these responses are strongly structured by stress type and further shaped by plant-species specific effects, adding an additional layer of complexity. Root fungal trophic mode richness responded differently to drought and warming, suggesting that these climate stressors impose distinct constraints on root-associated microbial communities (Hoefle et al., 2024; Lozano et al., 2021). Although pots were maintained under relatively well-watered conditions before treatment initiation, this regime was intended to reflect natural establishment conditions and to allow plants to establish before exposure to experimental drought and warming. Under ambient temperature, drought promoted higher richness across several root fungal trophic modes, whereas elevated temperature reduced fungal trophic mode richness regardless of moisture treatments. Although drought effects on root fungal richness vary among ecosystems and guilds (Alzarhani et al., 2019; Fu et al., 2022; Fuchslueger et al., 2014; Lagueux et al., 2021), warming has more consistently been shown to constrain fungal diversity, particularly for symbiotic fungi, through enhanced environmental filtering and dominance (Anthony et al., 2021; Devesa-Aranguren et al., 2025; Edwards et al., 2025; Laine & Leino, 2025; Yuste et al., 2011). Together these patters suggest that drought and warming influence root-associated fungal communities through fundamentally different ecological mechanisms.

The increase in fungal guild richness under drought likely reflects restructuring of the root microbiome rather than uniformly enhanced microbial function. Water limitation can alter plant physiological status, including shifts in carbon allocation and defence regulation,

potentially relaxing host filtering and allowing colonisation by a broader range of fungal taxa (Claeys & Inzé, 2013; Huot et al., 2014). Drought-induced changes in root carbon allocation may preferentially support fungal metabolism, particularly saprotrophs, as drought has been shown to reduce carbon flow to bacteria while maintaining or even increasing fungal carbon use (Fuchslueger et al., 2014; Hasibeder et al., 2015). Fine roots likely interact intensively with saprotrophs by releasing easily degradable carbohydrates, which can prime saprotrophic activity in the rhizosphere (Kuzyakov et al., 2000). Plant species with thin roots may therefore attract diverse saprotrophic communities (Semchenko et al., 2018), highlighting potential trait-mediated associations between fungal groups and root morphology. Drought may also increase plant susceptibility to pathogens (Mayek-PÉrez et al., 2002), potentially contributing to higher pathotroph richness. In addition, plants may recruit or maintain diverse symbiotic partners under drought as a risk-spreading strategy, consistent with the insurance hypothesis, whereby host-associated microbial diversity buffers plant performance under fluctuating environmental conditions (Jousset et al., 2017). Enhanced signalling pathways such as strigolactone biosynthesis under drought may further promote associations with mycorrhizal fungi (Ruiz-Lozano et al., 2016). Together, these findings suggest that while drought may stimulate recruitment or persistence of root-associated fungi, such shifts do not necessarily confer strong functional benefits to host plants under concurrent warming stress.

The decline of root fungal richness under warming stress in this study, with the strongest reductions occurring when warming was combined with drought, align with findings of other alpine ecosystems (Kou et al., 2024; Moravcová et al., 2023; Wang et al., 2023; Yin et al., 2024). Elevated temperature likely imposes strong environmental filtering on root-associated fungal communities (Devesa-Aranguren et al., 2025; Laine & Leino, 2025) by constraining fungal metabolism and the enzymatic processes required for root colonisation (Kubicek et al., 2014; Trivedi et al., 2020). These effects are intensified under drought, as water limitation further suppresses fungal extracellular enzyme activity and reduces plant-mediated buffering through carbon allocation and root exudation (A'Bear et al., 2014; Badri & Vivanco, 2009). As a result, fewer fungal taxa are able to persist within root tissues, leading to reduced richness under combined warm and drought stress. Similar patterns have been reported for alpine root endophytic fungi, where temperature effects on fungal richness were stronger under dry

conditions, highlighting the sensitivity of root-associated fungal communities to interacting climatic stressors (Moravcová et al., 2023; Wang et al., 2023). These contrasting responses highlight that future climate extremes may erode belowground biodiversity not through single stressors, but through their interaction, with implications for plant resilience under global change.

Drought and warming, individually and in combination, had pronounced effects on fungal functional richness and guild composition. In contrast, multivariate ordination, specificity–occupancy (SPEC–OCCU), and co-occurrence network analyses indicated relatively weak or non-significant restructuring of root fungal and arbuscular mycorrhizal fungal (AMF) communities across climatic treatments. This suggests that under controlled conditions where the soil microbial pool is shared, plant species identity does not exert a dominant influence on root endophyte community structure, contrasting with patterns reported in other systems (Aguilar-Trigueros & Rillig, 2016; Geisen et al., 2021). The predominance of generalist taxa suggests that most root-associated fungi in this system are capable of colonising multiple hosts, rather than forming highly host-specific associations (Kuang et al., 2021; Wang et al., 2021). Such generalist behaviour has been widely reported in grassland and alpine systems, where plants often share similar root traits and are exposed to similar environmental constraints (Chen et al., 2022; Michalska-Smith et al., 2022; Ni et al., 2018). While the SPEC–OCCU analysis used here identifies taxa that are consistently and disproportionately associated with particular host species, it does not capture potential interactions among microbial taxa within communities. We therefore complemented this analysis with co-occurrence network analysis to assess whether taxa exhibited coordinated association patterns, thereby providing additional insight into community organisation beyond host specificity alone.

Consistent with the SPEC–OCCU results, network analyses revealed no strong or systematic restructuring of microbial associations across host species or treatments. We found fungal communities under drought may slightly reorganize themselves through enhanced cooperative or shared-stress interactions, perhaps because drying soil concentrates fungal activity near roots, increasing co-occurrence (Gao et al., 2022), whereas warming disrupts these relationships, potentially constraining community stability and ecosystem functioning

under future climate warming (Wang et al., 2024). Stability of microbial interaction networks despite compositional change has also been observed in experimental grasslands and mesocosms, suggesting that network architecture can be buffered by functional redundancy and overlapping ecological roles among microbial taxa (in ‘t Zandt et al., 2023; Pacheco et al., 2021). In root-associated systems, such buffering may be particularly strong because microbial assembly is shaped simultaneously by plant-derived resources, soil conditions, and temporal succession, often leading to partial decoupling between microbial structure and host performance (Dibner et al., 2021; Reese et al., 2017). Collectively, these findings suggest that weak host specificity and stable interaction networks are intrinsic properties of root fungal communities under shared soil inoculum, rather than evidence of limited microbial responsiveness to climate stress. Although network analysis is a powerful tool to understand the assembly process of the microbial community, it also faces enormous challenges in linking statistical inferences to reliable biotic interactions (Blanchet et al., 2020). Therefore, further work should make efforts to identify real fungal species interactions in situ. Together, these results indicate that root fungal communities responded to environmental change primarily through shifts in taxonomic representation rather than through strong host filtering or reorganisation of interaction structure.

Limited coordination between root fungal communities and plant performance

The weak coordination observed between plant performance and root fungal community richness is consistent with broader evidence that biological compartments can become partially decoupled without necessarily compromising system stability. For example, long-term grassland experiments have shown that stable plant communities are associated with decoupled microbial networks, whereas tightly coupled microbial interactions may promote instability (in ’t Zandt et al., 2025). Experimental studies further demonstrate that plant community identity and soil conditions can exert independent controls on microbial community assembly, often without strong interactive effects or proportional impacts on plant performance (Reese et al., 2017). Similarly, evidence from rhizosphere experiments indicates that microbial community composition may be shaped more strongly by elapsed

time and successional processes than by host developmental stage (Dibner et al., 2021), highlighting that microbial assembly dynamics can proceed independently of host growth responses. Conceptual and experimental work in microbial ecology also shows that substantial shifts in taxonomic diversity can occur without proportional changes in community-level output, reflecting strong metabolic interactions, environmental filtering, and functional redundancy (Pacheco et al., 2021). In addition, fluctuating environments strongly shape microbial behaviour and community structure in non-linear and often non-additive ways that do not necessarily translate into proportional ecosystem- or host-level responses (reviewed in Nguyen et al., 2020). Together, these findings provide a coherent framework for interpreting the present results, suggesting that pronounced restructuring of root fungal communities under climate stress may occur without strong or consistent effects on plant performance. Rather than indicating functional failure, limited coordination between plants and microbial communities may reflect intrinsic structural properties of plant–soil systems operating under variable and complex environmental conditions. Despite clear positive effects of drought on root fungal community richness (Figure 4.5), root biomass remained unchanged (Figure 4.4), and the relative abundance of major functional guilds including saprotrophs, pathotrophs, and symbiotrophs was largely stable in roots (Figure 4.6). This suggests that key functional roles associated with organic matter turnover, nutrient cycling, and symbiotic support were maintained under drought, potentially buffering plant belowground growth against water limitation (Fu et al., 2022). In contrast, warming, and combined warming and drought stress significantly reduced fungal richness (Figure 4.5) and root biomass (Figure 4.4), and were accompanied by pronounced shifts in functional guild composition (Figure 4.6), indicating functional destabilisation. Together, these patterns indicate that while drought responses were characterised by functional stability and resilience, warming, particularly when combined with drought disrupted root fungal functional balance, coinciding with reduced root biomass and suggesting a loss of functional buffering capacity under more extreme climate stress.

Conclusions and future directions

Our results indicate that warming, rather than drought, was the dominant constraint on plant growth, with combined warming and drought producing the strongest reductions. The limited effect of soil inoculum indicates that plant growth under climate stress is largely driven by inherent resilience of Australian alpine vegetation. Likewise, weak host specificity and stable network structure indicate that generalist fungi dominate root communities, maintaining interaction stability under climate stress. Despite this stability, root fungal richness showed notable shifts, either increasing or remaining stable under drought but declining under warming and combined stress, accompanied by a shift toward saprotrophic and symbiotrophic guilds and a reduction in pathotrophs. Differential sensitivity among functional groups further suggests that ongoing warming and increasing climate extremes may drive community transitions from forb-dominated systems toward more stress-tolerant grasses or sedges. Overall, this chapter demonstrates that temperature and host identity exert stronger influences on plant performance than soil inoculum, suggesting that in natural settings, climate change is likely to impose strong direct constraints on plant performance that local microbial communities may not readily offset. Microbial contributions to stress tolerance are therefore, unlikely to act as a universal buffer, instead depending on prolonged exposure, repeated stress events, and the gradual reassembly of functionally compatible communities. While root fungal diversity and functional composition are responsive to climate stress, community assembly patterns and interaction networks remain buffered, leading to partial decoupling between microbial community change and plant performance. These findings underscore the importance of considering multiple dimensions of microbial community organisation when predicting alpine ecosystem responses to future climate change. Specifically further consideration is needed of plant-microbe responses under more realistic field conditions, where climatic stress interacts with natural soil and root macro and microbial communities, seasonal dynamics, and site-specific constraints. Evidence from long-term climate change experiments indicates that microbial buffering effects may be transient, context-dependent, or overridden by chronic abiotic stress. Bridging mechanistic greenhouse studies with field-scale manipulations will therefore be essential for predicting alpine vegetation persistence under future climate scenarios.

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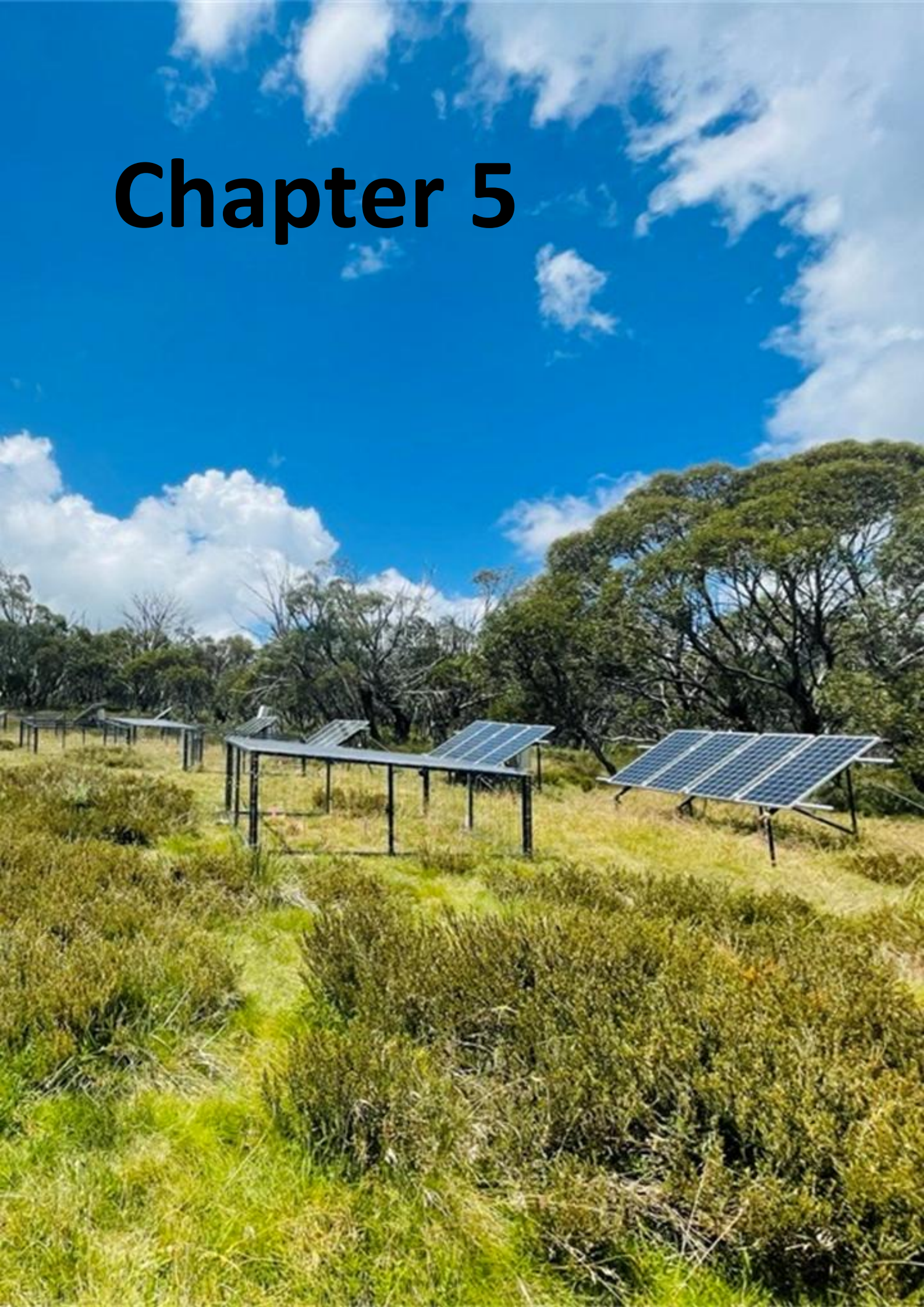
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Chapter 5



Chapter 5: Climate-driven filtering/ shifts in fungi colonising roots of sub-alpine plants

Abstract

Climate warming and increasing drought frequency are expected to alter sub-alpine ecosystems, and impact on growth and establishment of plants under these conditions are likely to depend on root-associated microbial symbioses. However, how climate change influences the assembly of these microbial communities within native vegetation remains poorly understood. Using a field-based transplant climate manipulation experiment, we examined how experimental warming and drought influence the assembly of root-associated fungal (ITS) and arbuscular mycorrhizal fungal (AMF) communities across three sub-alpine plant species (*Poa*, *Celmisia*, and *Podolepis*). Climate treatments significantly altered community composition, with strong host-dependent structuring of colonising communities. Temperature primarily influenced fungal diversity in a plant-specific manner, whereas moisture exerted stronger control over AMF richness. Community differences were driven primarily by species replacement rather than simple species loss, indicating that climate treatments reshuffled which taxa were able to colonise plant roots. Microbial assembly was largely structured by environmental conditions under climate treatments, suggesting strong environmental control over colonising taxa. Despite this restructuring, host-specialist taxa remained rare, with specialisation largely stable in fungi and declining modestly in AMF under combined warming and drought. Concurrently, shifts in taxon abundance were strongly host-dependent, with broadly distributed taxa responding differently across hosts and often showing stronger responses under combined climate stress. Together, these findings show that climate change reshapes root microbial communities during plant establishment by altering which taxa successfully colonise plant roots, with potential consequences for plant performance and implications for the management of sub-alpine ecosystems.

Introduction

Root-associated microbial communities are integral components of plant ecology (Lareen et al., 2016; Odelade & Babalola, 2019), functioning as an extended phenotype (de la Fuente Cantó et al., 2020; Vandenkoornhuyse et al., 2015) that influences nutrient acquisition, stress tolerance, and plant performance (Baron & Rigobelo, 2022; Poveda et al., 2022). Filamentous fungi, including arbuscular mycorrhizal fungi (AMF), colonise plant roots and mediate key processes including phosphorus uptake, organic matter decomposition, and plant–soil feedbacks (Crowther et al., 2012; Smith et al., 2011; Veen et al., 2019). Fungal symbionts can regulate plant responses and adaptation to abiotic stress by modifying root system architecture, patterns of nutrient uptake and allocation, and hormone-mediated signalling pathways (Ameen et al., 2024; Branco et al., 2022; Usman et al., 2021). Consequently, root-associated microbial communities contribute not only to individual plant fitness but also to plant coexistence and community dynamics in natural ecosystems (Lin et al., 2015; Zhou et al., 2018).

The composition and structure of root fungal microbial communities are shaped by multiple interacting factors (Andreo-Jimenez et al., 2019; Brigham et al., 2023; Hartman & Tringe, 2019). Host identity is a major determinant of fungal assemblages, as plant traits such as root morphology, carbon allocation patterns, and secondary metabolites influence fungal recruitment (Kivlin et al., 2022). Environmental factors, including soil temperature and moisture, further regulate microbial growth, survival, and competitive interactions (Finestone et al., 2022; Pietikäinen et al., 2005). Community assembly theory, grounded in niche and neutral frameworks, suggests that microbial communities are structured by a combination of deterministic processes, such as environmental filtering and host selection (Vellend, 2010), and stochastic processes including dispersal limitation and ecological drift (Hubbell, 2001; Zhou & Ning, 2017). While both processes operate simultaneously, their relative importance is context-dependent and may shift under environmental stress (Dini-Andreote et al., 2015; Yuan et al., 2019). These processes may be particularly important during root colonisation, where environmental conditions and host traits determine which microbial taxa successfully establish within plant roots.

Previous studies have documented climate-induced shifts in fungal diversity and composition (Moravcová et al., 2023; Yang et al., 2023). However, microbial communities often show variable responses across ecosystems (Delavaux et al., 2021; Duchesneau et al., 2024; Liu et al., 2024) with some studies indicating plant-specific patterns (Lozano et al., 2021; Wei et al., 2021) and others finding weak host specificity (Kennedy et al., 2020; Lorberau et al., 2017). For instance, studies along elevation gradients, which capture natural temperature variation, have shown contrasting host-specific responses of root endophytic fungi: with fungal diversity decreasing with increasing elevation in *Kobresia pygmaea* but increasing in *Elymus nutans* (Wei et al., 2021). Grasses also show contrasting patterns, with root endophytic fungal communities and diversity exhibiting either little response to warming (Lyons et al., 2021), or that warming primarily alters fungal community composition, whereas drought increases fungal diversity and modify community structure, with the combined effects of warming and drought influencing both diversity and composition (de Oliveira et al., 2020).

Despite a growing body of work on climate effects on soil and root microbiomes (Fuchslueger et al., 2014; López-Ráez, 2016; Sheik et al., 2011; Xie et al., 2021), relatively little is known about the interactive effects of warming and drought on root-associated microbial communities (in ’t Zandt et al., 2025; Tiziani et al., 2022; Wipf et al., 2021). In natural systems, these factors often co-vary, making it difficult to disentangle their individual and combined effects, while experimental studies frequently manipulate only one factor at a time (Catarecha et al., 2025; Devesa-Aranguren et al., 2025; Pan et al., 2024). As a result, how warming and drought interact to shape microbial community assembly remains poorly understood. Moreover, while many studies report changes in diversity metrics, fewer explicitly disentangle whether compositional shifts arise from species loss (nestedness) or taxon replacement (turnover), or whether climate change strengthens deterministic environmental filtering relative to stochastic processes. In addition, most studies have been conducted in Arctic, temperate, or tropical ecosystems, and relatively little is known about how root fungal communities respond to climate change in alpine or sub-alpine ecosystems.

While the effects of climate change on microbial communities are well studied, including in Chapter 2 and 3, comparatively little attention has been paid to the influences of these climate change effects on patterns of root colonisation. This gap is particularly relevant for

understanding plant establishment in natural system, where assembly of root-associated microbial communities from the surrounding soil microbial pool plays a key role in shaping plant performance under changing environmental conditions. It is also highly relevant in the context of natural recruitment, as well as ecological restoration and revegetation, where successful plant establishment depends on the recruitment of suitable microbial partners (Shu et al., 2023; Watson et al., 2022). Early-stage root colonisation plays a critical role in plant performance, influencing nutrient acquisition, resistance to biotic and abiotic stress, and overall ecosystem recovery (Aleklett et al., 2022; Bai et al., 2022; Hartman & Tringe, 2019).

Microbial colonisation is shaped by both environmental filtering, particularly temperature and soil moisture and host-specific traits, which together determine which taxa successfully establish within plant roots (Dastogeer et al., 2020; Devesa-Aranguren et al., 2025; Frindte et al., 2019; in ’t Zandt et al., 2025; Li et al., 2018; Rasmussen et al., 2020). However, despite growing interest in microbial responses to environmental stress, it remains unclear how these communities assemble and reorganise during plant establishment under climate change. This knowledge gap is especially critical in alpine and sub-alpine ecosystems, where climate change is expected to strongly influence both plant performance and microbial assembly processes, as outlined in the previous four chapters. Identifying which microbial taxa successfully colonise plant roots under altered climatic conditions is therefore critical for predicting future plant-microbe interactions in these systems.

While Chapter 4 examined how root-associated fungal and AMF communities responded to climate treatments under controlled conditions, this Chapter uses a transplant experiment to investigate which fungal and AMF taxa can successfully colonise plant roots after transplantation into field climate manipulation plots. This field-based approach allows us to assess how experimental warming and drought shape root-associated fungal and AMF communities during establishment, such as in natural recruitment and revegetation events, in an Australian sub-alpine grassland. Using co-occurring sub-alpine plant species, we examine how climate stressors shape fungal diversity, community structure, and host-microbe associations. Specifically, we aimed to determine (1) how climate change influences fungal and AMF root colonisation of different host species in a transplant experiment; (2)

whether climate-induced compositional shifts of colonising fungi and AMF are characterized primarily by taxon turnover or nested species loss; and (3) whether the assembly of root-colonising fungal and AMF communities under warming and drought is governed predominantly by deterministic processes.

Based on previous studies showing contrasting responses of fungal communities to warming and drought (de Oliveira et al., 2020; Lyons et al., 2021), we hypothesised that climate stress would restructure fungal and AMF communities in a host-dependent manner. We further expected that warming and drought would differentially influence community composition and diversity across plant species, and that their combined effects would be more pronounced than either factor alone. We also predicted that compositional shifts would be associated primarily with taxon replacement rather than species loss, and that community assembly would be structured by environmental conditions rather than increased host specialization. This knowledge improves our understanding of the colonisation potential of root-associated fungi and provides insights into how plant-microbe interactions may influence revegetation outcomes under future climate change.

Materials and methods

Study site, experimental design and root sample collection

This experiment was conducted at the sub-alpine grassland experimental site described in Chapter 2. Briefly, the study site is located in the Australian sub-alpine region within Kosciuszko National Park, New South Wales, Australia (−36.372°, 148.431°). The site represents a sub-alpine meadow ecosystem characterized by short growing seasons and strong climatic variability. To examine the effects of climate change on root-associated microbial communities, an in-field climate manipulation experiment altering temperature and moisture conditions was established (Brown et al., 2024; Chapter 2).

Three co-occurring sub-alpine plant species representing different functional groups were selected for the study: the grass *Poa costiniana* and the forbs *Celmisia costiniana* and *Podolepis robusta*. *Poa costiniana* individuals were propagated under controlled greenhouse conditions with potting mix at the ANU, whereas *Celmisia costiniana* and *Podolepis robusta*

were obtained as nursery-grown seedlings from a native plant nursery in Victoria, Australia. Three-month-old *Poa* seedlings and one-year-old *Celmisia* and *Podolepis* seedlings were transplanted into the experimental field plots in early November 2022 and exposed to the climate manipulation treatments described in Chapter 2. The experiment comprised four climate-treatment combinations replicated across five blocks, resulting in 20 experimental plots. Six individuals of each plant species were transplanted into each plot. The plot represented the experimental unit for the climate treatments, while individual plants within each plot represented within-plot biological subsamples. Replacement planting was carried out in mid-December 2022 to compensate for early transplant mortality.

Plants were harvested in April 2024 following two growing seasons in the field. Whole plants were carefully excavated and transported to the laboratory under cooled conditions with rhizosphere soil attached to the root systems in order to preserve root-associated microbial communities. For molecular analyses, three individuals per species per plot were selected for root sampling and DNA extraction, resulting in 180 root samples in total. In the laboratory, roots were washed with tap water to remove adhering soil following the procedure described in Chapter 3. Cleaned root samples were preserved in 70% ethanol and stored at 4°C prior to DNA extraction and amplicon sequencing.

DNA extraction and amplicon sequencing

DNA extraction and amplicon sequencing followed the protocol described in the greenhouse experiment (Chapter 4). Briefly, ethanol-preserved root samples were rinsed thoroughly with deionized water and stored at −80 °C until DNA extraction. Genomic DNA was extracted from approximately 100 mg of root tissue using the DNeasy Plant Pro Kit (Qiagen, USA) following the manufacturer's instructions. DNA quality and concentration were assessed using a NanoDrop 2000 spectrophotometer and Qubit Fluorometer (Thermo Fisher Scientific, USA). Root-associated fungal and arbuscular mycorrhizal fungal (AMF) communities were characterized using amplicon sequencing targeting the fungal ITS1 region and the AMF 18S rRNA gene. The fungal ITS region was amplified using the primer pair ITS1F/ITS2R (Caporaso et al., 2012), and AMF were amplified using the primers AMV4.5NF/AMDGR (Sato et al., 2005). Sequencing libraries were prepared and sequenced using 2 × 300 bp paired-end reads

on an Illumina MiSeq platform (v3 chemistry) at the Biomolecular Resource Facility, Australian National University.

Bioinformatics and statistical analysis

Amplicon sequences of fungal internal transcribed spacer (ITS), and arbuscular mycorrhizal fungal 18S ribosomal RNA (18S r RNA) were processed following the workflow described in chapter 2, briefly using the DADA2 (Callahan et al., 2016) pipeline v1.28.0 in R (R Core Team, 2025). Demultiplexed paired-end reads were trimmed using Cutadapt (Martin, 2011) prior to DADA2 processing. Reads were dereplicated, denoised, and merged with a minimum overlap of 12 bp and up to 1 mismatch to allow longer amplicons to have substantial overlap. Amplicon sequence variants (ASVs) were constructed, screened for target length, and filtered to remove chimeras. Final ASVs were assigned unique identifiers and exported in FASTA format. ASVs were subsequently clustered into operational taxonomic units (OTUs) at 97% similarity using QIIME2 v2024.2. Taxonomic classification was then performed on OTU representative sequences, with fungal ITS sequences classified against the UNITE v10.0 reference database (Abarenkov et al., 2024; Nilsson et al., 2019) using QIIME2's VSEARCH consensus taxonomy classifier (classify-consensus-vsearch) with a minimum sequence identity threshold of 90%. For AMF, taxonomy was assigned to OTU representative sequences against the MaarjAM 18S rDNA QIIME reference database (2021 release; Öpik et al., 2010) using a Naive Bayes classifier trained with fit-classifier-naive-bayes and applied using QIIME2's classify-sklearn method. Non-target sequences, including mitochondrial and chloroplast sequences, were removed prior to downstream analysis. OTU tables were further filtered to minimize sequencing artefacts and low-confidence detections. OTUs present in only a single sample across the dataset were removed. This step also eliminated globally observed singleton OTUs, which was subsequently verified. OTUs observed only twice (doubletons) or three times (triplets) across all samples were also excluded to reduce the influence of rare, low-confidence features. The final OTU tables, along with taxonomic annotations, phylogenetic trees, representative sequences, and sample metadata, were imported into phyloseq objects using phyloseq R package v1.52.0 (McMurdie & Holmes, 2013) for downstream ecological and statistical analyses in R v4.5.1 (R Core Team, 2025).

