Effects of aridity on the plant-soil interactions and microbial colonisation of the keystone grass, *Themeda triandra*

By

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DOCTOR OF PHILOSOPHY

Thesis
Submitted to Flinders University
for the degree of
Doctor of Philosophy (PhD)

College of Science and Engineering
13 June 2025

Flinders University

Themeda triandra

Acknowledgements

I am deeply grateful to my primary supervisor, A/Prof. Martin Breed, whose mentorship has been (using his favourite adjectives:) *critical, crucial, imperative, essential,* and *invaluable* to the completion of this thesis. Without Martin's guidance throughout every step of this research, but especially his writing advice, I would not have reached submission. I would also like to thank Dr. Craig Liddicoat, Dr. Sunita Ramesh, and Prof. Robert Edwards, each for their invaluable guidance and throughout the design, planning and writing of my thesis chapters. I would like to thank Prof. Huade Guan and Prof. Mike