Rarefaction and diversity estimation

To account for stochastic variation associated with subsampling, empirical rarefaction was performed for each amplicon dataset following the approach described by Schloss (2024). Each dataset was repeatedly rarefied 1,000 times using the rarefy_even_depth() function in the phyloseq R package. Rarefaction thresholds were selected to balance microbial diversity retention with adequate sample representation across treatments. Sequencing depths were set at 6,000 reads for fungal ITS datasets and 20,000 reads for AMF datasets (Figure D. 1). A fixed random seed was used to ensure reproducibility of rarefaction. Following rarefaction, eight fungal samples and three AMF samples were excluded due to insufficient sequencing depth. For each rarefaction iteration, alpha diversity metrics and Bray–Curtis dissimilarity matrices were calculated using the vegan R package (Oksanen et al., 2025). Distance matrices and diversity estimates from the 1,000 iterations were averaged for each sample to generate final estimates. These averaged values were then merged with sample metadata for downstream statistical analyses and visualisation.

Alpha diversity metrics were analysed following the approach described in Chapter 2. Briefly, diversity was quantified using taxon richness (Observed richness), Hill–Shannon diversity (q = 1), and Hill–Simpson diversity (q = 2), representing the effective number of taxa contributing to community diversity. Hill–Shannon and Hill–Simpson metrics were calculated as the exponential of the Shannon index and the inverse Simpson index, respectively (Hill, 1973). Treatment effects on alpha diversity were assessed using linear mixed-effects models, with temperature, moisture, and plant species included as fixed effects and experimental block included as a random effect. Model assumptions were evaluated using residual diagnostics, and the significance of fixed effects was tested using Type II analysis of variance followed by Tukey-adjusted post hoc comparisons.

To distinguish the mechanisms underlying treatment-specific root-colonising community structure, total β-diversity was partitioned into turnover and nestedness components. Species turnover represents taxonomic replacement between communities, whereas nestedness reflects differences in species richness where one community is largely a subset or superset of another, meaning taxa are lost or gained while the remaining taxa are shared

rather than replaced. Total dissimilarity was calculated using the Jaccard index based on presence–absence data. Partitioning of β-diversity was performed using the beta.multi function implemented in the betapart package in R. In this framework, total Jaccard dissimilarity (βJAC) is decomposed into species turnover (βJTU) and species nestedness (βJNE). In the context of this experiment, turnover represents the replacement of microbial taxa colonising plant roots under different climate treatments relative to the root-colonising communities of control plants at harvest, allowing treatment effects to be interpreted as either taxonomic replacement or differences in species numbers relative to the control colonisation community.

The relative contributions of stochastic and deterministic processes to microbial community assembly were quantified using the normalized stochasticity ratio (NST) implemented in the NST R package. The NST values range from 0 to 1, with values > 0.5 indicating stochastic dominance and values < 0.5 indicating deterministic assembly. NST was calculated separately for each plant species for both fungal and AMF communities. For each rarefaction replication (1000) and plant species, ASV abundance tables were converted to sample-by-taxon community matrices after removing taxa with zero total abundance. NST was calculated using the tNST() function with Bray–Curtis dissimilarity and 1000 randomizations, specifying treatment as the grouping variable. Pairwise NST values were extracted and pooled across rarefaction replicates, and NST distributions were visualised using boxplots. Co-occurrence network analyses were conducted separately for each plant species, following the approach described in Chapter 4, to assess potential shifts in root-associated fungal interactions under climatic treatments.

Host specialisation was quantified using a species–occupancy analysis, where ASVs were classified as specialists when both specificity and occupancy exceeded 0.7 within each climate treatment. To test whether climate manipulation and host identity influenced specialization probability, a binomial logistic regression was fitted using ASV-level data. The binary response variable indicated whether an ASV was classified as a specialist (1) or not (0), and fixed effects included Treatment (contol, drought, warming and combined warming and drought, Ambient × Non-droughted, Ambient × Droughted, Elevated × Non-droughted, Elevated × Droughted), Plant species (*Poa*, *Celmisia*, *Podolepis*), and their interaction. Model significance was

assessed using likelihood-ratio tests (analysis of deviance with χ^2 statistics). All analyses were conducted in R (R Core Team, 2025).

Potential differentially abundant microbial taxa were identified by the linear discriminate analysis (LDA) effect size (LEfSe) method with CPM normalization (Segata et al., 2011) at genus level. Briefly, the non-factorial parametric Kruskal Wallis (KW) sum-rank test was used to identify differences in the abundance of microbiome groups. Pairwise comparisons were performed for each plant between the control and each treatment group (drought, warming and warming and drought) to identify taxa differentially enriched after stress treatments. Significant taxa were identified using an LDA score > 2.0, combined with a Benjamini–Hochberg adjusted P-value (P_{adj}) < 0.05 to ensure both biological and statistical relevance.

Results

Diversity and structure of root-associated fungal communities

Climate stress altered not only how many microbial taxa colonised plant roots, but also how evenly they were distributed within established communities. The specific patterns differed among host plant species leading to significant species × treatment interactions (Table 5.1, Figure 5.1).

Table 5.1: Results of three-way ANOVA testing the effects of plant species, temperature, moisture, and their interactions on fungal (ITS) and AMF richness and diversity. Significant P-values are shown in bold.

Effects	Richness		Shannon		Simpson	
	Chisq	P value	Chisq	P value	Chisq	P value
			Fungi			
Plant	29.9	**<0.001**	21.0	**<0.001**	17.3	**<0.001**
Temperature	0.2	0.623	4.8	**0.029**	5.0	**0.025**
Moisture	0.9	0.333	0.2	0.625	0.1	0.767
Plant:Temperature	8.0	**0.018**	7.6	**0.022**	6.9	**0.031**
Plant:Moisture	3.6	0.169	1.2	0.557	0.9	0.647
Temperature:Moisture	0.3	0.590	0.1	0.722	0.2	0.650
Plant:Temperature:Moisture	1.1	0.573	0.9	0.644	1.5	0.470
			AMF			
Plant	7.0	**0.031**	19.0	**<0.001**	10.38	**0.006**
Temperature	0.0	0.991	0.002	0.965	0.03	0.874
Moisture	9.7	**0.002**	9.1	**0.003**	4.08	**0.043**
Plant:Temperature	5.5	0.063	1.5	0.467	0.76	0.684
Plant:Moisture	2.9	0.232	0.1	0.962	0.19	0.910
Temperature:Moisture	0.3	0.596	1.3	0.254	0.70	0.401
Plant:Temperature:Moisture	2.8	0.243	4.9	0.087	5.39	0.068

In *Poa*, the richness, relative abundance and dominance of fungal colonisation remained relatively stable across treatments. In *Celmisia*, warming significantly increased the richness and evenness of root colonising fungi (all $P < 0.05$, Table D. 1). In *Podolepis*, drought significantly influenced the diversity of AMF communities colonising plant roots by altering the relative abundance of common taxa ($P < 0.05$, Table D. 1). This indicates that overall, temperature structured the diversity of fungi colonising plant roots in a host-dependent manner, whereas AMF colonisation was shaped primarily by moisture and plant species, with no significant temperature-driven effects.

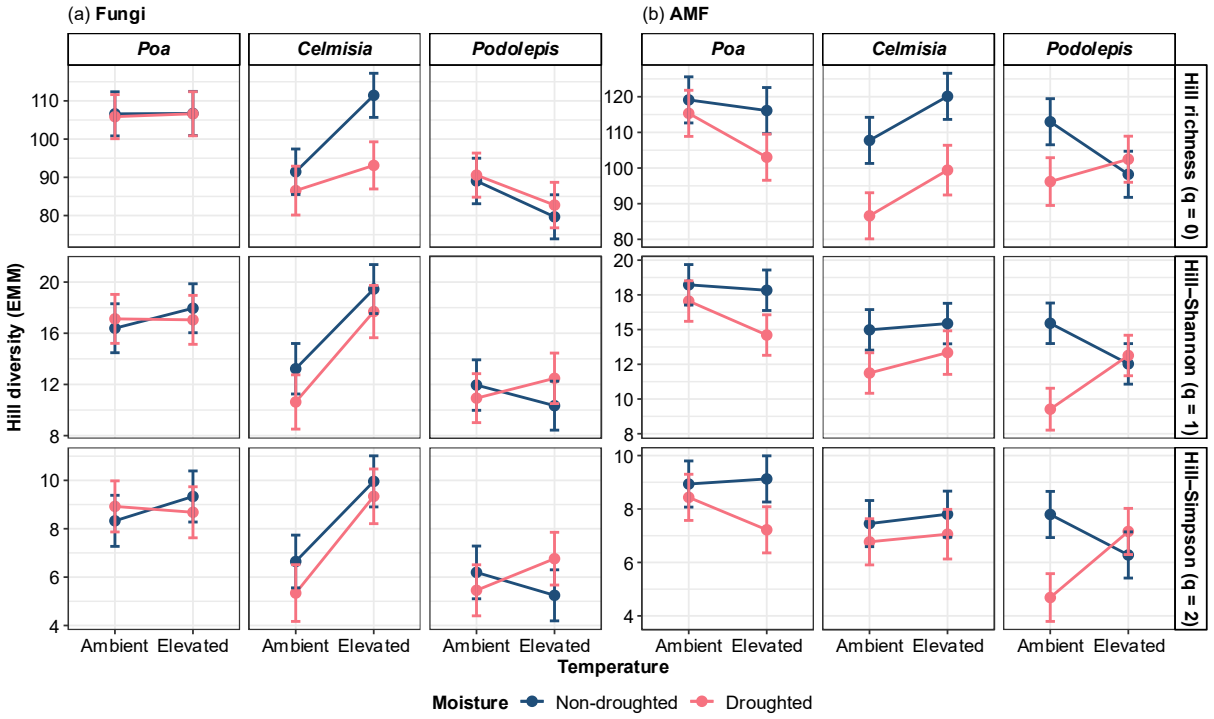


Figure 5.1: Climate-driven associations in root fungal and AMF diversity in *Poa*, *Celmisia* and *Podolepis*. Estimated marginal means (EMMs ± SE) were obtained from three-way factorial linear mixed-effects models (Temperature × Moisture × Soil inoculum), tested using Type II Wald χ^2 statistics.

Across all treatments, PCoA analyses revealed strong host-driven structuring of both fungal (ITS) and AMF communities associated with plant roots (Figure 5.2a and c). Community samples clustered primarily by plant species, with *Poa* forming a tight group characterised by low within-species compositional variability. In contrast, *Celmisia* and *Podolepis* showed greater dispersion and broad overlap, indicating more heterogeneous root-associated communities. PERMANOVA confirmed significant effects of temperature (T), moisture (M), plant species (P), and their interactions on community structure (all $P < 0.05$, Figure 5.2a and c) of root-colonising fungal and AMF communities.

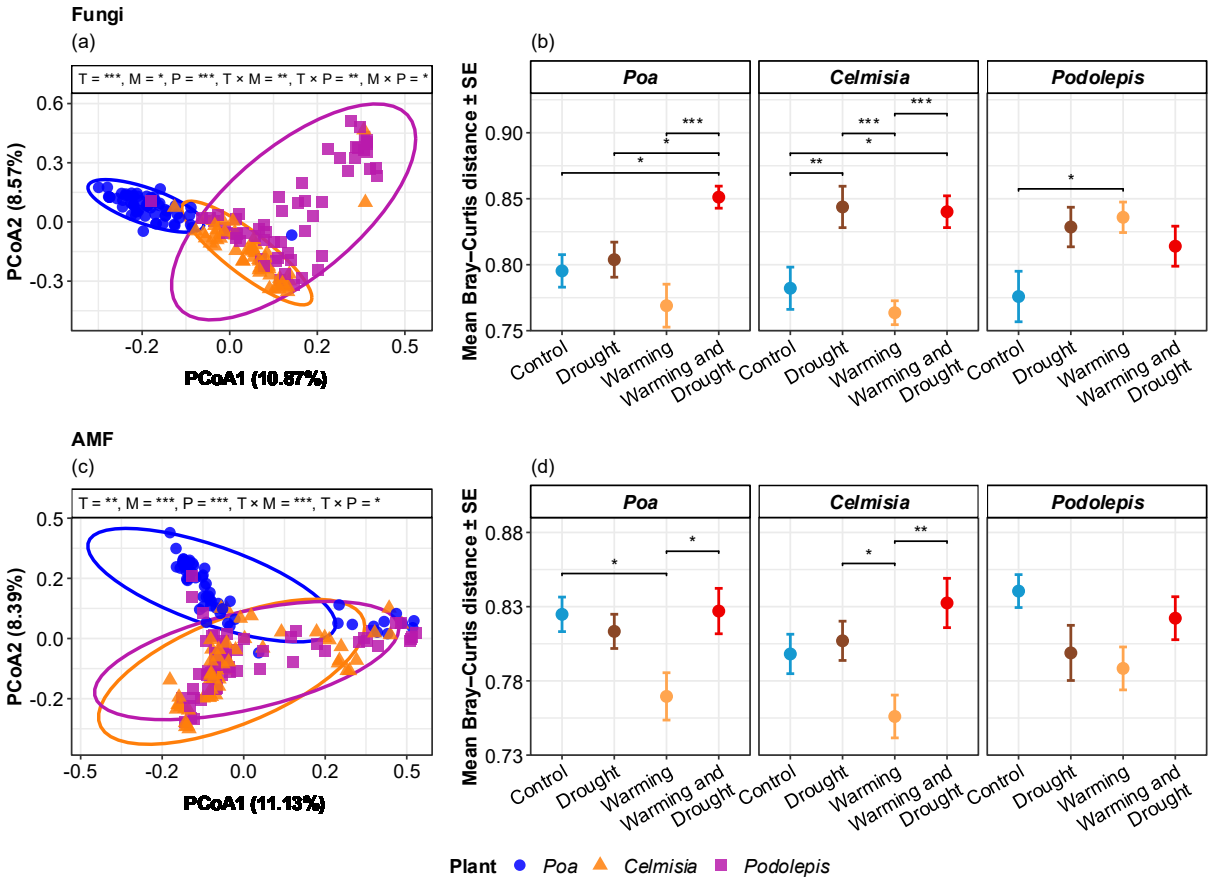


Figure 5.2: Differences in endophytic fungal communities among the three plant species. Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity is shown in panels (a, c). Within-plant mean pairwise Bray–Curtis distances (± SE) across treatments are presented in panels (b, d), with significant pairwise differences among treatments indicated based on Tukey's HSD post hoc comparisons (T, temperature; M, moisture; P, plant species; significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; post-hoc pairwise comparisons of mean distance with Tukey test and adjusted P-values).

In *Poa*, the combined warming and drought treatment is the primary driver of structural change in root-associated fungal communities, with significantly greater dissimilarity relative to control and single-stress treatments (all $P < 0.05$). In *Celmisia*, drought as well as combined warming and drought, exerted stronger effects on restructuring fungal communities colonising plant roots. In contrast, *Podolepis* exhibited weaker treatment differentiation, with modest compositional shifts in root-colonising fungal and AMF communities, although warming exerted a small but detectable effect ($P < 0.05$) compared to control. Overall, the

treatment-driven dissimilarity in fungal and AMF communities tended to increase under combined stress, but in a species-specific manner (Figure 5.2b and d).

Compositional differences were dominated by taxon turnover

Partitioning of total β-diversity showed that treatment-induced shifts in fungal (ITS) and AMF communities were driven primarily by species turnover (taxonomic replacement) rather than nestedness (differences in species richness where one community is largely a subset or superset of another) (Figure 3). Across all plant species, Jaccard dissimilarity was high (~0.75–0.81), indicating strong compositional divergence between treatment and control communities. In both ITS and AMF datasets, turnover accounted for most of this dissimilarity, whereas nestedness contributed only marginally. This pattern was consistent across *Poa*, *Celmisia*, and *Podolepis*, although *Celmisia* in the ITS dataset showed a slightly greater nestedness component under warming. Overall, these results indicate that climate treatments mainly altered which fungal and AMF taxa colonised plant roots, rather than simply reducing taxon richness. The dominance of turnover therefore suggests strong environmental filtering and selective replacement of colonising taxa under climate stress.

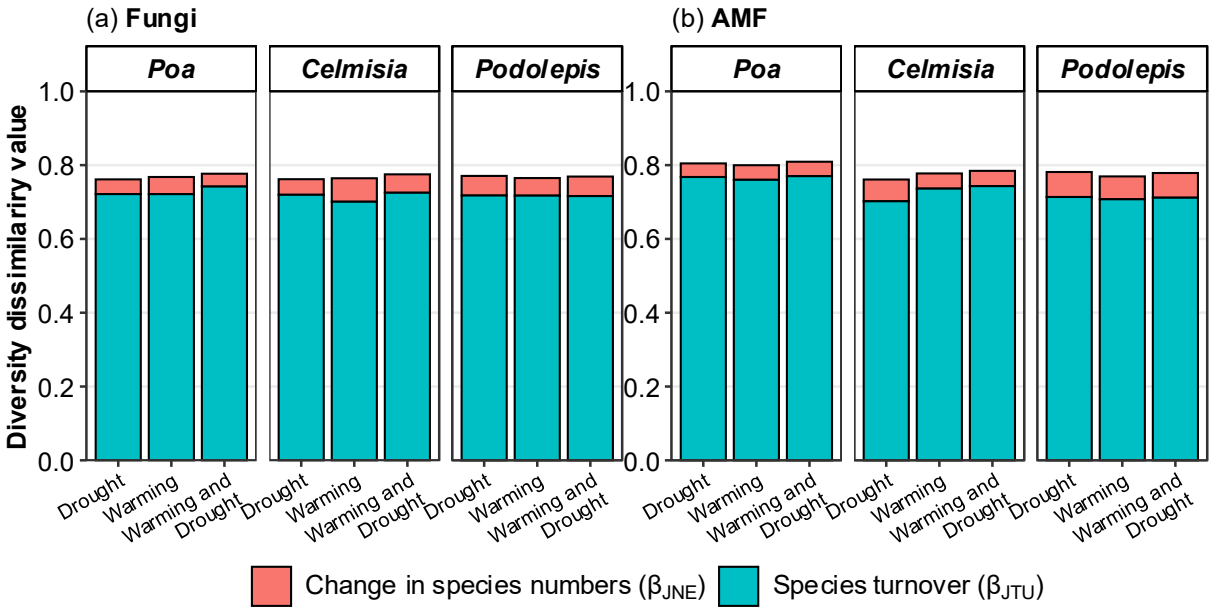


Figure 5.3: Partitioning of total β-diversity (Jaccard dissimilarity index) between control and climate treatments for (a) fungi and (b) AMF in *Poa*, *Celmisia*, and *Podolepis*. Jaccard β-diversity is decomposed into turnover (β_{JTU}; species replacement) and nestedness (β_{JNE}; richness differences due to species loss or gain).

Deterministic community assembly and network stability

The normalised stochasticity ratio (NST) was used to quantify the relative contributions of stochastic and deterministic processes in fungal community assembly. NST values greater than 0.5 indicate stochastic dominance, whereas values below 0.5 indicate deterministic dominance. Across all three host species, NST values were generally below 0.5 under all treatments, despite some variation. This suggests that deterministic processes, such as environmental filtering, predominantly governed the assembly of fungal communities associated with plant roots (Figure 5.4). Despite these climate-driven environmental filters, co-occurrence network topology remained relatively stable across treatments, with no significant differences detected in most network properties among treatments. However, a significant reduction in AMF network node number was observed in *Podolepis* under warming compared with the control treatment (Figure D. 2; Table D. 2).

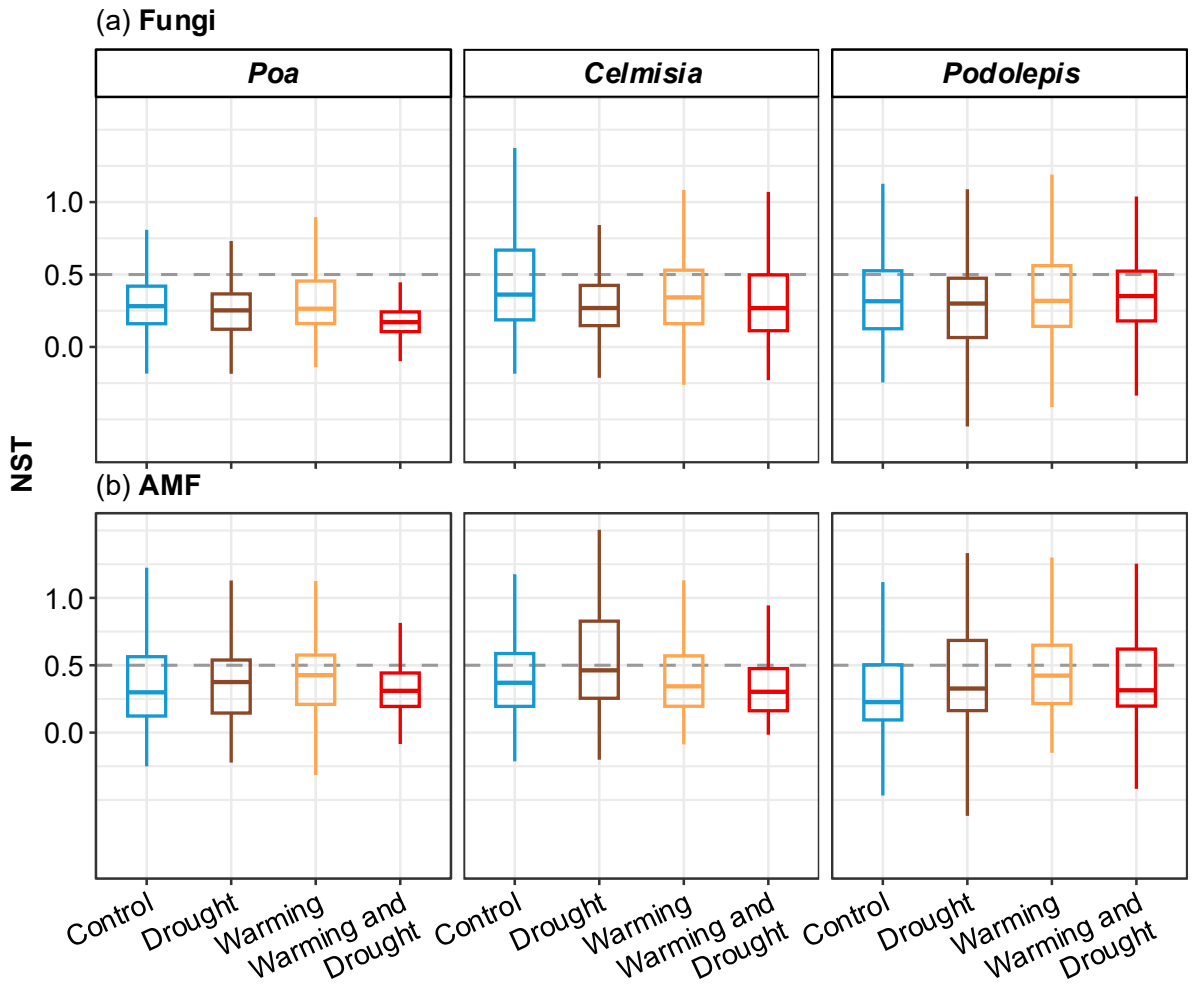


Figure 5.4: Normalised stochasticity ratio (NST) of endophytic (a) fungal and (b) arbuscular mycorrhizal fungal AMF communities under different treatments for each plant species. Values represent the relative contribution of stochastic versus deterministic assembly processes, with NST < 0.5 indicating deterministic assembly.

Host-specialist taxa comprised a small fraction of communities

Key fungal and AMF taxa were identified based on specificity and occupancy thresholds of ≥ 0.7, representing taxa strongly associated with particular climate treatments. Within the fungal communities, key taxa predominantly belonged to the phyla Ascomycota, Basidiomycota, and Mortierellomycota (Figure 5.5a). For AMF, key taxa were primarily affiliated with the orders Glomerales, Paraglomerales, and Diversisporales (Figure 5.5b).

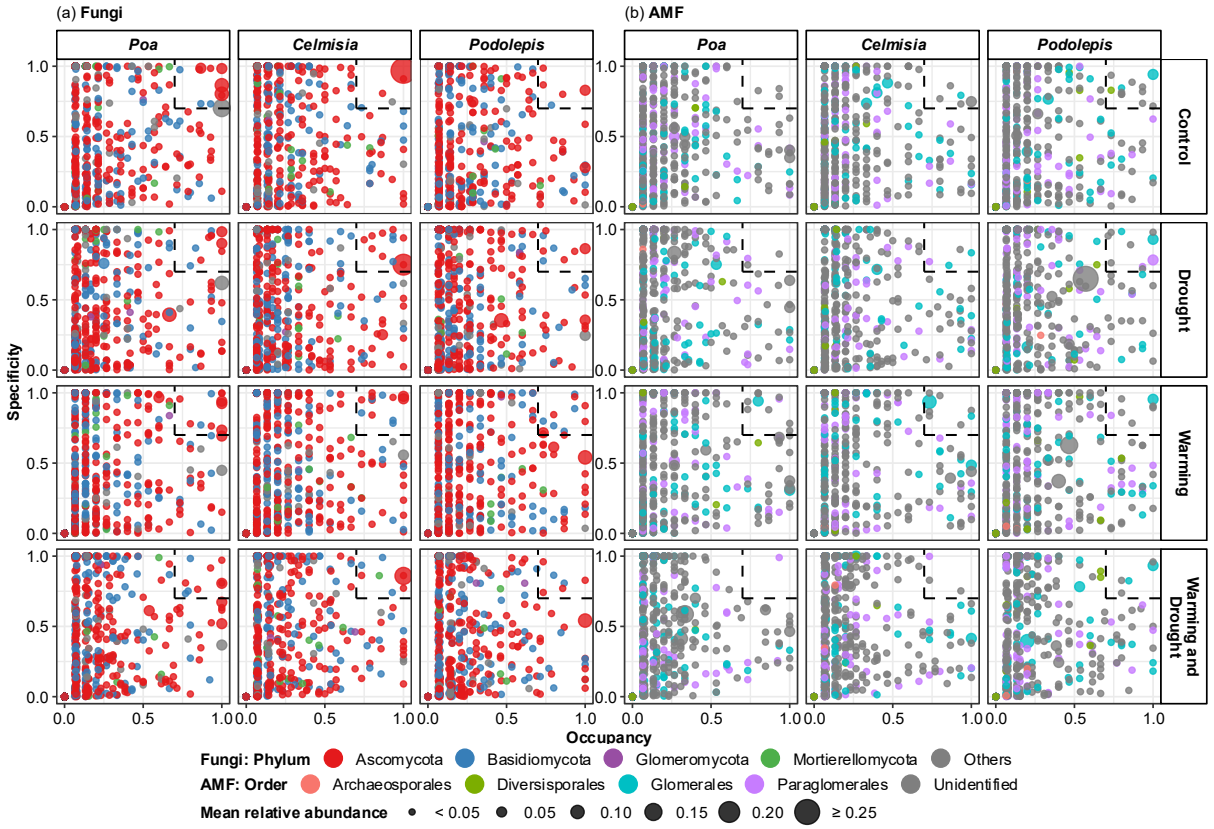


Figure 5.5: Host-specificity of root-associated fungal and AMF communities under different climatic stress. Specificity–occupancy plots show fungal ASVs associated with *Poa costiniana*, *Celmisia costiniana*, and *Podolepis robusta*. Each point represents an ASV, coloured by fungal phylum and for AMF coloured by order. The size of dots is scaled by mean relative abundance. Dashed lines indicate the specificity and occupancy thresholds (0.7), with ASVs exceeding both thresholds classified as host-specific specialists.

The proportion of specialist fungal ASVs was consistently low across treatments, ranging from 0.45% to 1.18% for fungi and 0.15% to 0.51% for AMF (Table D. 3). For fungal ASVs, binomial logistic regression revealed no significant effects of treatment, plant species, or their interaction on specialist occurrence (all $P > 0.05$; Table D. 4). In contrast, for AMF ASVs, the probability of being classified as specialists differed significantly among climate treatments ($P = 0.03$) and plant species ($P = 0.05$; Table D. 4). Post-hoc comparisons among climate treatments for AMF indicated a significant reduction under combined warming and drought relative to warming alone (BH-adjusted $P = 0.041$; Table D. 5). Among host species, *Podolepis* exhibited significantly higher specialisation probability than *Poa* (BH-adjusted $P = 0.039$; Table D. 5), with *Celmisia* showing intermediate values.

Differentially abundant taxa under climate treatments

The LEfSe analysis revealed that certain fungal taxa associated with plant roots were broadly sensitive to climatic treatments, independent of host identity (Figure 5.6). *Conocybe* was suppressed under climatic stress in all hosts, with the most consistent decline observed under combined warming and drought. *Endogonales* was suppressed exclusively under combined stress, whereas *Ophiosphaerella* exhibited consistent suppression under warming across hosts. In *Poa*, LEfSe analysis revealed clear compositional shift under climatic treatments. Drought, warming, and combined stress suppressed baseline saprotrophic taxa, including Mortierellaceae and *Subulicystidium*. In contrast, several stress-associated and root-colonising fungi were repeatedly enhanced, notably *Mycena*, *Oidiodendron*, Hypocreales, and *Chaetosphaeria*. Members of Hypocreales, including *Fusarium* and *Metapochonia*, as well as *Alternaria*, were also enriched, particularly under combined stress. In *Celmisia*, responses were characterised primarily by enhancement of fungal taxa, with warming and combined stress driving the strongest effects. *Talaromyces* was enhanced across all treatments, whereas Chaetothyriales and *Ceratobasidium* showed the strongest enrichment under warming, while *Penicillium* and Sordariales were preferentially enhanced under combined stress. In contrast, members of Helotiaceae and Sebacinales were repeatedly suppressed under warming and combined treatments. In *Podolepis*, responses were dominated by suppression of fungal taxa. Warming exerted the strongest effect and only Helotiales and *Conocybe* responded consistently across multiple treatments, both showing repeated suppression. AMF communities exhibited host specific responses to climatic treatments. In *Poa*, *Paraglomus* was enriched under both single stress of drought and warming, whereas Glomeromycete lineages were suppressed under warming and combined stress. In *Celmisia*, drought promoted *Acaulospora* but reduced *Glomus*, while *Ambispora* declined under combined stress. In *Podolepis*, *Claroideoglomus* was suppressed under drought.

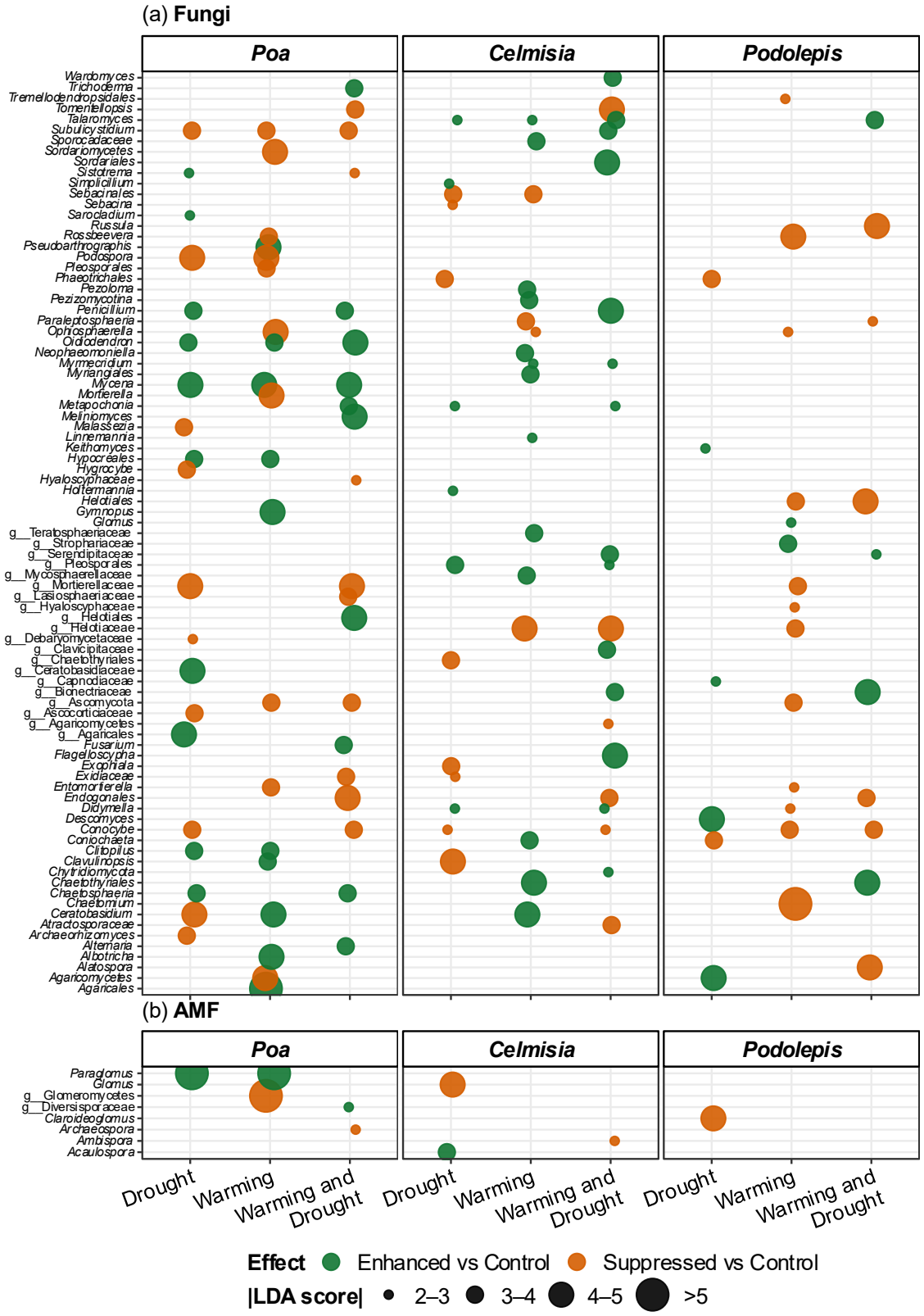


Figure 5.6: Differentially abundant fungal (a) and AMF (b) genera identified by LEfSe for each plant species. For each plant and pairwise comparison, up to 20 genera are visualised based on the highest absolute LDA scores (adjusted $p \leq 0.05$; $|LDA| \geq 2$).

Discussion

In this study we examined how drought, warming and their combined effects influence the assembly of the fungal and AMF communities colonising plant roots in a sub-alpine transplant experiment. Climate treatments altered fungal and AMF communities associated with plant roots, with responses varying strongly among host species. Differences in microbial diversity and community structure among *Poa*, *Celmisia*, and *Podolepis*, indicate that host identity plays a central role in shaping root microbial colonisation patterns. Experimental warming and drought modified community structure, with the strongest compositional shifts observed under combined stress. Beta-diversity partitioning showed that these shifts were primarily driven by species turnover rather than nested species loss, suggesting replacement of taxa during colonisation rather than simple reductions in diversity. The dominance of taxon turnover is consistent with normalized stochasticity ratio (NST) results indicating predominantly deterministic community assembly, suggesting that climate change treatments act as environmental filters that select for different microbial taxa during root colonisation. Despite these compositional shifts, host-specialist taxa remained rare, indicating that climate treatments reorganized broadly distributed taxa rather than promoting host specific colonisation. Differential abundance analysis (LEfSe) revealed that climatic treatments selectively enhanced several stress-associated fungal and AMF taxa while suppressing baseline taxa, with responses varying among host species.

Host identity and climate stress shape fungal and AMF colonisation patterns

Experimental warming and drought influenced the diversity of root-associated microbial communities, with responses differing between fungal and AMF assemblages and among host species. Temperature exerted a stronger influence on fungal diversity, whereas AMF responses were driven primarily by moisture. These patterns are partly consistent with soil microbial diversity responses in Chapter 2, particularly the strong sensitivity of AMF to moisture, while warming effects differed between soil- and root-associated communities, with consistent richness declines in all soil microbes but more variable responses in roots.

Responses were most pronounced in *Celmisia* compared with *Poa* and *Podolepis*. In *Celmisia* warming increased both fungal and AMF richness and evenness, indicating that more taxa successfully established within roots and that community dominance was reduced. The strong sensitivity of AMF to moisture is consistent with studies in temperate grasslands showing that mycorrhizal strategies shape microbial responses to environmental variability (Jian et al., 2022).

In contrast, microbial communities associated with *Poa* formed relatively tight clusters, indicating lower compositional variability, whereas *Celmisia* and *Podolepis* exhibited greater dispersion and heterogeneity. These patterns indicate that plant identity mediates microbial responses to climate treatments. This host-dependent structuring may reflect differences in plant functional traits and mycorrhizal associations, which influence microbial recruitment and community composition across diverse ecosystems (Jiang et al., 2021a; Wang et al., 2019). Similar host dependent responses have been reported in alpine and grassland ecosystems, where plant traits regulate microbial assembly under altered temperature and moisture regimes (Becklin et al., 2012; Kivlin et al., 2022; Kou et al., 2024; Xie et al., 2023). Such structuring is often attributed to variation in plant traits, including root morphology, nutrient acquisition strategies, mycorrhizal dependency and the composition of root exudate composition that regulate microbial recruitment (Compant et al., 2010; Rodríguez et al., 2025; Wicaksono et al., 2024).

The strongest structural shifts were observed under combined warming and drought conditions, more evident in *Poa* and *Celmisia* than *Podolepis*. This may reflect host-mediated regulation of microbial communities, where plants alter rhizosphere conditions and resource availability under stress, thereby influencing microbial composition and resilience (Hou et al., 2024; Veach et al., 2020). In addition, certain fungal taxa possess traits that enhance tolerance to climate stress, including thick cell walls, osmolytes production and melanin synthesis (Schimel et al., 2007; Treseder & Lennon, 2015). Combined stress may further intensify environmental filtering, altering microbial recruitment from the surrounding soil pool (Bérard et al., 2015; Catarecha et al., 2025). Together, these findings demonstrate that plant-microbe interactions mediate the selective colonisation of root-associated microbial communities under climate treatments, with implications for microbial assembly in sub-alpine ecosystems.

Environmental filtering drives microbial colonisation patterns

Although climate treatments had variable effects on fungal and AMF diversity, they strongly altered the composition of root-associated communities. Beta-diversity partitioning showed that these compositional shifts were driven primarily by taxon turnover rather than nestedness, indicating replacement of taxa during colonisation rather than through simple species loss (Hoefle et al., 2024; Jones et al., 2025; Stewart et al., 2026). Changes in temperature and soil moisture can modify the ecological niches available for microbial colonization, thereby favouring taxa adapted to altered conditions. Similar climate-driven filtering has been observed in alpine ecosystems, where warming alters fungal community structure through moisture-related niche selection (Yin et al., 2024). In addition to direct environmental effects, climate change may indirectly influence microbial community composition through plant-mediated mechanisms, as temperature and moisture can alter plant root exudation patterns that regulate microbial recruitment (Tiziani et al., 2022). Similar turnover-dominated responses have been reported in other ecosystems (Hoefle et al., 2024), although a weaker warming effect was reported, suggesting that responses may vary depending on host species and experimental context (Kazenel et al., 2019). Together, the dominance of taxon turnover and low NST values indicate that climate treatments strongly filter which microbial taxa successfully colonise plant roots. This filtering was most pronounced in *Poa* under combined warming and drought, suggesting that multiple stressors intensify selective colonisation and restrict establishment to stress-tolerant taxa. In contrast to Wei et al. (2024), where bacterial assembly shifted across the stochastic–deterministic threshold under combined climate treatments, fungal NST values in this study remained consistently below 0.5, indicating persistent deterministic assembly even under ambient conditions. This suggests that root-associated fungal communities in sub-alpine systems are inherently structured by strong environmental filtering and host-mediated selection. Given the low temperatures, nutrient limitation, and short growing seasons characteristic of sub-alpine environments, climate manipulation may strengthen deterministic filtering without fundamentally altering the dominant processes governing fungal colonisation. Despite

compositional turnover under climatic stress, co-occurrence network remained relatively stable across treatments, suggesting that fungal interaction patterns within plant roots were largely maintained. Similar decoupling between community composition and microbial association patterns has been reported in previous studies, where co-occurrence networks showed greater stability than taxonomic composition under environmental stress (Chen et al., 2026; Gao et al., 2022). In particular, root-associated endophytic microbial networks may exhibit greater resilience to environmental perturbation due to strong host-mediated selection and the maintenance of stress-tolerant core microbial associations (Banerjee et al., 2018; Chen et al., 2026). However, the reduced AMF network node number observed under warming in *Podolepis* suggests that some host-specific AMF associations may be sensitive to elevated temperature, potentially reflecting reduced complexity of microbial interactions in this species.

Host specialisation and taxonomic responses to climate treatments

Our findings suggest that root-associated fungal and AMF communities are dominated by generalist taxa capable of colonizing multiple host species in this sub-alpine ecosystem. Despite this dominance of generalist taxa, our alpha- and beta-diversity analyses showed that microbial community diversity and structure differed significantly among host plant species, indicating that plant identity mediates the establishment and relative abundance of broadly shared taxa. The dominance of generalist microbial taxa is widely reported in plant-associated microbiomes, where many microbial species can colonize a broad range of hosts (Albracht et al., 2023; Jiang et al., 2021b; Lee et al., 2013). Such generalist strategies may be advantageous under environmentally variable conditions, as microbes with broader ecological niches can colonise diverse habitats and persist under changing environments (Sriswasdi et al., 2017; Wang et al., 2021), although this advantage is likely context dependent. Under climate stress, generalist microbes may therefore play an important role in maintaining microbial community stability despite environmental fluctuations (Xu et al., 2022).

Despite the dominance of generalist taxa revealed by the species–occupancy analysis, climatic treatments altered the abundance of several fungal and AMF lineages across host species, including taxa associated with opportunistic or stress-tolerant lifestyles. This indicates that environmental filtering reshaped which taxa successfully colonised plant roots under warming and drought. These responses were most pronounced under combined warming and drought, suggesting that climatic stress favours taxa capable of rapidly exploiting altered rhizosphere conditions (Pan et al., 2024; Xun et al., 2024). Several stress-responsive fungal taxa increased under climatic treatments, including members of Hypocreales and other saprotrophic or root-associated groups. For example, taxa such as *Fusarium*, *Alternaria*, and *Metapochonia* increased in *Poa*, especially under combined warming and drought. Similar drought-associated increases in fungal taxa, including *Fusarium*, have been reported in tropical grasslands (Moravcová et al., 2023). Although overall diversity appeared stable in *Poa*, the community underwent substantial turnover-driven compositional reorganisation, reflecting potentially altering the functional balance of the microbiome rather than enhancing plant-microbe benefits. In *Celmisia*, these shifts were particularly evident. Warming increased fungal and AMF richness and evenness; however, taxon-specific responses indicate that this shift does not necessarily reflect an increase in beneficial associations. Members of the order Helotiales, which may contribute to nutrient acquisition (Bruyant et al., 2026), declined under climate stress, while several saprotrophic or opportunistic fungi (e.g., *Wardomyces*, *Penicillium*, *Talaromyces*, Clavicipitaceae) significantly increased in abundance. This suggests that climate-induced changes in root-associated communities may shift the community towards a more opportunistic assemblage, with unclear implications for host performance and may also have consequences for plant health, as climate-driven environmental filtering can favour opportunistic or potentially pathogenic taxa, aligning with broader evidence that climate change can increase the establishment and spread of plant-associated pathogens (Chaloner et al., 2021). The recurrence of particular taxa across treatments further indicates that fungal assembly under climatic stress is not random but reflects deterministic filtering driven by environmental conditions and host identity. Together, these findings indicate that climatic treatments promote selective establishment of fungal taxa during colonisation, highlighting the combined roles of environmental filtering and plant-mediated selection in structuring root-associated fungal communities.

Taken together, the results demonstrate that climate change alters the composition and assembly of root-associated fungal and AMF communities in sub-alpine plants. Community responses were strongly influenced by host plant identity, while warming and drought modified patterns of microbial colonisation and turnover, particularly when acting in combination. These shifts reflected changes in the taxa occupying root habitats rather than simple reductions in diversity, indicating that climatic stress reshapes root microbiomes through changes in community assembly processes. Collectively, these findings highlight the importance of considering both host identity and environmental change when predicting belowground responses to future climate conditions.

Conclusion and future directions

Experimental warming and drought significantly restructure sub-alpine root-associated fungal and AMF communities, primarily through deterministic, turnover-driven replacement of broadly shared taxa rather than widespread changes in host specialization. Community composition is structured by host plant identity, with temperature shaping fungal (ITS) diversity in a plant-dependent manner and moisture primarily influencing AMF diversity. Combined warming and drought produce pronounced compositional shifts, particularly in *Poa*, whereas *Celmisia* and *Podolepis* exhibit more heterogeneous and species-specific responses. Despite clear structural differentiation, specialist taxa remain rare, indicating that climate stress reorganises generalist-dominated communities rather than intensifying host exclusivity. Overall, environmental filtering emerges as the dominant mechanism governing which microbial taxa successfully colonise sub-alpine roots under future climate scenarios, rather than stochastic drift or shifts in specialisation. The insights gained here about patterns of microbial colonisation and community composition raise interesting questions about the functional consequences of these shifts for plant performance or ecosystem processes. These findings suggest that future climate change will not simply reduce microbial diversity in sub-alpine plant roots but will drive host-dependent, deterministic reassembly of broadly distributed taxa, with potential consequences for plant stress responses and belowground ecosystem functioning. However, colonisation in this study was inferred from sequence-based relative abundance rather than directly measured. Future studies combining

sequencing with direct assessments of root colonisation, such as microscopy-based staining or quantification of fungal structures within roots, would help determine whether shifts in microbial abundance reflect active colonisation under climate stress. These findings also have important implications for ecological restoration in sub-alpine systems. As microbial communities establish through deterministic, climate-driven filtering during colonisation, restoration success will depend on which taxa can establish under future conditions. Although the dominance of generalist microbes may support plant establishment across hosts, turnover-driven restructuring suggests that restored communities will differ from current ones, potentially affecting plant performance and ecosystem functioning. Incorporating microbial colonisation processes into restoration planning may therefore improve resilience under climate change.

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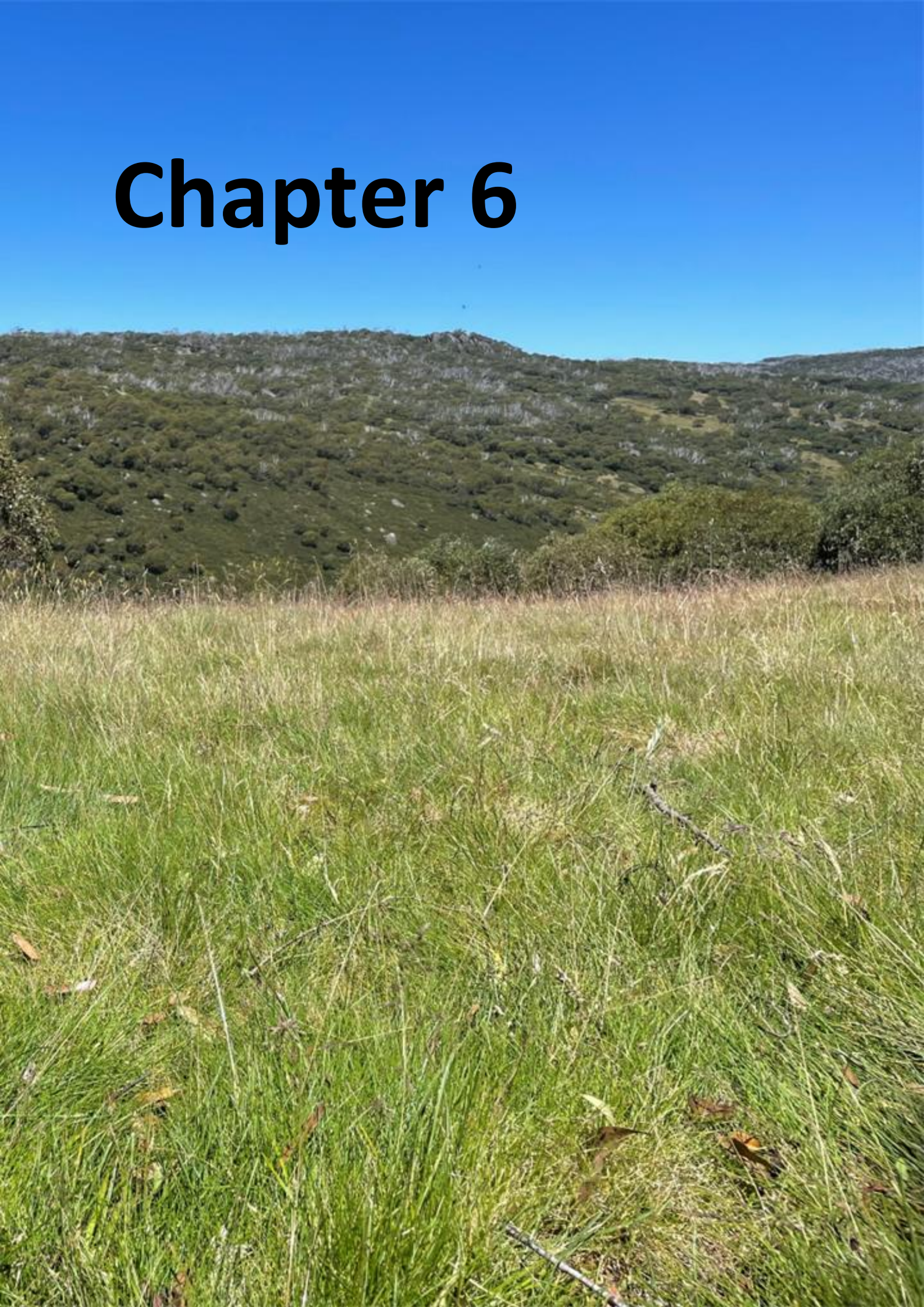
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Chapter 6



Chapter 6: Discussion

Overview

In this thesis, I sought to develop a comprehensive understanding of how climate change influences soil- and root-associated microbial communities and plant-microbe interactions in Australian sub-alpine ecosystems. To address this, I used an integrated experimental manipulation of temperature and moisture in field as well as in controlled greenhouse condition. While each chapter focused on a distinct aspect of climate-driven ecological change, several common themes emerged. Here, I first summarise the major findings of each experimental chapter before synthesising them to develop a broader understanding of how climate stress reshapes belowground ecological interactions in sub-alpine ecosystems.

In Chapter 2, I investigated how long-term warming and drought influenced soil microbial communities across multiple seasons in a sub-alpine grassland ecosystem. I found that, climate stress reduced the diversity and altered the community structure of soil fungi, AMF, bacteria, and archaea, although the magnitude and direction of responses differed among microbial groups. Combined warming and drought exerted stronger effects on all soil microbial communities, whereas AMF and bacterial communities were often more strongly influenced by drought-associated moisture limitation. Moreover, seasonal patterns strongly influenced microbial diversity and community structure across treatments. I found fungal and archaeal richness generally peaked during summer, likely reflecting increased temperature and plant productivity, whereas bacterial richness increased progressively over time, suggesting possible acclimation to repeated climate fluctuations, indicating potential consequences for ecosystem functioning under future climate scenarios.

In Chapter 3, I further investigated how microbial community composition, function and interaction affected under long-term warming and drought in the same sub-alpine grassland ecosystem. Since Chapter 2 demonstrated that microbial responses were strongest during summer, this chapter focused specifically on this biologically active period to better understand how climate stress influences belowground microbial functioning and interactions under peak environmental conditions. I found an alignment with the former

reduction of microbial diversity under climate stress. Climate stress reshaped microbial compositions, and the interaction and their potential functional roles of microbes within the ecosystem. However, the magnitude of these responses differed among microbial groups and respective stressors. Fungal communities responded most strongly to combined warming and drought, whereas bacterial communities were more strongly influenced by drought. Across microbial groups, climate stress generally promoted stress-tolerant and saprotrophic taxa while reducing groups associated with symbiosis and anaerobic processes, indicating substantial restructuring of belowground community compositions. I further demonstrated that climate stress altered not only microbial composition, but also microbial ecological interactions and predicted metabolic functions. Fungal and AMF co-occurrence networks consistently showed reduced complexity and connectivity under combined warming and drought, whereas bacterial networks remained comparatively stable despite strong compositional responses to drought. Predicted functional analyses revealed reductions in pathways associated with carbon metabolism, nutrient cycling, biosynthesis, and energy production across multiple microbial groups under prolonged stress. However, the extent to which these belowground changes translate into altered plant performance and root-associated microbial dynamics remained unclear, providing the basis for the greenhouse experiment explored in Chapter 4.

In Chapter 4, I investigated how warming and drought influenced plant performance and root-associated fungal communities under controlled greenhouse conditions using three functionally distinct native sub-alpine plant species. I found that plant responses to climate stress were strongly species-specific, although combined warming and drought generally reduced plant growth and biomass across treatments. I also found that climatic stress altered fungal trophic mode composition, with saprotrophic fungi becoming increasingly dominant under warming and drought treatments, whereas symbiotrophic and pathotrophic groups generally declined or remained stable under stress. However, relationships between root-associated fungal diversity and plant growth were often weak, suggesting that climate stress may partially decouple plant performance from belowground microbial community structure.

In Chapter 5, I investigated how long-term warming and drought influenced the fungal and AMF communities colonising the roots of transplanted native sub-alpine plants under field

conditions. I found that, root-associated microbial diversity and community composition are strongly influenced by both host identity and climate stress. Community shifts were driven primarily by taxonomic replacement rather than species loss, and microbial assembly appeared largely deterministic rather than random, reflecting strong environmental filtering under altered climatic conditions. Despite significant restructuring across treatments, root-associated communities were largely dominated by broadly distributed generalist taxa. Nevertheless, climatic stress frequently promoted saprotrophic and stress-tolerant fungal taxa, whereas several symbiotic fungal groups and AMF lineages showed repeated suppression under warming and combined stress treatments, suggesting shifts in root-associated fungal ecological strategies under environmental stress.

Microbial diversity alone is insufficient to explain ecological responses

Diversity indices are widely used to assess microbial responses to environmental change (Finn, 2024; Wagner et al., 2018), yet diversity patterns alone often fail to capture the broader ecological mechanisms or community dynamics underlying those response (Hughes et al., 2001; Shade, 2017). In my study, diversity metrics did not always align with shifts in community composition, networks stability, or functional attributes. However, in Chapter 2 and 3, I demonstrated clear cases where changes in soil fungal diversity were reflected in corresponding shifts in community composition, species interactions and functional pathways. Under combined warming and drought, fungal diversity reduced significantly, accompanied by strong suppression or enrichment of numerous taxa and functional pathways. These changes resulted in simplified co-occurrence networks, indicating reduced structural complexity. In contrast, bacterial networks in Chapter 3 remained comparatively stable under drought, even though bacterial diversity declined, community composition shifted, and functional pathways were reduced. Notably, during the second summer, bacterial networks became more complex under drought than under control conditions, suggesting compensatory or stress-induced restructuring. Similarly, in the transplant experiment (Chapter 5), root-associated fungal and AMF communities of *Poa* maintained relatively stable diversity under climatic stress. However, beta diversity partitioning revealed that community

changes were primarily driven by taxonomic turnover, indicating replacement of existing taxa by stress-tolerant groups under climatic stress. This pattern was also supported by Linear Discriminant Analysis Effect Size (LEfSe) and Normalized Stochasticity Ratio (NST) analyses.

Taxonomic alpha diversity and functional alpha diversity are often only weakly correlated, or even entirely decoupled, across alpine and sub alpine ecosystems (Nemergut et al., 2005; Wang et al., 2019). Thus, communities can look very different in terms of "who is there," yet perform similar overall functions, a pattern attributed to functional redundancy (Chen et al., 2022; Louca et al., 2018; Nemergut et al., 2005). Such redundancy can enable microbial communities to maintain broader/key ecological processes (Chen et al., 2022) and preserve interaction stability even when diversity declines or community composition shifts (Nemergut et al., 2005; Ramond et al., 2025; Shade, 2017). This framework helps explain the discrepancy in bacterial networks in my study: despite reduced bacterial diversity and altered community composition, network structure remained stable under drought, suggesting that drought acted as a selective filter that preserved a core set of stress-tolerant, functionally redundant taxa with strong interaction links (Gao et al., 2022; Wu et al., 2021).

Importantly, several studies have shown that the drivers of taxonomic structure (e.g., pH, moisture, dispersal limitation) often differ from those shaping functional structure (Cao et al., 2020; Louca et al., 2016; Qingqing et al., 2024; Wang et al., 2019). As a result, environmental gradients may shift richness and composition in one direction while functional traits or process rates follow another trajectory (Siles & Margesin, 2016; Sveen et al., 2025). Supporting this, a global meta-analysis of 1,235 studies found that soil functionality under climate change is better explained well by microbial community structure and biomass rather than by alpha diversity alone (Zhou et al., 2020). Therefore, focusing solely on diversity patterns may provide an incomplete or even misleading picture of microbial responses to climate stress. A more comprehensive understanding requires combining multiple analytical approaches and integrating e.g. taxonomic composition, functional traits, network structure, and environmental filtering to capture how climate change reshapes sub-alpine microbial communities.

Warming emerges as a stronger ecological driver than drought in sub-alpine ecosystems

Across my thesis, warming generally imposed broader and more consistent negative effects on plants and on both soil- and root-associated microbial communities than drought alone. Warming frequently reduced microbial (both soil and root) richness and diversity, altered fungal trophic mode composition, disrupted fungal network stability, and reduced plant growth and biomass across experiments. In contrast, drought effects were often more variable and appeared particularly important for bacterial community restructuring. The patterns likely reflect the fact that alpine and sub-alpine organisms are adapted to relatively cool conditions. Consequently, warming can therefore directly influence metabolic rates (Körner & Kèorner, 1999), shift seasonal phenology (Wolkovich et al., 2012), or impose substantial physiological stress on plants (Steinbauer et al., 2022). Such plant-level changes may be particularly important for fungal and AMF communities, which depend strongly on plant-derived carbon inputs to maintain symbiotic associations and ecological functioning (Ballhausen & de Boer, 2016; De Deyn et al., 2011).

Combined climatic stressors amplify ecological disruption

Across my thesis, combined warming and drought consistently produced the strongest negative impacts on soil and root microbial communities as well as plant performance, aligning with the broader literature (Cuartero et al., 2024; Hoefle et al., 2024; Montoya et al., 2025; von Rein et al., 2016). This likely reflects interacting physiological and resource limitations imposed by elevated temperature and reduced moisture availability for plants (David et al., 2017; Dos Santos et al., 2022; Rehschuh & Ruehr, 2022) and microbes (Guan et al., 2017; Nielsen & Ball, 2015). Experimental studies have similarly showed that drought strongly weakens the ability of alpine plants to cope with experimental warming conditions (Buchner et al., 2017; Kuo et al., 2026). Similarly, a global meta-analysis of 85 climate manipulation studies demonstrated that warming generally increased plant biomass under conditions of increased precipitation, whereas warming combined with drought frequently reduced biomass responses (Wu et al., 2011). Consistent with my findings, experimental

studies in alpine ecosystems have shown that warming and drought can alter multiple dimensions of microbial functioning, including growth, carbon-use efficiency, enzyme activity, and community dynamics, highlighting the strong sensitivity of alpine soil microbiomes to climate change (Liu et al., 2024; Ruan et al., 2024; Zhu et al., 2021).

Elevated temperatures can stimulate microbial biomass (Waghmode et al., 2019) metabolism and soil respiration (Song et al., 2019), accelerating soil carbon turnover and CO_2 efflux from soils (Allison & Treseder, 2008; Nissan et al., 2023). Moreover, warming can increase atmospheric evaporative demand and reduce soil moisture availability, thereby intensifying water stress for plants and soil microorganisms (David et al., 2017). Drought, in turn, can limit substrate diffusion, nutrient availability, and plant-derived carbon inputs to belowground systems (Ficken & Warren, 2019). Simultaneous exposure to warming and drought stressors may constrain microbial functioning and plant physiological performance (Ficken & Warren, 2019). Increasing numbers of global change drivers have been shown to exert non-additive effects on microbial communities, leading to declines in microbial diversity and shifts in community composition towards stress-tolerant taxa (Rillig et al., 2019). Such multifactor interactions often generate stronger responses than individual stressors alone for both plants and microbes (Fuggle et al., 2025; Zandalinas & Mittler, 2022).

Climatic extremes can also act as environmental filtering, favouring microbial taxa capable of dormancy, reduced metabolic activity, and other stress-tolerance strategies (Bardgett & Caruso, 2020; Jones & Lennon, 2010; Knight et al., 2024). Given that sub-alpine ecosystems are typically characterised by cool temperatures and relatively high moisture availability (Andrus et al., 2018; Bailey et al., 2021), the strong responses observed in my thesis likely reflect the limited historical exposure of resident communities to prolonged warming and drought stress (Hou et al., 2024; Kuo et al., 2026). Such restructuring of microbial communities and plant-microbial associations may have important long-term implications for nutrient cycling, decomposition processes, ecosystem stability, and plant resilience within sub-alpine environments (Broadbent et al., 2024; Hafeez et al., 2023; Hagedorn et al., 2019; Pandey et al., 2025; Seaton et al., 2022).

Climate-driven restructuring of fungal functional groups

Consistent with the findings of my thesis, recent studies show that drought and other climatic stressors can restructure microbial communities through environmental filtering, altering microbial diversity and functional groups, while reducing mutualistic microbial capacity and increasing the dominance of opportunistic or disease-associated taxa (Allison, 2023; Qiao et al., 2026). Similar patterns emerged throughout my thesis, where prolonged climatic stress reduced symbiotrophic fungi and favoured saprotrophic and opportunistic taxa across both soil- and root-associated communities, indicating a shift away from mutualistic associations towards communities better adapted to environmental stress and resource limitation. One potential mechanism underlying these shifts is the disruption of plant-microbe resource exchange under climatic stress (Wang et al., 2021). Reduced plant productivity under warming and drought may limit the carbon available to support root-associated symbiotic fungi (Hawkins et al., 2023; Ruehr et al., 2019). Recent syntheses suggest that carbon transfer to mycorrhizal fungi is strongly influenced by plant carbon availability, making symbiotic fungi particularly sensitive to reductions in host carbon supply (Bunn et al., 2024). In addition, reduced soil moisture can constrain nutrient mobility, particularly phosphorus, thereby altering the costs and benefits of plant-fungal mutualisms (Karlowsky et al., 2018). Climatic stress may also act as a strong environmental filter, favouring taxa with traits that enhance persistence under resource limitation and environmental disturbance (Allison, 2023). Another possible explanation is that climatic stress alters host physiology and root resource availability, thereby reshaping root fungal communities. Indeed, shifts in root fungal assemblages have been linked to plant physiological responses to abiotic stress, highlighting the important role of host condition in structuring root-associated fungi (Martínez-Arias et al., 2020). Such changes may favour opportunistic saprotrophic and pathotrophic fungi while reducing the competitive advantage of symbiotic fungi. Similar shifts have been observed following declines in mycorrhizal dominance, where opportunistic fungal groups rapidly increase and occupy newly available niches (Kohout et al., 2021; Lindahl et al., 2010).

However, these changes did not necessarily indicate a collapse of root-associated microbial communities. Despite substantial shifts in fungal ecological strategies and community

composition, root fungal networks remained comparatively stable under climatic stress. Furthermore, beta-diversity partitioning indicated that community responses were driven primarily by species turnover rather than nested species loss, suggesting replacement of fungal taxa and ecological functions rather than wholesale disruption of plant-microbe associations. The relatively low host specificity observed across both greenhouse and transplant experiments, where many fungal taxa were shared among host species, may have facilitated this reorganisation by allowing replacement among taxa without major disruption of root microbial interactions. Such shifts may weaken the protective and nutritional functions provided by root-associated microbial communities, potentially increasing plant vulnerability to environmental stress and disease pressure under future climate change (Singh et al., 2023). The decline of mutualistic fungi may also weaken positive plant-microbe feedback. Reduced nutrient acquisition and plant performance can decrease root-derived carbon inputs to soil, further constraining beneficial microbial groups and reinforcing the dominance of opportunistic taxa, thereby creating a feedback loop that accelerates microbiome degradation (Qiao et al., 2026).

Species-specific plant responses to climatic stress

Many climate change studies focus on a single plant species, particularly when testing physiological or developmental responses to stress (de Oliveira et al., 2020; Gray & Brady, 2016), which may limit broader understanding of ecosystem-level responses to climatic stress (Becklin et al., 2016; Kooyers et al., 2025). In this thesis, I have demonstrated that plant responses to climatic stress are strongly species-specific. The sedge *Carex capillacea* showed comparatively greater tolerance to warming and drought, maintaining higher growth and biomass, whereas the forb *Celmisia pugioniformis* was substantially more sensitive, exhibiting marked reductions in growth and biomass accumulation under stress. These findings broadly align with those of Gerwin et al. (2025), who reported that warming altered community composition in Australian subalpine meadows by increasing sedge abundance while reducing the abundance of several forb and grass groups. The greater tolerance of *C. capillacea* relative to *C. pugioniformis* observed in the present study is therefore consistent with the tendency for warming to favour sedges over forbs under changing climatic conditions. These

contrasting responses suggest that plant species differ considerably in their sensitivity to climatic stress, even within the same ecosystem. Similar species-specific drought responses have been reported in alpine grasslands, where drought reduced biomass production in some forb and graminoid species but had little effect on the dominant sedge *Carex curvula*, leading to shifts in community composition (Möhl et al., 2023). Consequently, predictions of vegetation change under future climate scenarios must account for species-level variation rather than assuming uniform responses across plant communities (Dahle et al., 2024; Mok et al., 2012).

Contrasting community responses of soil and root dwelling fungi to climate stress in sub-alpine grasslands

Soil- and root-associated microbial communities responded differently to climatic stress in respect of diversity and community interactions, suggesting host-mediated buffering of root-associated communities (Jones et al., 2019). Under both individual and combined stress treatments, soil fungal communities showed pronounced reductions in richness and diversity, whereas root microbial communities exhibited plant specific responses that also varied depending on the experimental environment. While soil fungal and AMF networks showed strong declines in network complexity under warming and combined warming and drought, root-associated fungal co-occurrence networks showed comparatively limited topological restructuring in both greenhouse and field conditions. This stability likely reflects the more protected habitat of root endophytes, which remain closely linked to host carbon allocation and physiological processes, potentially reducing their direct exposure to climatic stress (von Rein et al., 2016). Furthermore, host identity is often a major determinant of root microbiome assembly (Morella et al., 2020; Xiong et al., 2021), which may constrain community restructuring under environmental stress. Consistent with this interpretation, Lagueux et al. (2021) proposed that fungi inhabiting plant roots may are be less sensitive to drought than free-living soil fungi. Together, these findings suggest that climatic stress acts as a stronger environmental filter on free-living soil fungal communities, whereas root-associated fungal communities are partially buffered by host-mediated processes, resulting in greater structural stability under climatic stress.

Field conditions reveal ecological responses not captured under greenhouse experiments

The greenhouse and field experiments revealed different patterns of root-associated microbial responses to climatic stress, highlighting the importance of environmental context in shaping plant-microbiome interactions. Under controlled greenhouse conditions, root-associated fungal and AMF communities responded relatively similarly among host species exposed to climatic stress. In contrast, the field transplant experiment showed species-specific microbial responses, where microbial diversity remained relatively stable in *Poa*, increased in *Celmisia*, and declined in *Podolepis* under the same climatic stress. Such discrepancies between greenhouse and field outcomes are consistent with previous work. For example, Chung et al. (2019) found that root-associated fungal communities differed substantially between greenhouse and field environments, with field plants showing greater variability in microbial composition. Similar differences have been reported in plant-soil feedback studies, where ecological responses observed under controlled conditions often differ from those measured in natural field environments due to greater environmental complexity and biotic interactions (Heinze et al., 2016).

Environmental heterogeneity is a defining feature of natural sub-alpine ecosystems and plays a major role in shaping microbial community assembly through variation in seasonal conditions, soil properties, vegetation composition, and microbial dispersal (Huang et al., 2026; Liu et al., 2026). Field conditions may also strengthen host-mediated microbial selection under climatic stress, resulting in more distinct and context-dependent microbial responses among plant species. Furthermore, access to larger and more diverse native microbial pools may provide greater opportunities for plants to recruit stress-adapted microbial taxa (Liu et al., 2020). Recent work in subalpine grasslands suggests that plant-soil relationships may weaken under more severe climate change, leading to a partial decoupling of plant and microbial dynamics (Terry et al., 2025). Greenhouse experiments, while valuable for isolating mechanism, simplify natural variability and interactions, thus they may not fully capture field-scale ecosystem responses (Brien et al., 2013; Karitter et al.). Indeed, some field studies in sub-alpine grasslands have reported relatively stable or even increased plant productivity

under moderate warming conditions (Li et al., 2018; Volk et al., 2021). Synthesis of several long-term field studies in alpine and sub-alpine ecosystems further show that climatic warming may substantially alter plant community composition and biomass allocation patterns without necessarily causing immediate declines in overall productivity (Liu et al., 2018). Together, these findings highlight the importance of evaluating plant-microbiome responses under realistic field conditions rather than relying solely on controlled greenhouse experiments.

Future directions

While this thesis has advanced our understanding of how warming and drought influence soil- and root-associated microbial communities many of the mechanisms linking microbial responses to ecosystem functioning remain unresolved. Future research would benefit from linking climate-induced changes in microbial community composition with direct measurements of ecosystem functioning. Such studies should therefore quantify processes such as soil respiration, extracellular enzyme activities, decomposition rates, nutrient mineralisation, plant nutrient uptake, and mycorrhizal colonisation to determine how climate-driven shifts in microbiomes influence nutrient cycling, carbon turnover, and ecosystem resilience.

A major priority for future studies is to move beyond taxonomic characterisation and predicted functional profiling by applying shotgun metagenomic and metatranscriptomic approaches to directly assess microbial functional potential and activity under warming and drought. Although approaches such as PICRUSt2 provided valuable insights into potential functional responses of microbial communities, future studies could employ shotgun metagenomic sequencing of soil and root samples to directly characterise genes involved in carbon cycling, nutrient acquisition, stress tolerance, and plant-microbe interactions. Metatranscriptomic analyses conducted during periods of warming and drought could further identify which of these genes are actively expressed under climatic stress, providing direct evidence of microbial functional responses that cannot be inferred from amplicon sequencing alone.

Additionally, future work should integrate microbial community analyses with detailed measurements of soil physicochemical properties, microbial biomass, and ecosystem processes. In addition to total carbon, nitrogen, and phosphorus, future studies could quantify dissolved organic carbon, mineral nitrogen pools (NH_4^+ and NO_3^-), soil pH, moisture dynamics, and extracellular enzyme activities involved in carbon, nitrogen, and phosphorus cycling. Phospholipid fatty acid (PLFA) analysis, and neutral lipid fatty acid (NLFA) analyses, were originally planned as part of this study but could not be completed due to logistical and time constraints. Analyses such as these may potentially provide an independent assessment of microbial biomass, broad community structure and arbuscular mycorrhizal fungal (AMF) abundance (Olsson, 1999). Integrating PLFA/NLFA measurements with amplicon sequencing and functional analyses would help determine whether climate-induced shifts in microbial community composition are accompanied by changes in microbial biomass and activity. Measurements of soil respiration, litter decomposition rates, greenhouse gas fluxes, and carbon sequestration could then be used to establish direct links between microbial responses and ecosystem functioning. Such integration is particularly important in sub-alpine ecosystems, where large soil carbon stores may be especially vulnerable to future warming and drought (Chen et al., 2024; García-Palacios et al., 2024; Walker et al., 2022).

Another important research direction is to directly link climate-induced shifts in microbial communities with plant performance in field environments. Although this thesis quantified plant growth and biomass responses in greenhouse experiments, comparable measurements were not available alongside the field-based microbiome analyses. Future studies should therefore integrate microbial community assessments with measurements of plant growth, biomass, nutrient status, reproductive output, and survival within long-term climate manipulation experiments. This would allow direct testing of whether microbial shifts observed under warming and drought are associated with improved plant performance, reduced plant performance, or neutral outcomes for host plants. Such information would provide critical insight into whether altered plant-microbe interactions contribute to plant resilience or vulnerability under future climatic conditions. Extending these measurements across multiple alpine and sub-alpine sites would further allow assessment of whether

observed plant-microbiome responses are consistent across environmental gradients or are influenced by local site conditions.

Finally, future research should address a central challenge in soil ecology: resolving ecological processes across the spatial and temporal scales at which microorganisms operate, rather than at scales imposed by sampling convenience (Chu et al., 2020; Ladau & Eloe-Fadrosh, 2019). Microbial communities function within highly heterogeneous soil microhabitats, experience rapid turnover, and can remain dormant for extended periods (Joergensen & Wichern, 2018; Jones & Lennon, 2010). Future studies should therefore combine high-frequency seasonal sampling with fine-scale rhizosphere, root-surface, and bulk-soil sampling to better capture microbial dynamics through time and space (Philippot et al., 2013). Quantitative stable isotope probing (qSIP) could further be used to identify microorganisms actively assimilating carbon and nutrients under warming and drought conditions, thereby distinguishing active microbial populations from dormant members of the community (Greenlon et al., 2022; Hungate et al., 2015). Such approaches would provide a more mechanistic understanding of microbial adaptation to climatic stress and improve our capacity to predict how microbe-microbe and plant-microbe interactions contribute to ecosystem responses under future climate change.

Conclusion

By integrating greenhouse and field approaches to examine both soil- and root-associated microbiomes, this thesis provides new evidence that climate change is likely to drive substantial changes in belowground community structure and function, which has important implications for the future stability and functioning of sub-alpine ecosystems. I have shown that interacting climatic stressors (i.e. warming and drought) have greater ecological consequences than either in isolation, highlighting the need to consider multiple climate drivers when predicting ecosystem responses to future environmental change. Moreover, as microbial groups do not respond uniformly to climatic stress and different components of the microbiome are sensitive to different aspects of climate change, climate change is likely to reorganise, rather than simply reduce, belowground biodiversity. On this basis, future climate change may alter nutrient cycling, plant–microbe mutualisms, and soil carbon dynamics in

sub-alpine ecosystems. Ultimately, the resilience of sub-alpine ecosystems will depend on both individual plant-microbe responses, and the capacity for these interactions to persist under increasingly warmer and drier conditions.

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Appendix A: Supplementary materials of Chapter 2

Table A. 1: Overview of primers used to amplify target regions of microbial groups of interest.

Microbial target	Primer pair	Sequence (5'-3')	Target region	Reference
Fungi	ITS1f	CTTGGTCATTTAGAGGAAGTAA	ITS1	Gardes and Bruns (1993);
	ITS2	GCTGCGTTCTTCATCGATGC		White et al. (1990)
AMF	AMF4.5NF	AAGCTCGTAGTTGAATTTCG	18S	Sato et al. (2005)
	AMDGR	CCCAACTATCCCTATTAATCAT		
Bacteria	341F	CCT ACG GGN GGC WGC AG	16S	Caporaso et al. (2011);
	806R	GGACTACHVGGGTWTCTAAT		Herlemann et al. (2011)
Archaea	519F	CAGYMGCCR CGGKAA HACC	16S	Coolen et al. (2004)
	915R	GTGCTC CCC CGC CAA TTCCT		

Table A. 2: PCR reagents concentration and amount for each primer pair.

Reagents	Primer Pair			
	ITS1f/ITS2	AMF4.5NF/AMDGR	341F/806R	519F/915
5X High fidelity Phusion buffer (Thermo Scientific)	4 µl			
Phusion Hot Start II Polymerase	0.3µl			
50 mM $MgCl_2$	0.2µl	0.2µl		
dNTPs (2.5 nM)	1.6µl			
2X High fidelity Phusion Master Mix	-	10µl	12.5µl	12.5µl
100% DMSO	-	0.75µl	0.75µl	0.75µl
Primer F (2.0 nM)	3.75 µl	1.25µl	1.5µl	1.25µl
Primer R (2.0 nM)	3.75 µl	1.25µl	1.5µl	1.25µl
gDNA (2.5 ng/µl)	2.5 µl	2.5µl	2.5µl	2.5µl
Milli-Q water	3.9µl	4.05µl	1.25µl	1.75µl
Total	20 µl	20µl	20µl	20µl

Table A. 3: Summary of ANOVA results for treatment effects on soil chemical properties.

Fixed effects	df	Total Carbon		Total Nitrogen		Available Phosphorus	
		χ^2	P-value	χ^2	P-value	χ^2	P value
Time	1	164.4	**<0.001**	530.2	**<0.001**	41.6	**<0.001**
Temperature	1	0.0002	0.99	0.1	0.79	0.1	0.78
Moisture	1	1.0	0.33	1.6	0.21	1.3	0.25
Time: Temperature	1	11.1	**0.001**	0.1	0.76	0.9	0.34
Time: Moisture	1	0.1	0.80	7.6	0.01	3.0	0.08
Temperature: Moisture	1	0.04	0.84	0.8	0.38	1.1	0.28
Time: Temperature: Moisture	1	10.3	**0.001**	12.4	**<0.001**	2.7	0.10

Table A. 4: Results of pairwise post-hoc tests showing seasonal differences in richness (Observed index) and diversity (Hill–Shannon index) for (a) fungal, (b) AMF, (c) bacterial, and (d) archaeal communities. Columns show estimated mean differences (Estimate), standard error (SE), degrees of freedom (df), t-ratio, and corresponding p-values obtained from linear mixed-effects models.

	Species Richness (Observed index)					Species diversity (Hill Shannon index)				
(a) Fungi										
Contrast	**Estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Estimate**	**SE**	**df**	**t.ratio**	**P value**
Summer 2023 – Autumn 2023	25.08	6.78	255	3.70	0.002	7.05	2.18	255	3.23	**0.01**
Summer 2023 – Spring 2023	29.38	6.78	255	4.33	**<0.001**	4.00	2.18	255	1.83	0.36
Summer 2023 – Summer 2024	22.37	6.88	255	3.25	0.01	1.06	2.21	255	0.48	0.99
Summer 2023 – Autumn 2024	30.02	6.94	255	4.33	**<0.001**	10.75	2.23	255	4.81	**<0.001**
Autumn 2023 – Spring 2023	4.30	6.75	255	0.64	0.97	-3.04	2.17	255	-1.40	0.63
Autumn 2023 – Summer 2024	-2.71	6.85	255	-0.40	0.99	-5.99	2.20	255	-2.72	0.05
Autumn 2023 – Autumn 2024	4.93	6.91	255	0.71	0.95	3.70	2.22	255	1.66	0.46
Spring 2023 – Summer 2024	-7.01	6.85	255	-1.02	0.84	-2.95	2.20	255	-1.34	0.67
Spring 2023 – Autumn 2024	0.63	6.91	255	0.09	1.00	6.75	2.22	255	3.03	0.02
Summer 2024 – Autumn 2024	7.65	7.01	255	1.09	0.81	9.69	2.25	255	4.30	**<0.001**
(b) AMF										
Contrast	**Estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Estimate**	**SE**	**df**	**t.ratio**	**P value**
Summer 2023 – Autumn 2023	24.94	6.41	263	3.89	**0.001**	5.62	2.11	263	2.66	0.06
Summer 2023 – Spring 2023	31.32	6.38	263	4.91	**<0.001**	7.18	2.10	263	3.42	**0.01**
Summer 2023 – Summer 2024	26.32	6.38	263	4.13	**<0.001**	6.72	2.10	263	3.20	**0.01**
Summer 2023 – Autumn 2024	25.06	6.38	263	3.93	**0.001**	2.94	2.10	263	1.40	0.63
Autumn 2023 – Spring 2023	6.38	6.41	263	0.99	0.86	1.56	2.11	263	0.74	0.95
Autumn 2023 – Summer 2024	1.38	6.41	263	0.22	1.00	1.10	2.11	263	0.52	0.99
Autumn 2023 – Autumn 2024	0.13	6.41	263	0.02	1.00	-2.68	2.11	263	-1.27	0.71

(b) AMF

Contrast	Estimate	SE	df	t.ratio	p.value	Estimate	SE	df	t.ratio	P value
Spring 2023 – Summer 2024	-4.99	6.38	263	-0.78	0.94	-0.46	2.10	263	-0.22	1.00
Spring 2023 – Autumn 2024	-6.25	6.38	263	-0.98	0.86	-4.24	2.10	263	-2.02	0.26
Summer 2024 – Autumn 2024	-1.26	6.38	263	-0.20	1.00	-3.78	2.10	263	-1.80	0.38

(c) Bacteria

Contrast	Estimate	SE	df	t.ratio	p.value	Estimate	SE	df	t.ratio	P value
Summer 2023 – Autumn 2023	-18.40	15.22	249	-1.21	0.75	-1.72	3.86	245.66	-0.45	0.99
Summer 2023 – Spring 2023	-26.63	14.18	245	-1.88	0.33	-5.90	3.58	244.21	-1.64	0.47
Summer 2023 – Summer 2024	-75.22	14.18	245	-5.31	**<0.001**	-12.61	3.58	244.21	-3.52	0.010
Summer 2023 – Autumn 2024	-107.30	14.53	248	-7.38	**<0.001**	-18.40	3.68	245.18	-5.00	**<0.001**
Autumn 2023 – Spring 2023	-8.24	15.15	247	-0.54	0.98	-4.18	3.84	246.07	-1.09	0.81
Autumn 2023 – Summer 2024	-56.82	15.15	247	-3.75	0.002	-10.89	3.84	246.07	-2.83	0.04
Autumn 2023 – Autumn 2024	-88.91	15.51	247	-5.73	**<0.001**	-16.69	3.94	247.12	-4.23	0.00
Spring 2023 – Summer 2024	-48.58	14.05	244	-3.46	0.01	-6.72	3.55	244.02	-1.89	0.32
Spring 2023 – Autumn 2024	-80.67	14.40	247	-5.60	**<0.001**	-12.51	3.64	244.82	-3.43	0.01
Summer 2024 – Autumn 2024	-32.09	14.40	247	-2.23	0.17	-5.79	3.64	244.82	-1.59	0.51

(d) Archaea

Contrast	Estimate	SE	df	t.ratio	p.value	Estimate	SE	df	t.ratio	P value
Summer 2023 – Autumn 2023	11.62	1.76	250	6.61	**<0.001**	1.07	0.22	249	4.93	**<0.001**
Summer 2023 – Spring 2023	4.92	1.69	249	2.92	0.03	-0.26	0.21	249	-1.22	0.73784
Summer 2023 – Summer 2024	-5.26	1.67	249	-3.15	0.02	-1.63	0.21	249	-7.88	**<0.001**
Summer 2023 – Autumn 2024	-0.23	1.68	249	-0.14	1.00	-0.60	0.21	249	-2.88	0.03492
Autumn 2023 – Spring 2023	-6.71	1.77	249	-3.78	0.002	-1.33	0.22	249	-6.05	**<0.001**
Autumn 2023 – Summer 2024	-16.89	1.76	250	-9.60	**<0.001**	-2.70	0.22	249	-12.40	0

(d) Archaea										
Contrast	**Estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Estimate**	**SE**	**df**	**t.ratio**	**P value**
Autumn 2023 – Autumn 2024	-11.86	1.77	250	-6.71	**<0.001**	-1.67	0.22	249	-7.64	**<0.001**
Spring 2023 – Summer 2024	-10.18	1.69	249	-6.04	**<0.001**	-1.37	0.21	249	-6.57	**<0.001**
Spring 2023 – Autumn 2024	-5.15	1.69	249	-3.04	**0.02**	-0.34	0.21	249	-1.63	0.4783
Summer 2024 – Autumn 2024	5.03	1.68	249	3.00	**0.02**	1.03	0.21	249	4.96	**<0.001**

Table A. 5: Results of pairwise post-hoc tests showing the effects of temperature and moisture treatments on richness (Observed index) in (a) fungal, (b) arbuscular mycorrhizal fungal (AMF), (c) bacterial, and (d) archaeal communities. Columns show estimated mean differences (Estimate), standard error (SE), degrees of freedom (df), t-ratio, and corresponding p-values from linear mixed-effects models.

	Species richness (Observed index)					**Species diversity (Hill Shannon index)**				
(a) Fungi										
Contrast	**Estimate**	**SE**	**df**	**t.ratio**	**P value**	**Estimate**	**SE**	**df**	**t.ratio**	**P value**
Ambient: Non-droughted – Elevated: Non-droughted	17.67	11.25	12	1.57	0.43	3.18	4.97	12	0.64	0.92
Ambient: Non-droughted – Ambient: Droughted	13.01	11.24	12	1.16	0.66	1.87	4.97	12	0.38	0.98
Ambient: Non-droughted – Elevated: Droughted	35.42	11.24	12	3.15	**0.04**	4.35	4.97	12	0.88	0.82
Elevated: Non-droughted – Ambient: Droughted	-4.66	11.25	12	-0.41	0.97	-1.31	4.97	12	-0.26	0.99
Elevated: Non-droughted – Elevated: Droughted	17.74	11.25	12	1.58	0.43	1.18	4.97	12	0.24	1.00
Ambient: Droughted – Elevated: Droughted	22.41	11.24	12	1.99	0.24	2.48	4.97	12	0.50	0.96
(b) AMF										
Contrast	**Estimate**	**SE**	**df**	**t.ratio**	**P value**	**Estimate**	**SE**	**df**	**t.ratio**	**P value**
Ambient: Non-droughted – Elevated: Non-droughted	56.48	14.50	12	3.90	**0.01**	11.24	5.0	12	2.24	0.17
Ambient: Non-droughted – Ambient: Droughted	64.77	14.49	12	4.47	**0.004**	13.71	5.0	12	2.73	0.07
Ambient: Non-droughted – Elevated: Droughted	57.93	14.49	12	4.00	**0.01**	12.44	5.0	12	2.48	0.11
Elevated: Non-droughted – Ambient: Droughted	8.28	14.50	12	0.57	0.94	2.47	5.0	12	0.49	0.96
Elevated: Non-droughted – Elevated: Droughted	1.44	14.50	12	0.10	1.00	1.21	5.0	12	0.24	0.99

(b) AMF

Contrast	Estimate	SE	df	t.ratio	P value	Estimate	SE	df	t.ratio	P value
Ambient: Droughted – Elevated: Droughted	-6.84	14.49	12	-0.47	0.96	-1.27	5.0	12	-0.25	0.99

(c) Bacteria

Contrast	Estimate	SE	df	t.ratio	P value	Estimate	SE	df	t.ratio	P value
Ambient: Non-droughted – Elevated: Non-droughted	58.74	13.04	12	4.50	**0.004**	8.52	5.4	12	1.57	0.43
Ambient: Non-droughted – Ambient: Droughted	63.32	13.09	12	4.84	**0.002**	18.46	5.4	12	3.39	**0.02**
Ambient: Non-droughted – Elevated: Droughted	54.96	13.25	12	4.15	**0.01**	15.93	5.5	12	2.91	**0.05**
Elevated: Non-droughted – Ambient: Droughted	4.58	12.94	11	0.35	0.98	9.94	5.4	12	1.83	0.31
Elevated: Non-droughted – Elevated: Droughted	-3.78	13.11	12	-0.29	0.99	7.41	5.4	12	1.36	0.55
Ambient: Droughted – Elevated: Droughted	-8.36	13.15	12	-0.64	0.92	-2.53	5.5	12	-0.46	0.97

(d) Archaea

Contrast	Estimate	SE	df	t.ratio	P value	Estimate	SE	df	t.ratio	P value
Ambient: Non-droughted – Elevated: Non-droughted	1.16	2.52	13	0.46	0.97	-0.04	0.4	12	-0.09	1.00
Ambient: Non-droughted – Ambient: Droughted	-1.25	2.48	12	-0.51	0.96	0.66	0.4	12	1.60	0.41
Ambient: Non-droughted – Elevated: Droughted	4.50	2.47	12	1.82	0.31	0.57	0.4	12	1.38	0.54
Elevated: Non-droughted – Ambient: Droughted	-2.41	2.52	13	-0.96	0.77	0.70	0.4	12	1.68	0.37
Elevated: Non-droughted – Elevated: Droughted	3.34	2.51	13	1.33	0.56	0.61	0.4	12	1.45	0.49
Ambient: Droughted – Elevated: Droughted	5.75	2.47	12	2.33	0.15	-0.09	0.4	12	-0.23	1.00

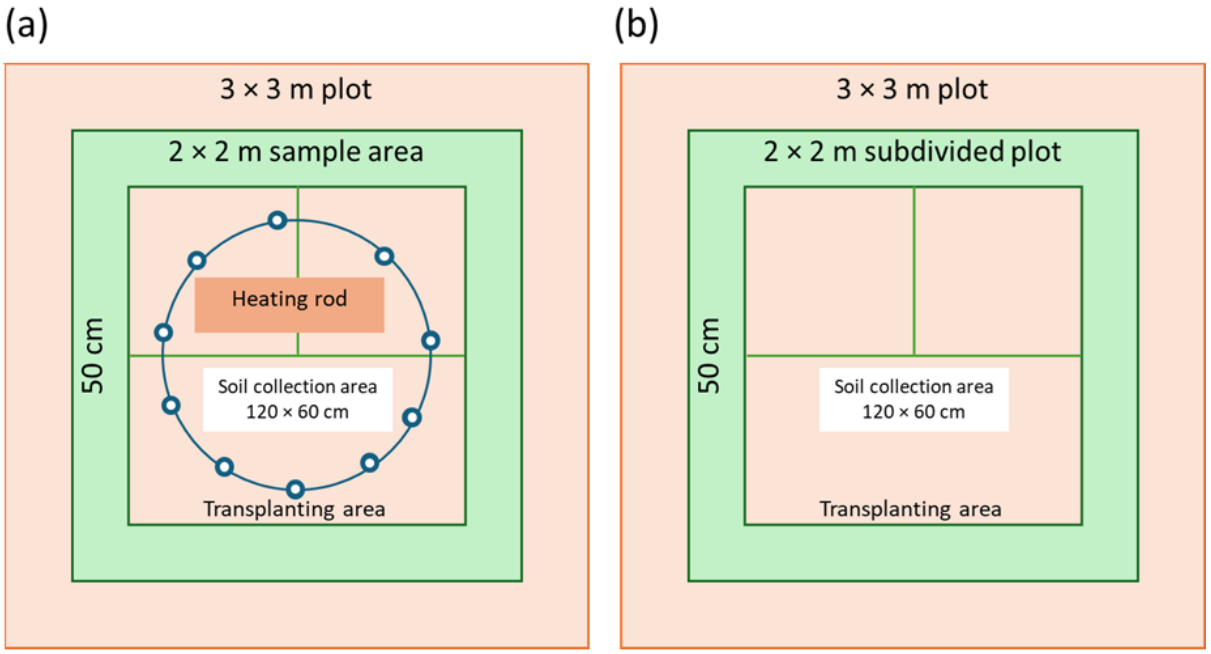


Figure A. 1: Experimental Design (a) Heating plot (b) Control plot.

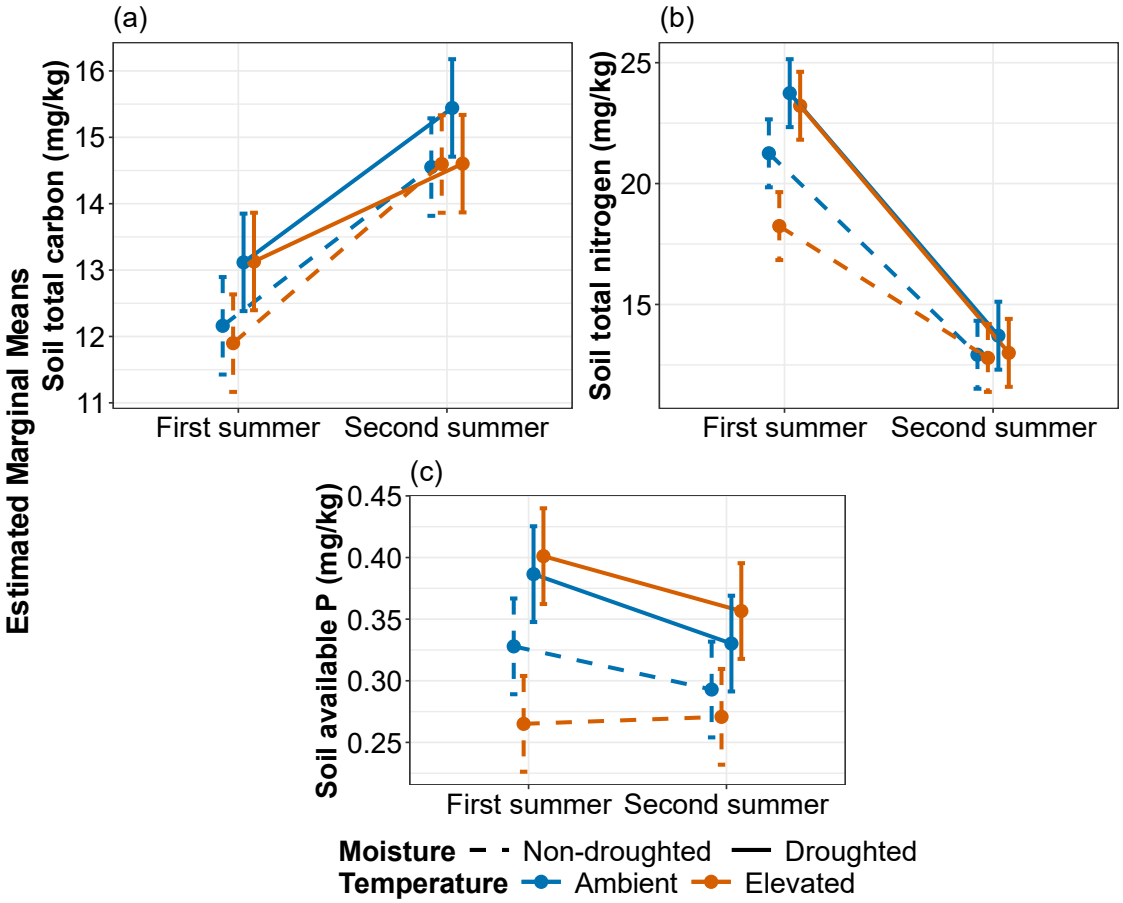


Figure A. 2: Differences in soil chemical properties among experimental treatments between the initial spring 2022 soil sample (pre-treatment) and the final autumn sample in May 2024. Estimated marginal means (± SE) of (a) total carbon, (b) total nitrogen, and (c) available phosphorus for combinations of temperature (Ambient, Elevated) and moisture (Non-Droughted, Droughted) treatments.

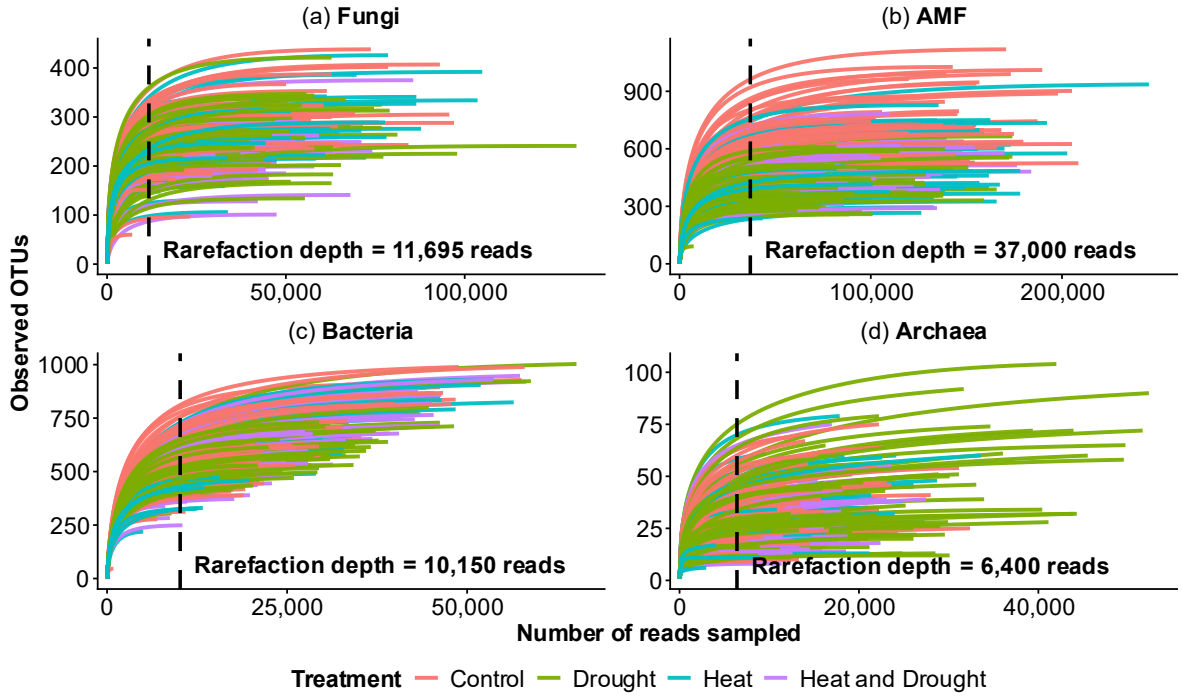


Figure A. 3: Rarefaction curves of soil fungal, arbuscular mycorrhizal fungal, bacterial and archaeal communities across treatments.

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Appendix B: Supplementary materials of Chapter 3

Table B. 1: Network topology metrics of microbiome (fungi, AMF, bacteria and archaea) OTU-level co-occurrence networks across treatments and sampling periods. Networks were constructed using significant pairwise Spearman correlations ($|\rho| \geq 0.60$, $q \leq 0.05$) at the OTU level and coloured by taxonomic order. Permutation-tested differences in microbial co-occurrence network metrics across treatments vs control (significance: * < 0.05, ** < 0.01, *** < 0.001).

Treatment	Sampling period	Node	Edge	Transitivity	Avg. path length	Modularity	Graph density	Network diameter	Avg. degree	Clustering coefficient	Negative	Positive
						Fungi						
Control	First summer	151	789	0.5	3.5	0.3	0.07	12	10.5	0.4	253	536
Control	Second summer	113	139	0.3	4.4	0.7	0.02	12	2.5	0.3	50	89
Drought	First summer	82 **(P<0.05)**	79 **(P<0.001)**	0.4	2.4	0.9	0.02	6	1.9 **(P<0.001)**	0.4	17	62
Drought	Second summer	91 **(P<0.05)**	95	0.4	7.0 **(P<0.05)**	0.8	0.02	18	2.1	0.3	8	87
Warming	First summer	19 **(P<0.001)**	10 **(P<0.001)**	0.0	1.1 **(P<0.05)**	0.9	0.06	2 **(P<0.05)**	1.1 **(P<0.001)**	0.0	1	9
Warming	Second summer	51	30 **(P<0.05)**	0.0	1.4 **(P<0.05)**	0.9	0.02	4 **(P<0.05)**	1.2 **(P<0.01)**	0.0	2	28
Warming and Drought	First summer	9 **(P<0.001)**	5 **(P<0.001)**	0.0	1.2	0.7	0.14	2 **(P<0.05)**	1.1 **(P<0.001)**	0.0	0	5
Warming and Drought	Second summer	15 **(P<0.001)**	9 **(P<0.01)**	0.0	1.4 **(P<0.05)**	0.8	0.09	3 **(P<0.05)**	1.2 **(P<0.05)**	0.0	1	8

Treatment	Sampling period	Node	Edge	Transitivity	Avg. path length	Modularity	Graph density	Network diameter	Avg. degree	Clustering coefficient	Negative	Positive
						AMF						
Control	First summer	146	442	0.4	3.6	0.5	0.04	13	6.1	0.4	95	347
Control	Second summer	76	80	0.5	3.0	0.8	0.03	9	2.1	0.4	4	76
Drought	First summer	149	384 **(P<0.01)**	0.4	4.1	0.5	0.03	12	5.2 **(P<0.01)**	0.3	17	367
Drought	Second summer	28	23	0.4	1.6	0.8	0.06	4	1.6	0.4	2	21
Warming	First summer	111	166 **(P<0.01)**	0.4	6.1	0.7	0.03	19	3.0 **(P<0.01)**	0.4	4	162
Warming	Second summer	156	657	0.5	3.5 **(P<0.01)**	0.4	0.05	10 **(P<0.01)**	8.4	0.4	27	630
Warming and Drought	First summer	13 **(P<0.001)**	7 **(P<0.001)**	0.0	1.1	0.8	0.09	2 **(P<0.05)**	1.1 **(P<0.01)**	0.0	0	7
Warming and Drought	Second summer	25	22	0.5	1.9	0.7	0.07	5	1.8	0.3	1	21
						Bacteria						
Control	First summer	152	367	0.4	4.8	0.6	0.03	13	4.8	0.4	125	242
Control	Second summer	99	116	0.3	4.1	0.8	0.02	11	2.3	0.4	41	75
Drought	First summer	84	72	0.4	1.7	0.9	0.02	4	1.7	0.5	28	44

Treatment	Sampling period	Node	Edge	Transitivity	Avg. path length	Modularity	Graph density	Network diameter	Avg. degree	Clustering coefficient	Negative	Positive
Bacteria												
Drought	Second summer	146	321	0.4	4.5	0.6	0.03	13	4.4	0.4	136	185
Warming	First summer	127	232	0.4	4.4	0.6	0.03	11	3.7	0.4	98	134
Warming	Second summer	166	604	0.4	3.7	0.6	0.04	9	7.3	0.4	249	355
Warming and Drought	First summer	113	157	0.3	7.0	0.7	0.02	21	2.8	0.3	40	117
Warming and Drought	Second summer	52	45	0.3	4.8	0.8	0.03	11	1.7	0.4	13	32
Archaea												
Control	First summer	22	44	0.6	2.6	0.4	0.19	7	4.0	0.6	17	27
Control	Second summer	36	73	0.5	3.1	0.5	0.12	8	4.1	0.6	12	61
Drought	First summer	29	61	0.7	2.3	0.6	0.15	5	4.2	0.6	13	48
Drought	Second summer	36	77	0.5	3.6	0.5	0.12	9	4.3	0.7	6	71
Warming	First summer	28	45	0.5	3.9	0.5	0.12	11	3.2	0.4	8	37

Treatment	Sampling period	Node	Edge	Transitivity	Avg. path length	Modularity	Graph density	Network diameter	Avg. degree	Clustering coefficient	Negative	Positive
					Archaea							
Warming	Second summer	29	60	0.4	2.9	0.5	0.15	7	4.1	0.6	22	38
Warming and Drought	First summer	33	96	0.6	2.6	0.5	0.18	5	5.8	0.7	10	86
Warming and Drought	Second summer	15 **(P<0.05)**	13	0.2	1.9	0.5	0.12	4	1.7	0.3	1	12

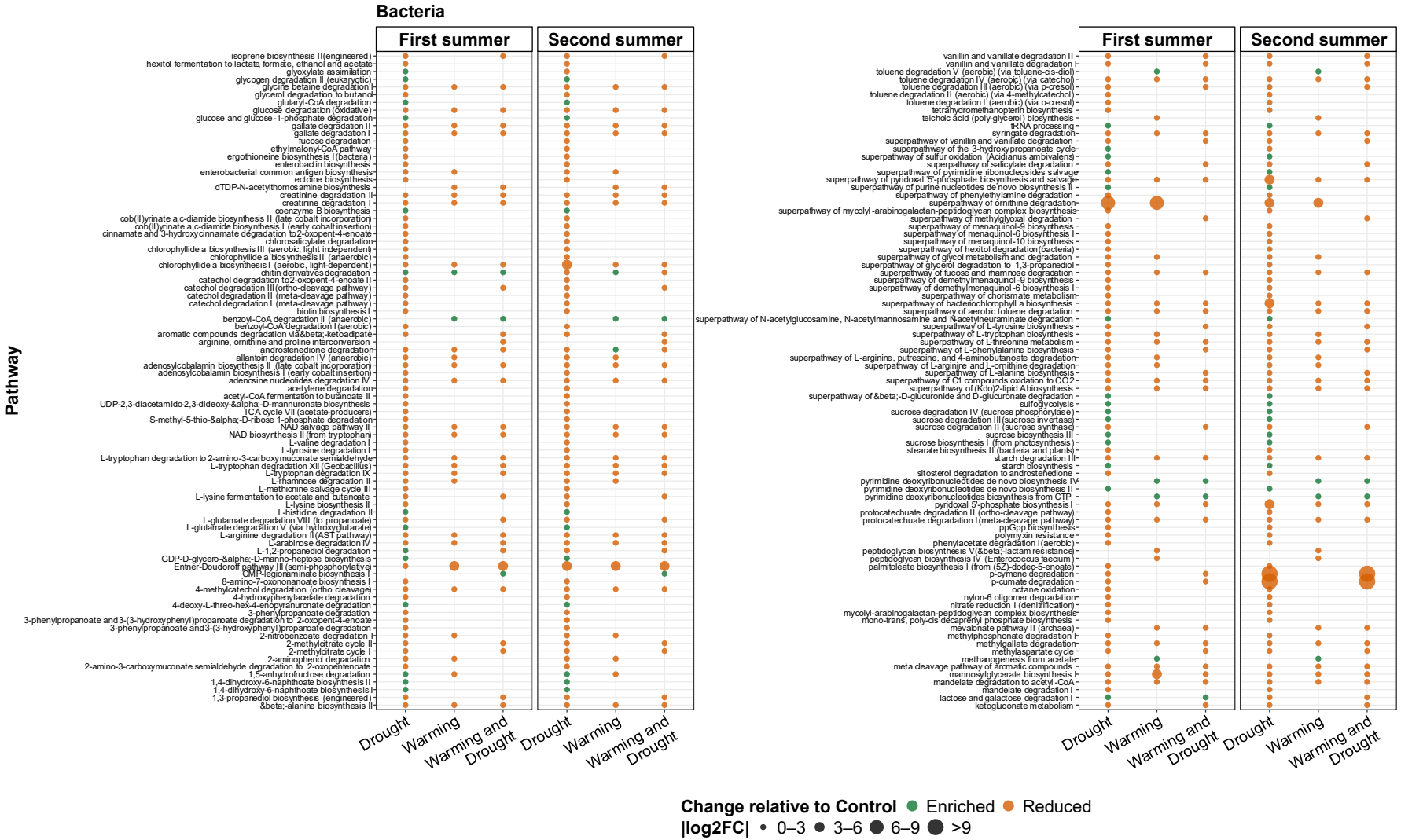


Figure B. 1: Differentially abundant functional pathways (PICRUSt2) for bacteria to drought, warming, and combined warming-drought across two summers. Bubble colour indicates enrichment or reduction relative to the control, and bubble size reflects $|\log_2 FC|$.

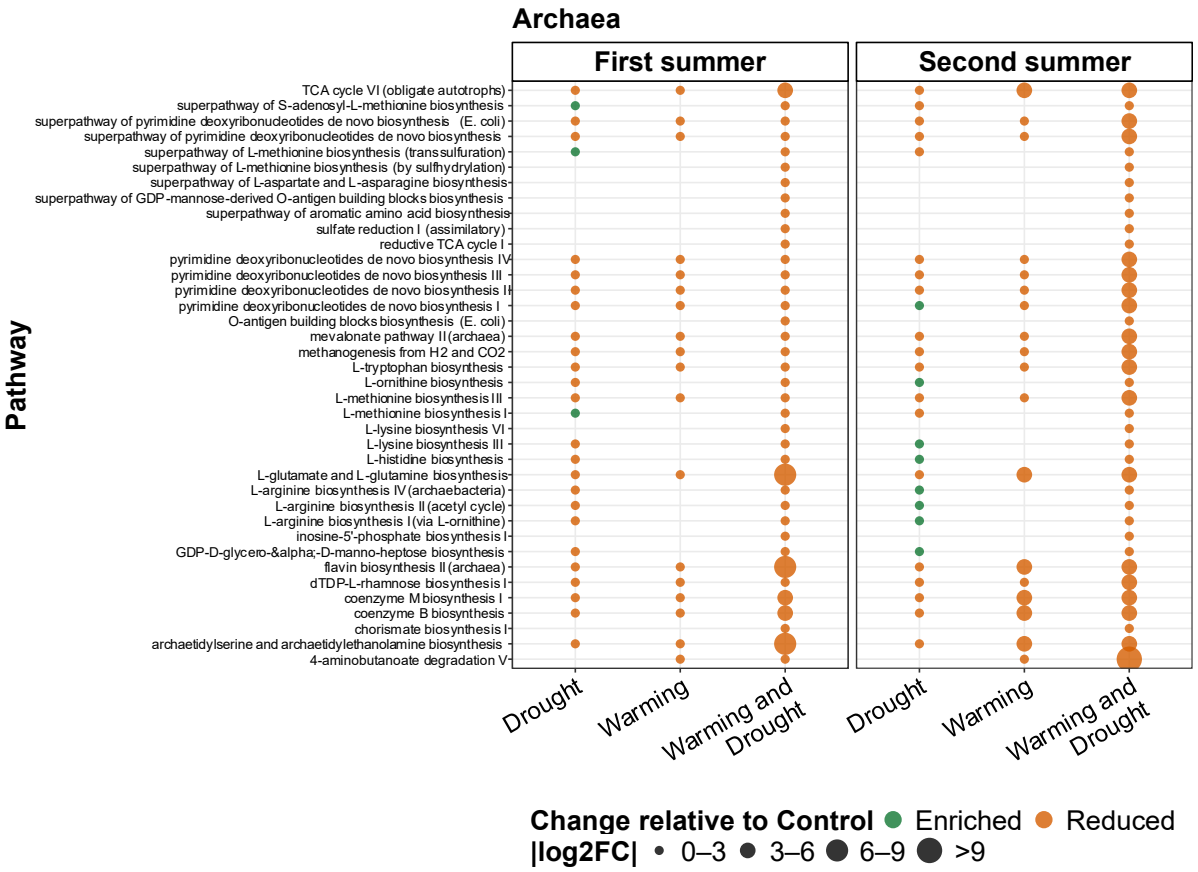


Figure B. 2: Differentially abundant functional pathways (PICRUSt2) for archaea to drought, warming, and combined warming-drought across two summers. Bubble colour indicates enrichment or reduction relative to the control, and bubble size reflects $|\log_2 FC|$.

Appendix C: Supplementary materials of Chapter 4

Table C. 1: Type III Wald chi-square tests from linear mixed-effects models evaluating the effects of temperature, Moisture availability, Species, soil inoculum, time (Day), and their interactions on digital biomass (Digital_biomass_mm_a3). Models included experimental unit as a random effect to account for repeated measurements. Significant effects indicate factors influencing temporal biomass trajectories under warming and drought treatments.

Effect	Chisq	Df	Pr(>Chisq)	Signif.
Intercept	5953.1	1	< 0.001	***
Temperature	1.0	1	0.318	
Moisture	0.9	2	0.636	
Species	52.8	2	0.000	***
Day	0.4	1	0.545	
Soil inoculum	2.4	1	0.124	
Temperature: Moisture	3.0	2	0.222	
Temperature: Species	1.5	2	0.473	
Moisture: Species	3.6	4	0.463	
Temperature: Day	7.2	1	0.007	**
Moisture: Day	5.2	2	0.073	.
Species: Day	59.8	2	< 0.001	***
Temperature: Soil inoculum	0.7	1	0.405	
Moisture: Soil inoculum	0.2	2	0.915	
Species: Soil inoculum	16.2	2	0.000	***
Day: Soil inoculum	0.0	1	0.874	
Temperature: Moisture: Species	0.9	4	0.921	
Temperature: Moisture: Day	3.1	2	0.209	
Temperature: Species: Day	42.3	2	< 0.001	***
Moisture: Species: Day	10.6	4	0.031	*
Temperature: Moisture: Soil inoculum	1.8	2	0.412	
Temperature: Species: Soil inoculum	9.4	2	0.009	**
Moisture: Species: Soil inoculum	1.9	4	0.747	
Temperature: Day: Soil inoculum	0.0	1	0.917	
Moisture: Day: Soil inoculum	0.3	2	0.874	
Species: Day: Soil inoculum	11.9	2	0.003	**
Temperature: Moisture: Species: Day	19.2	4	0.001	***
Temperature: Moisture: Species: Soil inoculum	3.0	4	0.554	
Temperature: Moisture: Day: Soil inoculum	0.3	2	0.865	
Temperature: Species: Day: Soil inoculum	5.5	2	0.063	.
Moisture: Species: Day: Soil inoculum	7.5	4	0.112	
Temperature: Moisture: Species: Day: Soil inoculum	1.8	4	0.766	

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table C. 2: Pairwise contrasts of temperature and moisture effects on log-transformed digital biomass. Results are from a full factorial linear model including moisture, temperature, soil inoculum, species, and their interactions. Significant P-values are shown in bold.

Within Temperature						
Contrast	**Water**	**Plant**	**Estimate**	**SE**	**z.ratio**	**P value**
Elevated - Ambient	Well-watered	*Poa*	0.007	0.001	5.0	**< 0.001**
Elevated - Ambient	50% Drought		0.007	0.001	5.1	**< 0.001**
Elevated - Ambient	70% Drought		0.005	0.001	3.2	**0.001**
Elevated - Ambient	Well-watered	*Celmisia*	-0.014	0.001	-13.9	**< 0.001**
Elevated - Ambient	50% Drought		-0.012	0.001	-12.5	**< 0.001**
Elevated - Ambient	70% Drought		-0.010	0.001	-10.3	**< 0.001**
Elevated - Ambient	Well-watered	*Carex*	0.006	0.001	4.3	**< 0.001**
Elevated - Ambient	50% Drought		-0.004	0.001	-2.9	**0.003**
Elevated - Ambient	70% Drought		-0.002	0.001	-1.4	0.149
Within Moisture						
Contrast	**Temperature**	**Plant**	**Estimate**	**SE**	**z.ratio**	**P value**
Well-watered - 50% Drought	Ambient	*Poa*	-0.003	0.001	-2.1	0.085
Well-watered - 70% Drought	Ambient		-0.002	0.001	-1.3	0.397
50% Drought - 70% Drought	Ambient		0.001	0.001	0.8	0.686
Well-watered - 50% Drought	Elevated		-0.003	0.001	-2.0	0.114
Well-watered - 70% Drought	Elevated		0.001	0.001	0.6	0.823
50% Drought - 70% Drought	Elevated		0.004	0.001	2.6	0.022
Well-watered - 50% Drought	Ambient	*Celmisia*	-0.0002	0.001	-0.3	0.965
Well-watered - 70% Drought	Ambient		0.0003	0.001	0.3	0.955
50% Drought - 70% Drought	Ambient		0.001	0.001	0.5	0.851
Well-watered - 50% Drought	Elevated		-0.002	0.001	-1.6	0.237
Well-watered - 70% Drought	Elevated		-0.003	0.001	-3.1	**0.006**
50% Drought - 70% Drought	Elevated		-0.001	0.001	-1.5	0.3
Well-watered - 50% Drought	Ambient	*Carex*	-0.002	0.001	-1.3	0.418
Well-watered - 70% Drought	Ambient		-0.001	0.001	-1.0	0.582
50% Drought - 70% Drought	Ambient		0.0004	0.001	0.3	0.957
Well-watered - 50% Drought	Elevated		0.008	0.001	6.0	**< 0.001**
Well-watered - 70% Drought	Elevated		0.007	0.001	4.7	**< 0.001**
50% Drought - 70% Drought	Elevated		-0.002	0.001	-1.2	0.457

Table C. 3: Type II analysis of variance (ANOVA) for log-transformed dry shoot and root biomass. Results are from a full factorial linear model including moisture, temperature, soil inoculum, species, and their interactions. Significant P-values are shown in bold.

Effect	Dry shoot biomass			Dry root biomass		
	Sum Sq	F value	P value	Sum Sq	F value	P value
Moisture	1.24	24.7	**< 0.001**	4.07	16.0	**< 0.001**
Soil inoculum	0.01	0.3	0.57	1.44	11.3	**0.001**
Temperature	1.71	68.5	**< 0.001**	20.16	158.1	**< 0.001**
Species	67.08	1340.6	**< 0.001**	97.30	381.6	**< 0.001**
Moisture: Soil inoculum	0.02	0.4	0.645	0.31	1.2	0.298
Moisture: Temperature	0.00	0.0	0.999	0.40	1.6	0.209
Soil inoculum: Temperature	0.01	0.2	0.634	0.49	3.8	0.051
Moisture: Species	0.61	6.1	**< 0.001**	0.13	0.3	0.906
Soil inoculum: Species	0.26	5.2	**0.006**	0.10	0.4	0.683
Temperature: Species	1.46	29.1	**< 0.001**	7.25	28.4	**< 0.001**
Moisture:Soil inoculum:Temperature	0.08	1.5	0.221	0.16	0.6	0.544
Moisture: Soil inoculum: Species	0.16	1.6	0.174	0.20	0.4	0.818
Moisture: Temperature: Species	0.16	1.6	0.165	1.77	3.5	**0.009**
Soil inoculum: Temperature: Species	0	0.1	0.922	0.07	0.3	0.759
Moisture: Soil inoculum: Temperature: Species	0.09	0.9	0.463	0.27	0.5	0.711
Residuals	6.96	NA	NA	35.44	NA	NA

Table C. 4: Summary of ANOVA Results for treatment effects on fungal community. Responses of fungal and AMF richness to experimentally altered temperature and moisture and soil inoculum in *Poa*, *Celmisia* and *Carex*. Significant P-values are shown in bold.

Effects	Plant	Saprotroph		Symbitroph		Pathotroph		AMF	
		Chisq	P value	Chisq	P value	Chisq	P value	Chisq	P value
Temperature	*Poa*	21.0	**<0.001**	8.5	**0.004**	15.4	**<0.001**	23.9	**<0.001**
Moisture		17.3	**<0.001**	10.0	**0.007**	12.6	**0.002**	5.0	0.084
Inoculum		15.0	**<0.001**	5.4	**0.02**	2.1	0.148	5.4	**0.020**
Temperature:Moisture		5.5	0.065	2.3	0.324	4.9	0.087	9.0	**0.011**
Temperature:Inoculum		6.1	**0.013**	5.8	**0.016**	1.7	0.189	2.9	0.090
Moisture:Inoculum		3.3	0.197	7.7	**0.022**	2.5	0.291	1.2	0.540
Temperature:Moisture:Inoculum		2.3	0.314	5.4	0.066	0.8	0.677	2.3	0.313
Temperature	*Celmisia*	10.2	**0.001**	0.0	0.877	2.0	0.162	0.1	0.704
Moisture		16.7	**<0.001**	8.1	**0.017**	20	**<0.001**	15.3	**<0.001**
Inoculum		1.5	0.228	0.8	0.364	9.6	**0.002**	12.0	**0.001**
Temperature:Moisture		1.0	0.605	0.1	0.958	0.5	0.769	0.1	0.949
Temperature:Inoculum		0.3	0.562	0.0	0.996	1.1	0.284	0.0	0.859
Moisture:Inoculum		1.3	0.532	2.1	0.347	0.5	0.764	2.9	0.231
Temperature:Moisture:Inoculum		0.3	0.869	1.0	0.615	1.1	0.568	0.7	0.692
Temperature	*Carex*	19.9	**<0.001**	12.9	**<0.001**	32.2	**<0.001**	11.7	**0.001**
Moisture		4.7	0.093	3.3	0.189	10.7	**0.005**	0.5	0.775
Inoculum		5.1	**0.024**	4.0	**0.046**	0.7	0.388	5.8	**0.016**
Temperature:Moisture		0.5	0.766	0.1	0.946	0.7	0.7	0.1	0.930
Temperature:Inoculum		1.4	0.239	1.2	0.273	0.4	0.541	2.9	0.088
Moisture:Inoculum		2.8	0.25	2.2	0.335	2.2	0.337	1.8	0.411
Temperature:Moisture:Inoculum		3.1	0.214	2.0	0.361	7.0	**0.031**	0.1	0.962

Table C. 5: Pairwise contrasts of fungal trophic mode and AMF richness across temperature and moisture treatments for each plant species. Significant P-values are shown in bold.

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Ambient Well-watered – Elevated Well water	Saprotroph	*Poa*	2.0	2.1	0.95	0.931	1.000
Ambient Well-watered – Ambient 50% drought	Saprotroph		-8.0	2.1	-3.82	0.004	**0.013**
Ambient Well-watered – Elevated 50% drought	Saprotroph		1.1	2.1	0.52	0.995	1.000
Ambient Well-watered – Ambient 70% drought	Saprotroph		-7.5	2.1	-3.58	0.009	0.023
Ambient Well-watered – Elevated 70% drought	Saprotroph		-2.5	2.1	-1.19	0.840	1.000
Elevated Well-watered – Ambient 50% drought	Saprotroph		-10.0	2.1	-4.79	0.000	**0.003**
Elevated Well-watered – Elevated 50% drought	Saprotroph		-0.9	2.1	-0.45	0.998	1.000
Elevated Well-watered – Ambient 70% drought	Saprotroph		-9.5	2.1	-4.55	0.000	**0.003**
Elevated Well-watered – Elevated 70% drought	Saprotroph		-4.5	2.1	-2.16	0.272	0.454
Ambient 50% drought – Elevated 50% drought	Saprotroph		9.1	2.0	4.46	0.001	**0.003**
Ambient 50% drought – Ambient 70% drought	Saprotroph		0.5	2.0	0.25	1.000	1.000
Ambient 50% drought – Elevated 70% drought	Saprotroph		5.5	2.0	2.70	0.092	0.197
Elevated 50% drought – Ambient 70% drought	Saprotroph		-8.6	2.0	-4.22	0.001	**0.005**
Elevated 50% drought – Elevated 70% drought	Saprotroph		-3.6	2.0	-1.76	0.500	0.749
Ambient 70% drought – Elevated 70% drought	Saprotroph		5.0	2.0	2.46	0.156	0.293
Ambient Well-watered – Elevated Well water	Symbiotroph		0.7	0.9	0.72	0.979	1.000
Ambient Well-watered – Ambient 50% drought	Symbiotroph		-2.3	0.9	-2.46	0.155	0.388
Ambient Well-watered – Elevated 50% drought	Symbiotroph		0.4	0.9	0.41	0.998	1.000
Ambient Well-watered – Ambient 70% drought	Symbiotroph		-2.3	0.9	-2.46	0.155	0.388
Ambient Well-watered – Elevated 70% drought	Symbiotroph		-1.1	0.9	-1.20	0.833	1.000
Elevated Well-watered – Ambient 50% drought	Symbiotroph		-3.0	0.9	-3.19	0.027	0.192

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Elevated Well-watered – Elevated 50% drought	Symbiotroph	*Poa*	-0.3	0.9	-0.33	0.999	1.000
Elevated Well-watered – Ambient 70% drought	Symbiotroph		-3.0	0.9	-3.19	0.027	0.192
Elevated Well-watered – Elevated 70% drought	Symbiotroph		-1.8	0.9	-1.94	0.391	0.837
Ambient 50% drought – Elevated 50% drought	Symbiotroph		2.7	0.9	2.95	0.051	0.192
Ambient 50% drought – Ambient 70% drought	Symbiotroph		0.0	0.9	0.00	1.000	1.000
Ambient 50% drought – Elevated 70% drought	Symbiotroph		1.2	0.9	1.29	0.790	1.000
Elevated 50% drought – Ambient 70% drought	Symbiotroph		-2.7	0.9	-2.95	0.051	0.192
Elevated 50% drought – Elevated 70% drought	Symbiotroph		-1.5	0.9	-1.66	0.565	1.000
Ambient 70% drought – Elevated 70% drought	Symbiotroph		1.2	0.9	1.29	0.790	1.000
Ambient Well-watered – Elevated Well water	Pathotroph		1.4	1.5	0.94	0.935	1.000
Ambient Well-watered – Ambient 50% drought	Pathotroph		-4.7	1.4	-3.27	0.022	0.067
Ambient Well-watered – Elevated 50% drought	Pathotroph		1.0	1.4	0.70	0.981	1.000
Ambient Well-watered – Ambient 70% drought	Pathotroph		-4.0	1.4	-2.80	0.073	0.182
Ambient Well-watered – Elevated 70% drought	Pathotroph		-1.6	1.4	-1.11	0.876	1.000
Elevated Well-watered – Ambient 50% drought	Pathotroph		-6.0	1.4	-4.22	0.001	**0.015**
Elevated Well-watered – Elevated 50% drought	Pathotroph		-0.4	1.4	-0.26	1.000	1.000
Elevated Well-watered – Ambient 70% drought	Pathotroph		-5.4	1.4	-3.76	0.005	**0.027**
Elevated Well-watered – Elevated 70% drought	Pathotroph		-2.9	1.4	-2.07	0.321	0.602
Ambient 50% drought – Elevated 50% drought	Pathotroph		5.7	1.4	4.07	0.002	0.015
Ambient 50% drought – Ambient 70% drought	Pathotroph		0.7	1.4	0.48	0.997	1.000
Ambient 50% drought – Elevated 70% drought	Pathotroph		3.1	1.4	2.22	0.247	0.530
Elevated 50% drought – Ambient 70% drought	Pathotroph		-5.0	1.4	-3.60	0.009	**0.033**

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Elevated 50% drought – Elevated 70% drought	Pathotroph	*Poa*	-2.6	1.4	-1.86	0.439	0.732
Ambient 70% drought – Elevated 70% drought	Pathotroph		2.4	1.4	1.74	0.514	0.771
Ambient Well-watered – Elevated Well water	Saprotroph	*Celmisia*	4.1	1.8	2.29	0.215	0.458
Ambient Well-watered – Ambient 50% drought	Saprotroph		-0.8	1.7	-0.48	0.997	1.000
Ambient Well-watered – Elevated 50% drought	Saprotroph		1.0	1.7	0.55	0.993	1.000
Ambient Well-watered – Ambient 70% drought	Saprotroph		-4.7	1.7	-2.75	0.083	0.248
Ambient Well-watered – Elevated 70% drought	Saprotroph		-1.0	1.7	-0.58	0.992	1.000
Elevated Well-watered – Ambient 50% drought	Saprotroph		-4.9	1.7	-2.82	0.070	0.248
Elevated Well-watered – Elevated 50% drought	Saprotroph		-3.1	1.8	-1.75	0.503	0.838
Elevated Well-watered – Ambient 70% drought	Saprotroph		-8.8	1.7	-5.02	0.000	**0.001**
Elevated Well-watered – Elevated 70% drought	Saprotroph		-5.1	1.7	-2.92	0.055	0.248
Ambient 50% drought – Elevated 50% drought	Saprotroph		1.8	1.7	1.05	0.897	1.000
Ambient 50% drought – Ambient 70% drought	Saprotroph		-3.8	1.6	-2.33	0.202	0.458
Ambient 50% drought – Elevated 70% drought	Saprotroph		-0.2	1.6	-0.10	1.000	1.000
Elevated 50% drought – Ambient 70% drought	Saprotroph		-5.6	1.7	-3.32	0.020	0.147
Elevated 50% drought – Elevated 70% drought	Saprotroph		-1.9	1.7	-1.15	0.857	1.000
Ambient 70% drought – Elevated 70% drought	Saprotroph		3.7	1.6	2.23	0.244	0.458
Ambient Well-watered – Elevated Well water	Symbiotroph		-0.2	1.0	-0.17	1.000	1.000
Ambient Well-watered – Ambient 50% drought	Symbiotroph		-0.9	0.9	-0.96	0.929	1.000
Ambient Well-watered – Elevated 50% drought	Symbiotroph		-0.6	0.9	-0.63	0.988	1.000
Ambient Well-watered – Ambient 70% drought	Symbiotroph		-2.0	0.9	-2.14	0.286	1.000
Ambient Well-watered – Elevated 70% drought	Symbiotroph		-1.9	0.9	-2.04	0.332	1.000

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Elevated Well-watered – Ambient 50% drought	Symbiotroph	*Celmisia*	-0.7	0.9	-0.76	0.973	1.000
Elevated Well-watered – Elevated 50% drought	Symbiotroph		-0.4	1.0	-0.45	0.998	1.000
Elevated Well-watered – Ambient 70% drought	Symbiotroph		-1.8	0.9	-1.90	0.413	1.000
Elevated Well-watered – Elevated 70% drought	Symbiotroph		-1.7	0.9	-1.81	0.466	1.000
Ambient 50% drought – Elevated 50% drought	Symbiotroph		0.3	0.9	0.31	1.000	1.000
Ambient 50% drought – Ambient 70% drought	Symbiotroph		-1.1	0.9	-1.21	0.831	1.000
Ambient 50% drought – Elevated 70% drought	Symbiotroph		-1.0	0.9	-1.12	0.873	1.000
Elevated 50% drought – Ambient 70% drought	Symbiotroph		-1.4	0.9	-1.48	0.676	1.000
Elevated 50% drought – Elevated 70% drought	Symbiotroph		-1.3	0.9	-1.39	0.731	1.000
Ambient 70% drought – Elevated 70% drought	Symbiotroph		0.1	0.9	0.09	1.000	1.000
Ambient Well-watered – Elevated Well water	Pathotroph		1.6	1.1	1.38	0.736	1.000
Ambient Well-watered – Ambient 50% drought	Pathotroph		-0.7	1.1	-0.67	0.984	1.000
Ambient Well-watered – Elevated 50% drought	Pathotroph		0.0	1.1	0.01	1.000	1.000
Ambient Well-watered – Ambient 70% drought	Pathotroph		-2.8	1.1	-2.63	0.109	0.408
Ambient Well-watered – Elevated 70% drought	Pathotroph		-2.3	1.1	-2.16	0.275	0.662
Elevated Well-watered – Ambient 50% drought	Pathotroph		-2.3	1.1	-2.07	0.317	0.662
Elevated Well-watered – Elevated 50% drought	Pathotroph		-1.5	1.1	-1.37	0.742	1.000
Elevated Well-watered – Ambient 70% drought	Pathotroph		-4.4	1.1	-3.97	0.003	**0.043**
Elevated Well-watered – Elevated 70% drought	Pathotroph		-3.9	1.1	-3.52	0.011	0.084
Ambient 50% drought – Elevated 50% drought	Pathotroph		0.7	1.1	0.68	0.983	1.000
Ambient 50% drought – Ambient 70% drought	Pathotroph		-2.1	1.0	-2.01	0.353	0.662
Ambient 50% drought – Elevated 70% drought	Pathotroph		-1.6	1.0	-1.53	0.650	1.000

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Elevated 50% drought – Ambient 70% drought	Pathotroph	*Celmisia*	-2.8	1.1	-2.64	0.106	0.408
Elevated 50% drought – Elevated 70% drought	Pathotroph		-2.3	1.1	-2.17	0.270	0.662
Ambient 70% drought – Elevated 70% drought	Pathotroph		0.5	1.0	0.48	0.997	1.000
Ambient Well-watered – Elevated Well water	Saprotroph	*Carex*	7.9	3.7	2.17	0.270	0.676
Ambient Well-watered – Ambient 50% drought	Saprotroph		-1.2	3.6	-0.34	0.999	0.999
Ambient Well-watered – Elevated 50% drought	Saprotroph		6.1	3.6	1.69	0.544	0.910
Ambient Well-watered – Ambient 70% drought	Saprotroph		-6.3	3.6	-1.76	0.502	0.910
Ambient Well-watered – Elevated 70% drought	Saprotroph		4.1	3.6	1.14	0.864	0.999
Elevated Well-watered – Ambient 50% drought	Saprotroph		-9.2	3.3	-2.80	0.074	0.277
Elevated Well-watered – Elevated 50% drought	Saprotroph		-1.8	3.3	-0.56	0.993	0.999
Elevated Well-watered – Ambient 70% drought	Saprotroph		-14.3	3.3	-4.35	0.001	**0.013**
Elevated Well-watered – Elevated 70% drought	Saprotroph		-3.8	3.3	-1.17	0.848	0.999
Ambient 50% drought – Elevated 50% drought	Saprotroph		7.3	3.2	2.30	0.214	0.641
Ambient 50% drought – Ambient 70% drought	Saprotroph		-5.1	3.2	-1.59	0.606	0.910
Ambient 50% drought – Elevated 70% drought	Saprotroph		5.3	3.2	1.67	0.556	0.910
Elevated 50% drought – Ambient 70% drought	Saprotroph		-12.4	3.2	-3.89	0.004	**0.028**
Elevated 50% drought – Elevated 70% drought	Saprotroph		-2.0	3.2	-0.63	0.988	0.999
Ambient 70% drought – Elevated 70% drought	Saprotroph		10.4	3.2	3.27	0.023	0.114
Ambient Well-watered – Elevated Well water	Symbiotroph		4.0	2.0	2.03	0.339	1.000
Ambient Well-watered – Ambient 50% drought	Symbiotroph		-0.1	1.9	-0.05	1.000	1.000
Ambient Well-watered – Elevated 50% drought	Symbiotroph		3.2	1.9	1.64	0.579	1.000
Ambient Well-watered – Ambient 70% drought	Symbiotroph		-2.2	1.9	-1.13	0.868	1.000

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Ambient Well-watered – Elevated 70% drought	Symbiotroph	*Carex*	1.8	1.9	0.95	0.933	1.000
Elevated Well-watered – Ambient 50% drought	Symbiotroph		-4.1	1.8	-2.32	0.205	0.767
Elevated Well-watered – Elevated 50% drought	Symbiotroph		-0.8	1.8	-0.47	0.997	1.000
Elevated Well-watered – Ambient 70% drought	Symbiotroph		-6.2	1.8	-3.51	0.012	0.174
Elevated Well-watered – Elevated 70% drought	Symbiotroph		-2.2	1.8	-1.23	0.821	1.000
Ambient 50% drought – Elevated 50% drought	Symbiotroph		3.3	1.7	1.90	0.411	1.000
Ambient 50% drought – Ambient 70% drought	Symbiotroph		-2.1	1.7	-1.22	0.825	1.000
Ambient 50% drought – Elevated 70% drought	Symbiotroph		1.9	1.7	1.12	0.869	1.000
Elevated 50% drought – Ambient 70% drought	Symbiotroph		-5.3	1.7	-3.13	0.033	0.248
Elevated 50% drought – Elevated 70% drought	Symbiotroph		-1.3	1.7	-0.78	0.969	1.000
Ambient 70% drought – Elevated 70% drought	Symbiotroph		4.0	1.7	2.34	0.196	0.767
Ambient Well-watered – Elevated Well water	Pathotroph		5.4	1.6	3.30	0.021	0.063
Ambient Well-watered – Ambient 50% drought	Pathotroph		-0.6	1.6	-0.37	0.999	0.999
Ambient Well-watered – Elevated 50% drought	Pathotroph		3.3	1.6	2.06	0.325	0.500
Ambient Well-watered – Ambient 70% drought	Pathotroph		-3.5	1.6	-2.18	0.266	0.498
Ambient Well-watered – Elevated 70% drought	Pathotroph		2.0	1.6	1.23	0.819	0.945
Elevated Well-watered – Ambient 50% drought	Pathotroph		-6.0	1.5	-4.09	0.002	**0.010**
Elevated Well-watered – Elevated 50% drought	Pathotroph		-2.1	1.5	-1.41	0.719	0.899
Elevated Well-watered – Ambient 70% drought	Pathotroph		-8.9	1.5	-6.08	0.000	**<0.001**
Elevated Well-watered – Elevated 70% drought	Pathotroph		-3.4	1.5	-2.32	0.204	0.438
Ambient 50% drought – Elevated 50% drought	Pathotroph		3.9	1.4	2.74	0.084	0.211
Ambient 50% drought – Ambient 70% drought	Pathotroph		-2.9	1.4	-2.04	0.333	0.500

Contrast	Trophic	Plant	Estimate	SE	t.ratio	P value	P_{adj}
Ambient 50% drought – Elevated 70% drought	Pathotroph	*Carex*	2.6	1.4	1.81	0.469	0.639
Elevated 50% drought – Ambient 70% drought	Pathotroph		-6.8	1.4	-4.79	0.000	**0.002**
Elevated 50% drought – Elevated 70% drought	Pathotroph		-1.3	1.4	-0.93	0.936	0.999
Ambient 70% drought – Elevated 70% drought	Pathotroph		5.5	1.4	3.85	0.004	**0.016**

Table C. 6: Pairwise contrasts of AMF richness across temperature and moisture treatments for each plant species.

Contrast	Plant	Estimate	SE	t. ratio	P value	P_{adj}
Ambient Well-watered – Elevated Well-watered	*Poa*	7.1	6.8	1.1	0.898	0.987
Ambient Well-watered – Ambient 50% drought		-20.5	6.6	-3.1	0.034	0.099
Ambient Well-watered – Elevated 50% drought		13.9	6.6	2.1	0.305	0.513
Ambient Well-watered – Ambient 70% drought		-13.5	6.6	-2.0	0.330	0.513
Ambient Well-watered – Elevated 70% drought		0.5	6.6	0.1	1.000	1.000
Elevated Well-watered – Ambient 50% drought		-27.7	6.8	-4.1	0.002	**0.010**
Elevated Well-watered – Elevated 50% drought		6.7	6.8	1.0	0.920	0.987
Elevated Well-watered – Ambient 70% drought		-20.7	6.8	-3.0	0.040	0.099
Elevated Well-watered – Elevated 70% drought		-6.7	6.8	-1.0	0.921	0.987
Ambient 50% drought – Elevated 50% drought		34.4	6.6	5.2	0.000	**0.001**
Ambient 50% drought – Ambient 70% drought		7.0	6.6	1.1	0.898	0.987
Ambient 50% drought – Elevated 70% drought		21.0	6.6	3.2	0.029	0.099
Elevated 50% drought – Ambient 70% drought		-27.4	6.6	-4.1	0.002	**0.010**
Elevated 50% drought – Elevated 70% drought		-13.4	6.6	-2.0	0.342	0.513
Ambient 70% drought – Elevated 70% drought		14.0	6.6	2.1	0.294	0.513
Ambient Well-watered – Elevated Well-watered	*Celmisia*	3.0	8.0	0.4	0.999	1.000
Ambient Well-watered – Ambient 50% drought		-12.5	8.0	-1.6	0.626	1.000
Ambient Well-watered – Elevated 50% drought		-12.8	8.0	-1.6	0.599	1.000
Ambient Well-watered – Ambient 70% drought		-21.5	8.0	-2.7	0.092	0.462
Ambient Well-watered – Elevated 70% drought		-19.0	8.0	-2.4	0.182	0.683
Elevated Well-watered – Ambient 50% drought		-15.5	8.0	-1.9	0.388	0.969
Elevated Well-watered – Elevated 50% drought		-15.8	8.0	-2.0	0.364	0.969
Elevated Well-watered – Ambient 70% drought		-24.6	8.0	-3.1	0.036	0.462
Elevated Well-watered – Elevated 70% drought		-22.0	8.0	-2.8	0.080	0.462
Ambient 50% drought – Elevated 50% drought		-0.3	8.0	0.0	1.000	1.000
Ambient 50% drought – Ambient 70% drought		-9.0	8.0	-1.1	0.865	1.000
Ambient 50% drought – Elevated 70% drought		-6.5	8.0	-0.8	0.964	1.000
Elevated 50% drought – Ambient 70% drought		-8.7	8.0	-1.1	0.882	1.000
Elevated 50% drought – Elevated 70% drought		-6.2	8.0	-0.8	0.971	1.000
Ambient 70% drought – Elevated 70% drought		2.5	8.0	0.3	1.000	1.000

Contrast	Plant	Estimate	SE	t. ratio	P value	P_{adj}
Ambient Well-watered – Elevated Well-watered	*Carex*	14.3	7.6	1.9	0.424	1.000
Ambient Well-watered – Ambient 50% drought		3.1	7.6	0.4	0.999	1.000
Ambient Well-watered – Elevated 50% drought		16.5	7.6	2.2	0.271	1.000
Ambient Well-watered – Ambient 70% drought		-2.6	7.6	-0.3	0.999	1.000
Ambient Well-watered – Elevated 70% drought		14.7	7.6	1.9	0.395	1.000
Elevated Well-watered – Ambient 50% drought		-11.3	7.6	-1.5	0.680	1.000
Elevated Well-watered – Elevated 50% drought		2.2	7.6	0.3	1.000	1.000
Elevated Well-watered – Ambient 70% drought		-17.0	7.6	-2.2	0.242	1.000
Elevated Well-watered – Elevated 70% drought		0.4	7.6	0.0	1.000	1.000
Ambient 50% drought – Elevated 50% drought		13.4	7.6	1.8	0.500	1.000
Ambient 50% drought – Ambient 70% drought		-5.7	7.6	-0.8	0.974	1.000
Ambient 50% drought – Elevated 70% drought		11.6	7.6	1.5	0.650	1.000
Elevated 50% drought – Ambient 70% drought		-19.1	7.6	-2.5	0.139	1.000
Elevated 50% drought – Elevated 70% drought		-1.8	7.6	-0.2	1.000	1.000
Ambient 70% drought – Elevated 70% drought		17.3	7.6	2.3	0.222	1.000

Table C. 7: Pairwise post-hoc contrasts (BH-adjusted P-values) for fungal trophic mode and AMF richness between sterile and live soil inoculum treatments.

Contrast	Estimate	SE	Trophic	Plant	t.ratio	P value	P_{adj}
Sterile - Live	-4.58	1.2	Saprotroph	*Poa*	-3.8	0.000	**< 0.001**
Sterile - Live	-1.25	0.5	Symbiotroph		-2.3	0.023	**0.023**
Sterile - Live	-1.18	0.8	Pathotroph		-1.4	0.154	0.154
Sterile - Live	1.19	1.0	Saprotroph	*Celmisia*	1.2	0.232	0.232
Sterile - Live	0.50	0.5	Symbiotroph		0.9	0.359	0.359
Sterile - Live	1.90	0.6	Pathotroph		3.1	0.004	**0.004**
Sterile - Live	-4.81	1.9	Saprotroph	*Carex*	-2.5	0.016	**0.016**
Sterile - Live	-2.29	1.0	Symbiotroph		-2.2	0.032	**0.032**
Sterile - Live	-0.94	0.9	Pathotroph		-1.1	0.283	0.283
Sterile - Live	-9.04	3.9	AMF	*Poa*	-2.3	0.023	**0.023**
Sterile - Live	-15.97	4.6	AMF	*Celmisia*	-3.5	0.001	**0.001**
Sterile - Live	-10.58	4.4	AMF	*Carex*	-2.4	0.020	**0.020**

Table C. 8: PERMANOVA results (adonis2, 999 permutations) testing the effects of host plant identity, experimental treatment, and inoculum on root fungal (ITS) and AMF community composition based on averaged Bray–Curtis dissimilarities. Experimental treatment represents the factorial combination of temperature and moisture conditions (Control, 50% Drought, 70% Drought, Warming, Warming and 50% Drought, Warming and 70% Drought). Separate models are shown for all samples combined and for live inoculum samples only.

Model scope	Factor tested	df	F	R^2	P value
		Fungi			
All samples	Plant	2	12.07	0.107	**0.001**
All samples	Treatment	10	2.71	0.109	**0.001**
All samples	Inoculum × Plant	2	2.28	0.02	**0.002**
Live only	Plant	2	9.34	0.159	**0.001**
		AMF			
All samples	Plant	2	5.9	0.053	**0.001**
All samples	Treatment	10	9.57	0.29	**0.001**
All samples	Inoculum × Plant	2	0.95	0.008	0.542
Live only	Plant	2	4.02	0.072	**0.001**

Table C. 9: Network topology metrics of microbiome (fungi and AMF) OTU-level co-occurrence networks across treatments. Networks were constructed using significant pairwise Spearman correlations ($|\rho| \geq 0.60$, $q \leq 0.05$) at the OTU level. Permutation-tested differences in microbial co-occurrence network metrics across treatments vs control (significance: * < 0.05, ** < 0.01, *** < 0.001).

Dataset	Treatment	Nodes	Edges	Density	Mean degree	Positive edges	Negative edges	Modularity	Transitivity
Fungi	Control (Live)	292	4098	0.10	28.1	4088	10	0.32	0.93
	Control (Sterile)	164	776	0.06	9.5	774	2	0.78	0.97
	50% Drought (Live)	253	1074	0.03	8.5	1069	5	0.89	0.86
	50% Drought (Sterile)	391	21663	0.28	110.8	21643	20	0.07	0.92
	70% Drought (Live)	376	7584	0.11	40.3	7578	6	0.38	0.73
	70% Drought (Sterile)	277	1498	0.04	10.8	1495	3	0.72	0.88
	Warming (Live)	113	271	0.04	4.8	271	0	0.89	0.99
	Warming (Sterile)	156	523	0.04	6.7	519	4	0.80	0.84
	Warming and 50% Drought (Live)	188	723	0.04	7.7	720	3	0.87	0.84

Dataset	Treatment	Nodes	Edges	Density	Mean degree	Positive edges	Negative edges	Modularity	Transitivity
Fungi	Warming and 50% Drought (Sterile)	158	636	0.05	8.1	635	1	0.83	0.90
	Warming and 70% Drought (Live)	193	733	0.04	7.6	731	2	0.80	0.89
	Warming and 70% Drought (Sterile)	184	622	0.04	6.8	622	0	0.88	0.92
AMF	Control (Live)	471	4283	0.04	18.2	4275	8	0.86	0.90
	Control (Sterile)	493	4643	0.04	18.8	4636	7	0.86	0.90
	50% Drought (Live)	553	6364	0.04	23.0	6360	4	0.84	0.88
	50% Drought (Sterile)	544	6415	0.04	23.6	6410	5	0.83	0.92
	70% Drought (Live)	597	6352	0.04	21.3	6344	8	0.85	0.88
	70% Drought (Sterile)	565	5876	0.04	20.8	5873	3	0.88	0.89
	Warming (Live)	416	3914	0.05	18.8	3912	2	0.83	0.91
	Warming (Sterile)	377	3149	0.04	16.7	3148	1	0.88	0.93
	Warming and 50% Drought (Live)	491	4717	0.04	19.2	4717	0	0.84	0.87
	Warming and 50% Drought (Sterile)	384	2852	0.04	14.9	2852	0	0.88	0.85
	Warming and 70% Drought (Live)	461	3472	0.03	15.1	3471	1	0.87	0.86
	Warming and 70% (Sterile)	441	3980	0.04	18.0	3979	1	0.88	0.92

Table C. 10: Pairwise Spearman correlation coefficients (ρ) and associated p-values describing relationships among fungal trophic mode richness (saprotroph, symbiotroph, pathotroph, AMF) and plant growth traits under control, drought, warming, and combined warming–drought treatments across three plant species.

		Poa								***Celmisia***								***Carex***							
		Control		**Drought**		**Warming**		**Warming and Drought**		**Control**		**Drought**		**Warming**		**Warming and Drought**		**Control**		**Drought**		**Warming**		**Warming and Drought**	
Variable 1	**Variable2**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**
Growth rate	Growth rate	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000
Digital biomass	Growth rate	-0.5	0.089	-0.3	0.189	0.2	0.576	0.6	0.006	1.0	0.000	0.9	0.000	0.8	0.006	0.7	0.001	0.7	0.037	0.2	0.425	0.8	0.004	0.6	0.003
Dry shoot biomass	Growth rate	-0.3	0.326	-0.5	0.026	-0.1	0.847	0.3	0.106	0.6	0.066	0.3	0.132	0.5	0.162	0.5	0.012	-0.7	0.081	-0.5	0.023	-0.2	0.467	-0.5	0.009
AMF	Growth rate	0.2	0.593	0.0	0.859	0.6	0.112	-0.4	0.091	-0.3	0.298	-0.2	0.257	-0.4	0.200	-0.1	0.684	-0.3	0.420	0.2	0.361	-0.3	0.401	0.0	0.900
Saprotroph	Growth rate	-0.3	0.322	-0.2	0.370	0.1	0.864	-0.2	0.465	-0.7	0.026	-0.1	0.705	-0.2	0.599	0.0	0.970	0.3	0.531	-0.2	0.402	-0.4	0.284	-0.1	0.654
Symbiotroph	Growth rate	-0.3	0.402	-0.2	0.477	0.1	0.741	0.0	0.957	-0.4	0.286	0.0	0.832	-0.1	0.724	0.1	0.648	0.4	0.346	-0.1	0.646	-0.3	0.420	0.0	0.897
Pathotroph	Growth rate	-0.3	0.332	-0.2	0.274	0.0	0.930	-0.1	0.623	-0.3	0.408	0.0	0.856	-0.3	0.469	0.0	0.866	0.0	1.000	0.0	0.923	-0.2	0.568	-0.2	0.312
Growth rate	Digital biomass	-0.5	0.089	-0.3	0.189	0.2	0.576	0.6	0.006	1.0	0.000	0.9	0.000	0.8	0.006	0.7	0.001	0.7	0.037	0.2	0.425	0.8	0.004	0.6	0.003
Digital biomass	Digital biomass	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000
Dry shoot biomass	Digital biomass	0.3	0.355	0.5	0.007	0.5	0.194	0.5	0.019	0.6	0.056	0.3	0.212	0.8	0.009	0.6	0.003	-0.6	0.096	0.0	0.844	-0.1	0.670	-0.1	0.524
AMF	Digital biomass	-0.2	0.502	0.1	0.485	0.6	0.099	0.0	0.986	-0.5	0.160	-0.4	0.054	-0.3	0.446	-0.4	0.102	0.1	0.736	0.1	0.541	-0.7	0.016	0.1	0.799
Saprotroph	Digital biomass	0.4	0.281	0.2	0.438	0.4	0.230	-0.1	0.672	-0.7	0.026	-0.1	0.634	0.0	0.987	0.0	0.847	0.7	0.047	0.2	0.376	-0.5	0.136	-0.1	0.549
Symbiotroph	Digital biomass	0.3	0.347	0.1	0.694	0.5	0.133	-0.1	0.741	-0.4	0.211	0.1	0.748	0.1	0.840	0.1	0.733	0.8	0.015	0.3	0.110	-0.3	0.313	0.1	0.704

		Poa								*Celmisia*								*Carex*							
		Control		Drought		Warming		Warming and Drought		Control		Drought		Warming		Warming and Drought		Control		Drought		Warming		Warming and Drought	
Variable 1	**Variable2**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**
Pathotroph	Digital biomass	0.4	0.209	0.1	0.623	0.3	0.407	0.1	0.746	-0.4	0.262	0.0	0.964	0.0	0.933	-0.2	0.379	0.4	0.387	0.2	0.448	-0.2	0.486	-0.3	0.157
Growth rate	Dry shoot biomass	-0.3	0.326	-0.5	0.026	-0.1	0.847	0.3	0.106	0.6	0.066	0.3	0.132	0.5	0.162	0.5	0.012	-0.7	0.081	-0.5	0.023	-0.2	0.467	-0.5	0.009
Digital biomass	Dry shoot biomass	0.3	0.355	0.5	0.007	0.5	0.194	0.5	0.019	0.6	0.056	0.3	0.212	0.8	0.009	0.6	0.003	-0.6	0.096	0.0	0.844	-0.1	0.670	-0.1	0.524
Dry shoot biomass	Dry shoot biomass	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000
AMF	Dry shoot biomass	-0.2	0.484	0.2	0.400	0.2	0.604	-0.1	0.533	-0.6	0.051	0.2	0.420	0.0	0.907	0.0	0.887	0.3	0.432	-0.1	0.812	-0.2	0.502	-0.2	0.256
Saprotroph	Dry shoot biomass	0.3	0.382	0.5	0.011	0.2	0.688	-0.1	0.703	-0.3	0.420	-0.2	0.403	0.1	0.800	0.2	0.376	-0.3	0.526	0.1	0.699	-0.5	0.127	-0.1	0.572
Symbiotroph	Dry shoot biomass	0.1	0.660	0.3	0.225	0.7	0.053	-0.1	0.604	-0.3	0.358	0.0	0.915	-0.1	0.880	0.2	0.308	-0.3	0.463	0.0	0.943	-0.4	0.285	-0.1	0.711
Pathotroph	Dry shoot biomass	0.2	0.486	0.4	0.049	0.2	0.643	0.0	0.899	0.0	0.935	-0.1	0.589	0.2	0.584	0.1	0.622	0.1	0.815	0.2	0.433	-0.5	0.122	-0.1	0.715
Growth rate	AMF	0.2	0.593	0.0	0.859	0.6	0.112	-0.4	0.091	-0.3	0.298	-0.2	0.257	-0.4	0.200	-0.1	0.684	-0.3	0.420	0.2	0.361	-0.3	0.401	0.0	0.900
Digital biomass	AMF	-0.2	0.502	0.1	0.485	0.6	0.099	0.0	0.986	-0.5	0.160	-0.4	0.054	-0.3	0.446	-0.4	0.102	0.1	0.736	0.1	0.541	-0.7	0.016	0.1	0.799
Dry shoot biomass	AMF	-0.2	0.484	0.2	0.400	0.2	0.604	-0.1	0.533	-0.6	0.051	0.2	0.420	0.0	0.907	0.0	0.887	0.3	0.432	-0.1	0.812	-0.2	0.502	-0.2	0.256
AMF	AMF	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000
Saprotroph	AMF	0.0	0.936	0.0	0.895	0.5	0.128	0.3	0.239	0.5	0.085	0.2	0.405	0.7	0.023	0.0	0.884	0.5	0.160	0.4	0.053	0.6	0.069	0.3	0.176
Symbiotroph	AMF	0.0	0.902	0.0	0.879	0.2	0.518	0.1	0.786	0.5	0.132	0.2	0.468	0.6	0.043	0.2	0.406	0.4	0.313	0.1	0.605	0.3	0.357	0.3	0.191

		Poa								*Celmisia*								*Carex*							
		Control		Drought		Warming		Warming and Drought		Control		Drought		Warming		Warming and Drought		Control		Drought		Warming		Warming and Drought	
Variable 1	**Variable2**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**
Pathotroph	AMF	0.2	0.512	0.0	0.827	0.1	0.726	0.2	0.293	0.7	0.025	-0.1	0.501	0.5	0.153	0.0	0.868	0.8	0.018	0.5	0.010	0.3	0.332	0.1	0.742
Growth rate	Saprotroph	-0.3	0.322	-0.2	0.370	0.1	0.864	-0.2	0.465	-0.7	0.026	-0.1	0.705	-0.2	0.599	0.0	0.970	0.3	0.531	-0.2	0.402	-0.4	0.284	-0.1	0.654
Digital biomass	Saprotroph	0.4	0.281	0.2	0.438	0.4	0.230	-0.1	0.672	-0.7	0.026	-0.1	0.634	0.0	0.987	0.0	0.847	0.7	0.047	0.2	0.376	-0.5	0.136	-0.1	0.549
Dry shoot biomass	Saprotroph	0.3	0.382	0.5	0.011	0.2	0.688	-0.1	0.703	-0.3	0.420	-0.2	0.403	0.1	0.800	0.2	0.376	-0.3	0.526	0.1	0.699	-0.5	0.127	-0.1	0.572
AMF	Saprotroph	0.0	0.936	0.0	0.895	0.5	0.128	0.3	0.239	0.5	0.085	0.2	0.405	0.7	0.023	0.0	0.884	0.5	0.160	0.4	0.053	0.6	0.069	0.3	0.176
Saprotroph	Saprotroph	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000
Symbiotroph	Saprotroph	0.7	0.015	0.8	0.000	0.6	0.073	0.5	0.018	0.8	0.005	0.8	0.000	0.8	0.012	0.8	0.000	1.0	0.000	0.8	0.000	0.8	0.005	0.7	0.001
Pathotroph	Saprotroph	0.8	0.001	0.9	0.000	0.5	0.150	0.9	0.000	0.8	0.003	0.7	0.000	0.6	0.078	0.8	0.000	0.8	0.008	0.8	0.000	0.8	0.002	0.8	0.000
Growth rate	Symbiotroph	-0.3	0.402	-0.2	0.477	0.1	0.741	0.0	0.957	-0.4	0.286	0.0	0.832	-0.1	0.724	0.1	0.648	0.4	0.346	-0.1	0.646	-0.3	0.420	0.0	0.897
Digital biomass	Symbiotroph	0.3	0.347	0.1	0.694	0.5	0.133	-0.1	0.741	-0.4	0.211	0.1	0.748	0.1	0.840	0.1	0.733	0.8	0.015	0.3	0.110	-0.3	0.313	0.1	0.704
Dry shoot biomass	Symbiotroph	0.1	0.660	0.3	0.225	0.7	0.053	-0.1	0.604	-0.3	0.358	0.0	0.915	-0.1	0.880	0.2	0.308	-0.3	0.463	0.0	0.943	-0.4	0.285	-0.1	0.711
AMF	Symbiotroph	0.0	0.902	0.0	0.879	0.2	0.518	0.1	0.786	0.5	0.132	0.2	0.468	0.6	0.043	0.2	0.406	0.4	0.313	0.1	0.605	0.3	0.357	0.3	0.191
Saprotroph	Symbiotroph	0.7	0.015	0.8	0.000	0.6	0.073	0.5	0.018	0.8	0.005	0.8	0.000	0.8	0.012	0.8	0.000	1.0	0.000	0.8	0.000	0.8	0.005	0.7	0.001
Symbiotroph	Symbiotroph	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000
Pathotroph	Symbiotroph	0.6	0.036	0.7	0.000	0.4	0.241	0.4	0.046	0.6	0.046	0.4	0.065	0.7	0.022	0.6	0.003	0.7	0.049	0.4	0.043	0.5	0.123	0.6	0.001
Growth rate	Pathotroph	-0.3	0.332	-0.2	0.274	0.0	0.930	-0.1	0.623	-0.3	0.408	0.0	0.856	-0.3	0.469	0.0	0.866	0.0	1.000	0.0	0.923	-0.2	0.568	-0.2	0.312
Digital biomass	Pathotroph	0.4	0.209	0.1	0.623	0.3	0.407	0.1	0.746	-0.4	0.262	0.0	0.964	0.0	0.933	-0.2	0.379	0.4	0.387	0.2	0.448	-0.2	0.486	-0.3	0.157

		Poa								*Celmisia*								*Carex*							
		Control		Drought		Warming		Warming and Drought		Control		Drought		Warming		Warming and Drought		Control		Drought		Warming		Warming and Drought	
Variable 1	**Variable2**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**	**(ρ)**	**P**
Dry shoot biomass	Pathotroph	0.2	0.486	0.4	0.049	0.2	0.643	0.0	0.899	0.0	0.935	-0.1	0.589	0.2	0.584	0.1	0.622	0.1	0.815	0.2	0.433	-0.5	0.122	-0.1	0.715
AMF	Pathotroph	0.2	0.512	0.0	0.827	0.1	0.726	0.2	0.293	0.7	0.025	-0.1	0.501	0.5	0.153	0.0	0.868	0.8	0.018	0.5	0.010	0.3	0.332	0.1	0.742
Saprotroph	Pathotroph	0.8	0.001	0.9	0.000	0.5	0.150	0.9	0.000	0.8	0.003	0.7	0.000	0.6	0.078	0.8	0.000	0.8	0.008	0.8	0.000	0.8	0.002	0.8	0.000
Symbiotroph	Pathotroph	0.6	0.036	0.7	0.000	0.4	0.241	0.4	0.046	0.6	0.046	0.4	0.065	0.7	0.022	0.6	0.003	0.7	0.049	0.4	0.043	0.5	0.123	0.6	0.001
Pathotroph	Pathotroph	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000	1.0	0.000

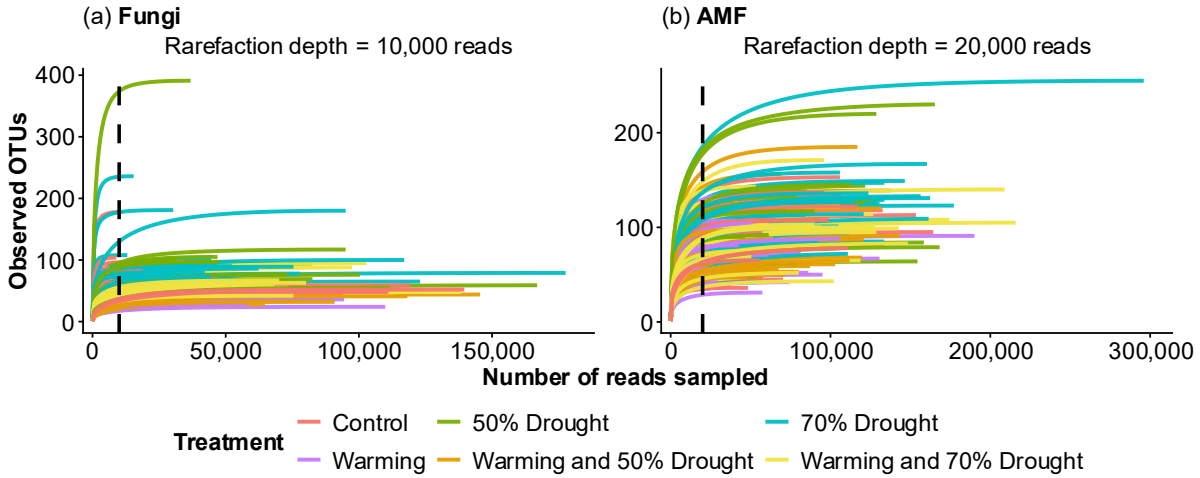


Figure C. 1: Rarefaction curves of root fungal (ITS) and arbuscular mycorrhizal fungal (AMF) communities across greenhouse treatments.

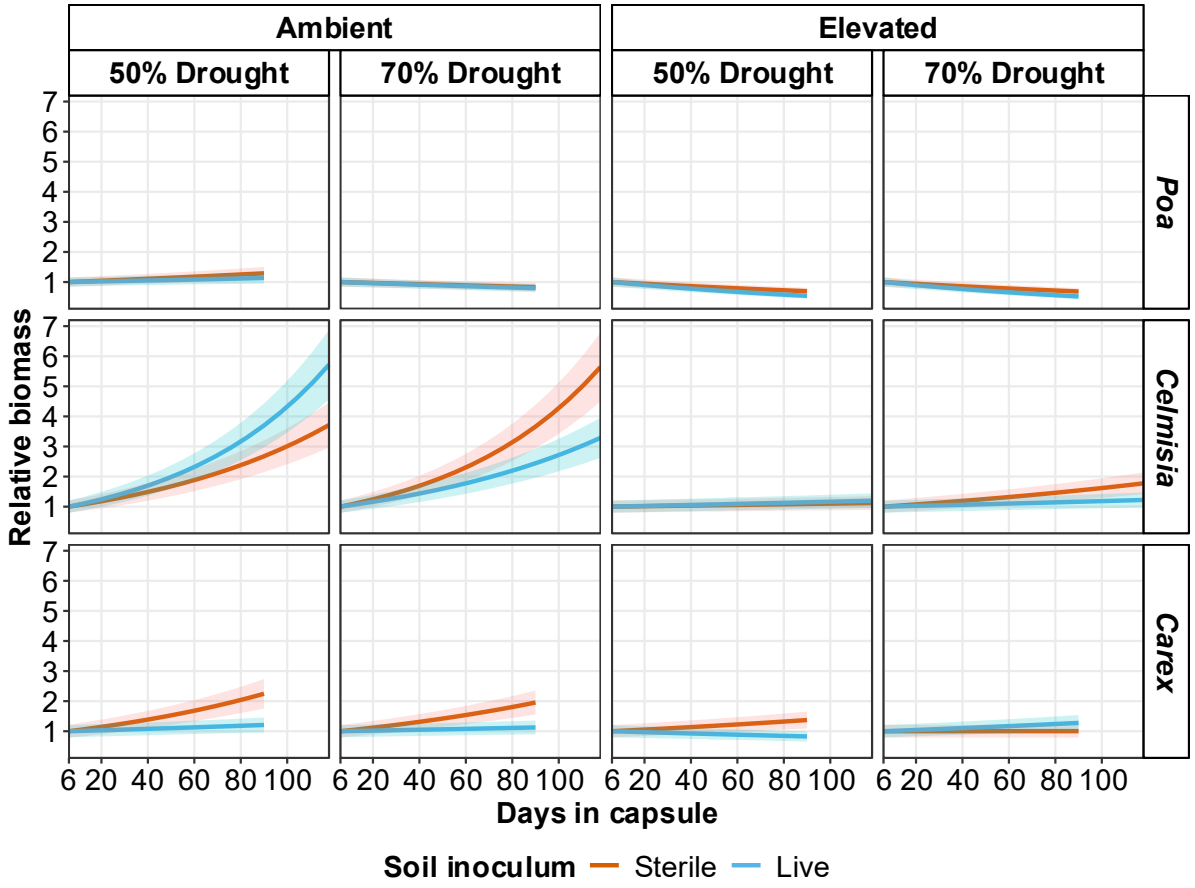


Figure C. 2: Relative digital biomass trajectories (Day-6 normalised) predicted by the mixed-effects model across Species, temperature, Moisture availability, and soil inoculum treatments. Lines represent model-predicted values, with shaded ribbons indicating 95% confidence intervals. Trajectories were derived from weekly Phenospex 3D scans and illustrate temporal patterns underlying the biomass growth-rate estimates presented in Figure 4.3.

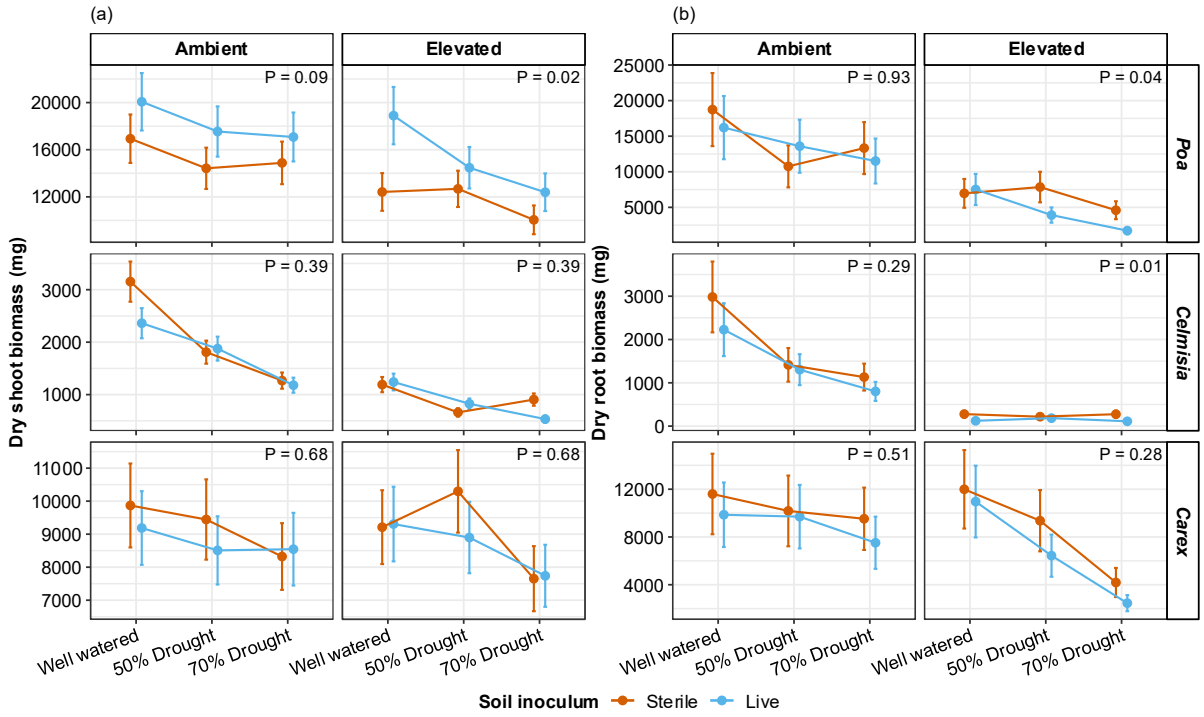


Figure C. 3: Dry shoot and root biomass of *Poa*, *Celmisia*, and *Carex* under different watering treatments and temperature regimes. Points represent back-transformed estimated marginal means ± SE derived from a linear model fitted to $\log_{10}$(dry_shoot_mg + 1) and $\log_{10}$(dry_root_mg + 1). Lines indicate trends across watering treatments. Colours denote soil inoculum (live vs sterile). P-values represent pairwise contrasts between live and sterile soil within each genotype × temperature combination, averaged across watering treatments.

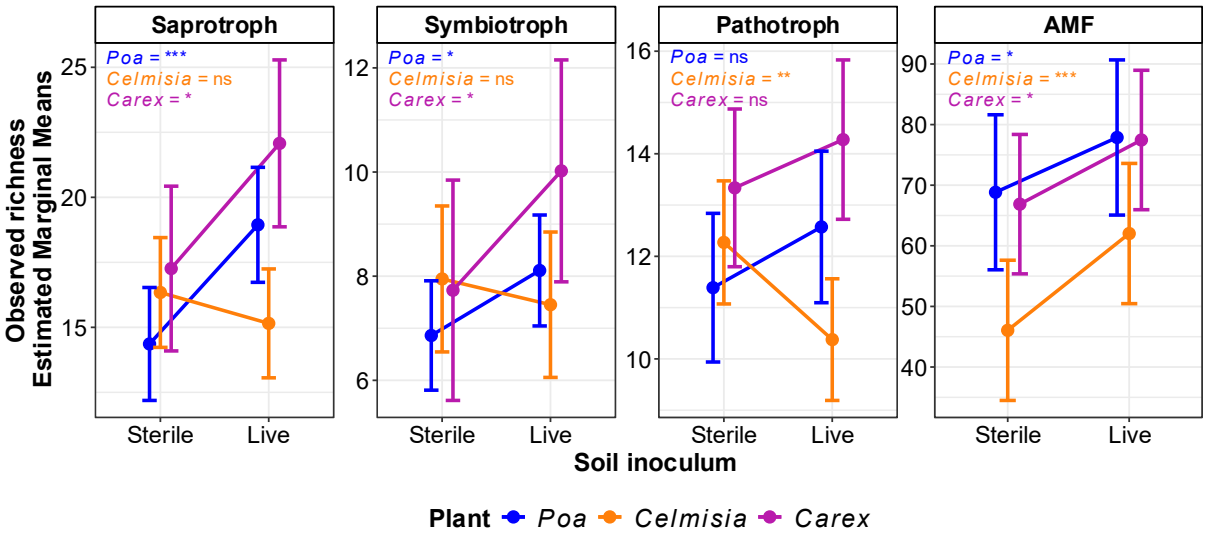


Figure C. 4: Responses of fungal richness to soil inoculum in *Poa*, *Celmisia* and *Carex*. Estimated marginal means (EMMs ± SE) were obtained from three-way factorial linear mixed-effects models (Temperature × Moisture × soil inoculum), tested using Type II Wald χ^2 statistics. (Significance levels: · $P_{adj} < 0.1$, * $P_{adj} < 0.05$, ** $P_{adj} < 0.01$, *** $P_{adj} < 0.001$; ns = not significant; post-hoc pairwise comparisons of EMMs with adjusted P-values).

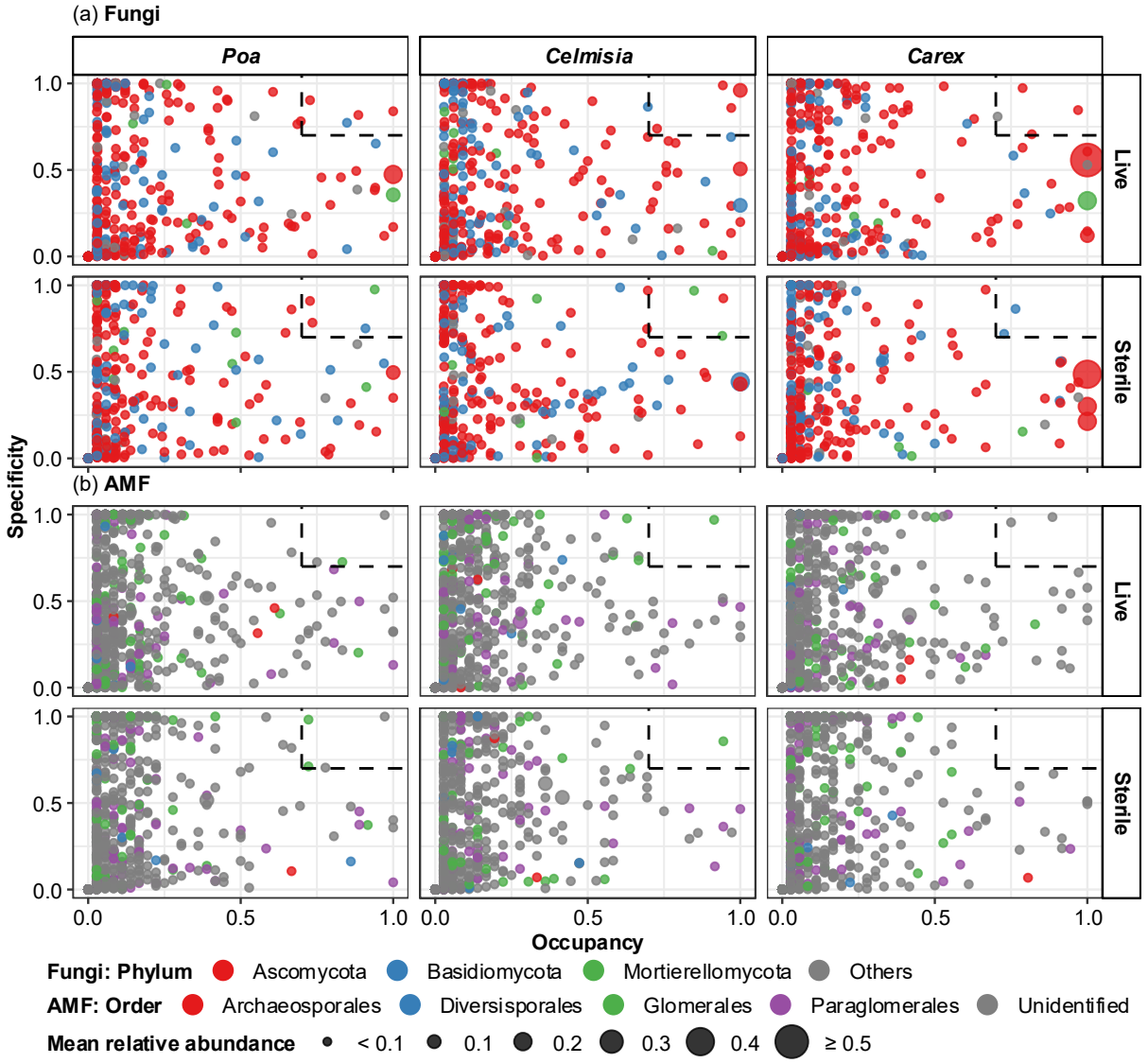


Figure C. 5: Host-specificity of root-associated (a) fungal and (b) AMF communities under live and sterile soil conditions. Specificity–occupancy plots show fungal ASVs associated with *Poa costiniana*, *Celmisia pugioniformis*, and *Carex capillacea*. Each point represents an ASV, coloured by phylum and order for fungi and AMF respectively and scaled by mean relative abundance. Dashed lines indicate the specificity and occupancy thresholds (0.7), with ASVs exceeding both thresholds classified as host-specific specialists.

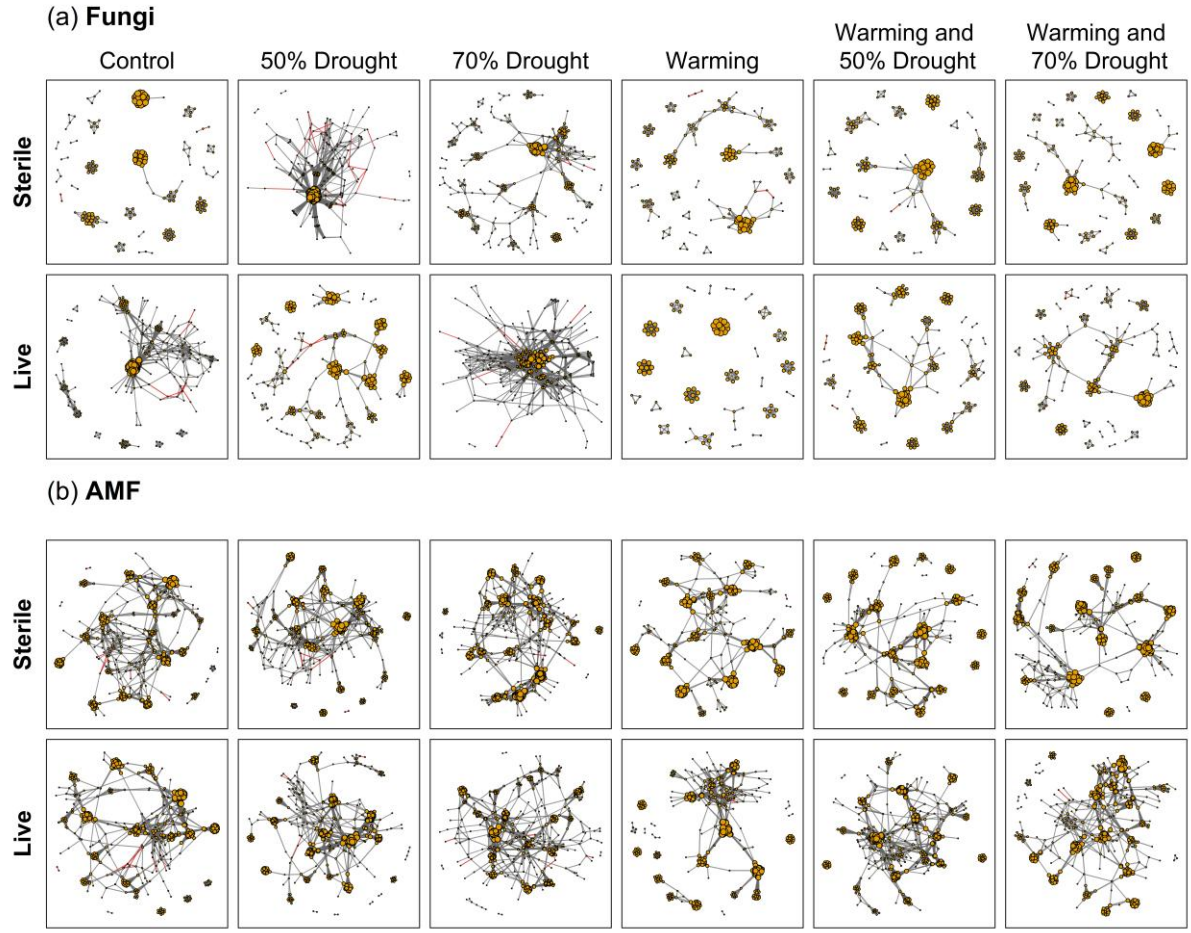


Figure C. 6: Treatment-specific co-occurrence networks inferred from significant Spearman correlations (|ρ| ≥ 0.6, FDR-corrected), showing positive (black) and negative (red) associations for root fungi and arbuscular mycorrhizal fungi (AMF).

Appendix D: Supplementary materials of Chapter 5

Table D. 1: Pairwise contrasts of temperature and moisture effects on fungal (ITS) and AMF richness and diversity. Results are derived from a full factorial linear model including temperature, moisture, plant species and their interactions. Significant P-values are shown in bold.

		Richness		Shannon		Simpson	
Contrast	**Plant**	**Estimate**	**P value**	**Estimate**	**P value**	**Estimate**	**P value**
		Fungi					
Ambient Non-droughted – Elevated Non-droughted	*Poa*	-0.10	0.998	-1.56	0.955	-1.01	0.864
Ambient Non-droughted – Ambient Droughted		0.74	0.998	-0.73	0.955	-0.60	0.864
Ambient Non-droughted – Elevated Droughted		-0.02	0.998	-0.66	0.955	-0.35	0.864
Ambient Non-droughted – Elevated Non-droughted	*Celmisia*	-19.99	**0.033**	-6.23	**0.034**	-3.31	**0.046**
Ambient Non-droughted – Ambient Droughted		4.93	0.658	2.60	0.412	1.31	0.474
Ambient Non-droughted – Elevated Droughted		-1.66	0.837	-4.47	0.146	-2.69	0.112
Ambient Non-droughted – Elevated Non-droughted	*Podolepis*	9.37	0.627	1.61	0.841	0.95	0.840
Ambient Non-droughted – Ambient Droughted		-1.53	0.843	1.02	0.841	0.74	0.840
Ambient Non-droughted – Elevated Droughted		6.30	0.638	-0.53	0.841	-0.57	0.840
		AMF					
Ambient Non-droughted – Elevated Non-droughted	*Poa*	3.00	0.892	0.39	0.847	-0.19	0.874
Ambient Non-droughted – Ambient Droughted		3.77	0.892	1.17	0.841	0.50	0.815
Ambient Non-droughted – Elevated Droughted		16.09	0.360	3.62	0.338	1.71	0.468
Ambient Non-droughted – Elevated Non-droughted	*Celmisia*	-12.35	0.216	-0.45	0.824	-0.35	0.819
Ambient Non-droughted – Ambient Droughted		21.16	0.062	3.11	0.378	0.68	0.819
Ambient Non-droughted – Elevated Droughted		8.35	0.381	1.64	0.582	0.40	0.819
Ambient Non-droughted - Elevated Non-droughted	*Podolepis*	14.75	0.326	2.92	0.225	1.52	0.314

			Richness		Shannon		Simpson	
Contrast		**Plant**	**Estimate**	**P value**	**Estimate**	**P value**	**Estimate**	**P value**
			AMF					
Ambient Non-droughted - Ambient Droughted		*Podolepis*	16.79	0.326	6.19	**0.018**	3.11	0.072
Ambient Non-droughted - Elevated Droughted			10.53	0.503	2.32	0.304	0.64	0.598

Table D. 2: Plant-specific network topology metrics of root-associated fungal OTU-level co-occurrence networks across climate treatments. Networks were constructed using significant pairwise Spearman correlations ($|\rho| \geq 0.60$, $q \leq 0.05$) at the OTU level. Permutation-tested differences in microbial co-occurrence network metrics among treatments were compared against the control treatment (significance: * < 0.05, ** < 0.01, *** < 0.001).

Plant	**Treatment**	**Nodes**	**Edges**	**Density**	**Mean degree**	**Positive edges**	**Negative edges**	**Modularity**	**Transitivity**
					Fungi				
Poa	Control	436	2019	0.02	9.26	2010	9	0.90	0.94
	Drought	441	1943	0.02	8.81	1932	11	0.91	0.96
	Warming	512	2933	0.02	11.46	2922	11	0.91	0.91
	Warming and drought	437	2417	0.03	11.06	2413	4	0.89	0.99
Celmisia	Control	346	1516	0.03	8.76	1512	4	0.90	1.00
	Drought	337	1517	0.03	9.00	1511	6	0.92	0.98
	Warming	463	2411	0.02	10.41	2398	13	0.91	0.97
	Warming and drought	364	2048	0.03	11.25	2043	5	0.89	0.95
Podolepis	Control	361	1993	0.03	11.04	1988	5	0.88	0.99
	Drought	541	7180	0.05	26.54	7152	28	0.73	0.94
	Warming	370	1802	0.03	9.74	1793	9	0.90	0.94
	Warming and drought	447	3229	0.03	14.45	3216	13	0.88	0.98
					AMF				
Poa	Control	558	3404	0.02	12.20	3394	10	0.92	0.95
	Drought	558	4084	0.03	14.64	4080	4	0.90	0.99
	Warming	517	3149	0.02	12.18	3128	21	0.91	0.95
	Warming and drought	473	2798	0.03	11.83	2794	4	0.92	0.97
Celmisia	Control	581	8395	0.05	28.90	8386	9	0.81	0.91
	Drought	406	2563	0.03	12.63	2561	2	0.91	0.96
	Warming	565	3532	0.02	12.50	3510	22	0.91	0.93
	Warming and drought	481	3847	0.03	16.00	3842	5	0.90	0.99

Plant	Treatment	Nodes	Edges	Density	Mean degree	Positive edges	Negative edges	Modularity	Transitivity
					AMF				
Podolepis	Control	566	4029	0.03	14.24	4026	3	0.91	0.99
	Drought	448	2999	0.03	13.39	2994	5	0.92	0.98
	Warming	408*	2265	0.03	11.10	2262	3	0.91	0.99
	Warming and drought	526	3730	0.03	14.18	3727	3	0.89	0.98

Table D. 3: Percentage of specialist fungal ASVs identified by specificity–occupancy analysis across plant species and climate treatments.

Treatments	Plant	Total ASVs	No. specialists	Percentage	Total ASVs	No. specialists	Percentage
			Fungi			**AMF**	
Control	*Poa*	1100	11	1.00	1680	3	0.18
Drought		1100	10	0.91	1680	3	0.18
Warming		1100	10	0.91	1680	11	0.65
Warming and drought		1100	9	0.82	1680	1	0.06
Control	*Celmisia*	1100	5	0.45	1680	5	0.30
Drought		1100	12	1.09	1680	4	0.24
Warming		1100	13	1.18	1680	8	0.48
Warming and drought		1100	8	0.73	1680	3	0.18
Control	*Podolepis*	1100	5	0.45	1680	12	0.71
Drought		1100	8	0.73	1680	10	0.60
Warming		1100	11	1.00	1680	7	0.42
Warming and drought		1100	6	0.55	1680	5	0.30

Table D. 4: Results of the binomial generalized linear model testing the effects of plant species and climate treatments on the probability of ASVs being classified as specialists. Significant P-values are shown in bold.

Effects	Df	Deviance	P value
	Fungi		
Treatment	3	4.1	0.25
Plant	2	1.6	0.45
Treatment: Plant	6	3.5	0.75
	AMF		
Treatment	3	8.9	**0.03**
Plant	2	6.1	**0.05**
Treatment: Plant	6	9.1	0.17

Table D. 5: Results of pairwise comparisons for AMF specialist probabilities across climate treatments and host plant species derived from binomial logistic regression models with Benjamini–Hochberg (BH) correction. Significant P-values are shown in bold.

Treatment comparison				
Contrast	**Odds ratio**	**SE**	**Z ratio**	**P value**
Control- Droughted	1.1	0.4	0.4	0.721
Control- Warming	0.7	0.2	-1.2	0.255
Control- Warming and droughted	2.3	1.1	1.7	0.217
Droughted- Warming	0.6	0.2	-1.6	0.217
Droughted- Warming and droughted	2.0	1.0	1.4	0.242
Warming- Warming and droughted	3.5	1.6	2.7	**0.041**
Host species comparison				
Poa -Celmisia	0.7	0.3	-0.97	0.33
Poa - Podolepis	0.4	0.1	-2.49	**0.04**
Celmisia - Podolepis	0.6	0.2	-1.81	0.10

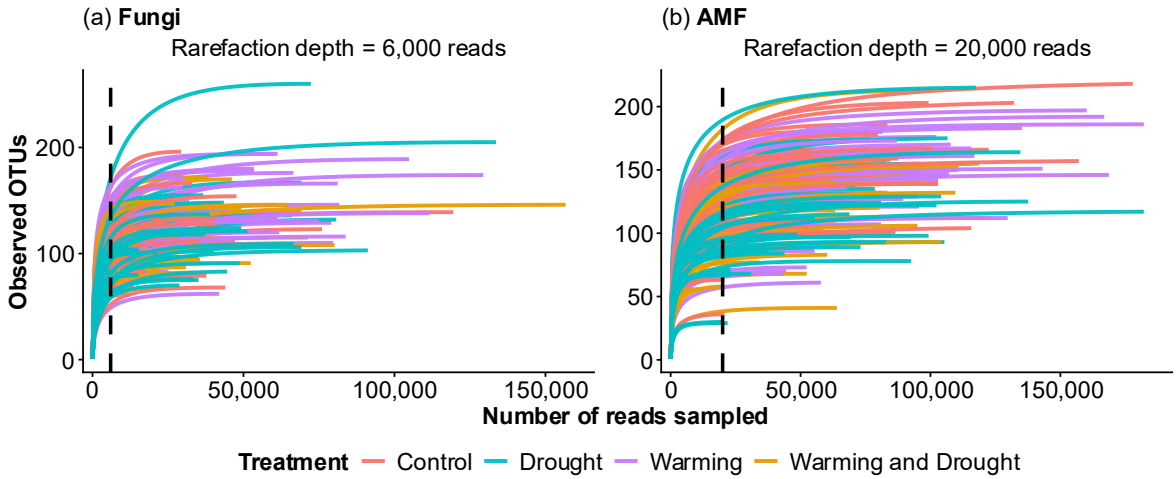


Figure D. 1: Rarefaction curves of root fungal (ITS) and arbuscular mycorrhizal fungal (AMF) communities across treatments.

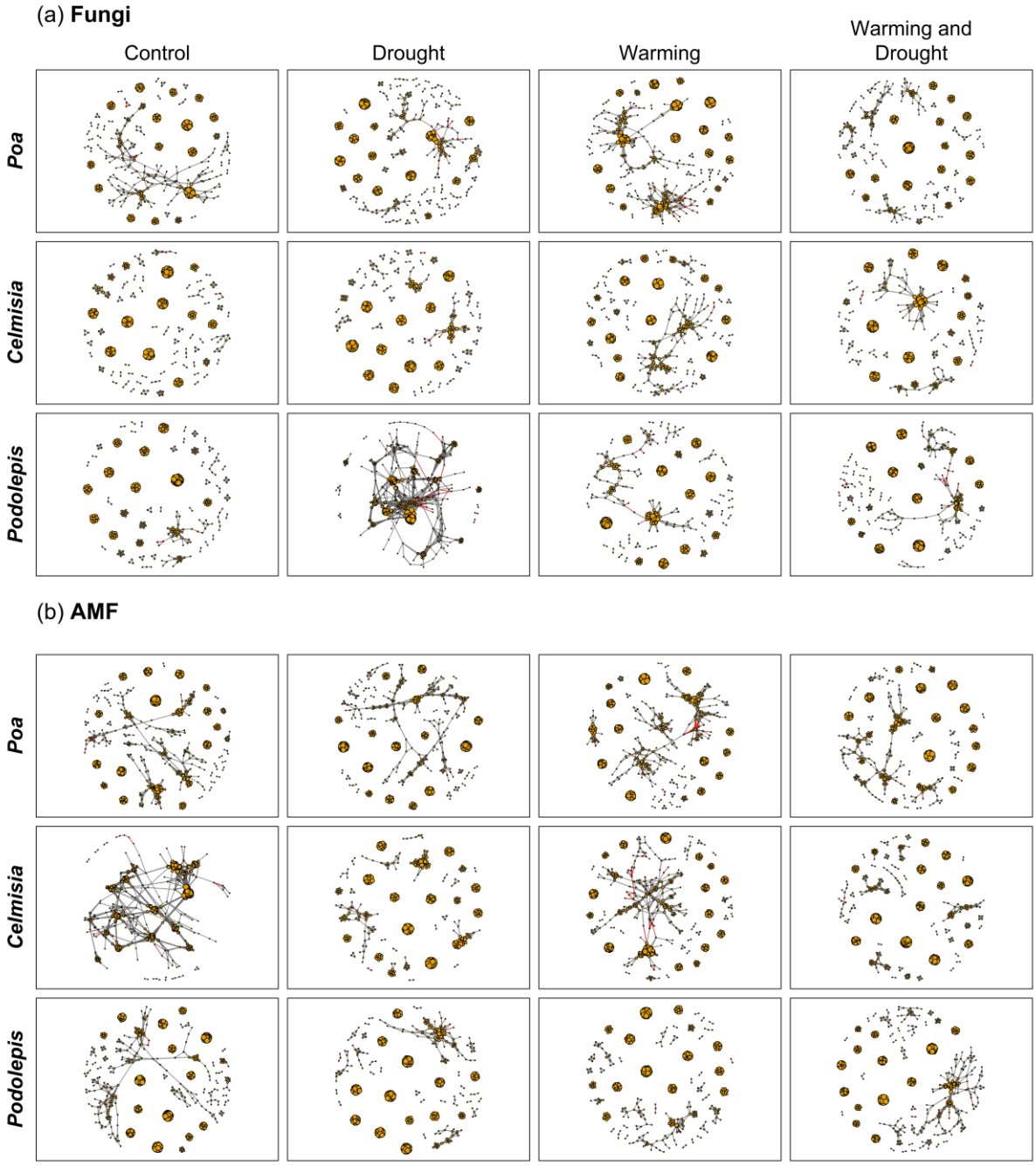


Figure D. 2: Co-occurrence networks of fungal and arbuscular mycorrhizal fungal (AMF) communities associated with the roots of *Poa*, *Celmisia*, and *Podolepis* under four climate treatments. Networks were constructed using Spearman's rank correlations among taxa, with edges retained at $|\rho| \geq 0.6$ and false discovery rate-adjusted $P \leq 0.05$.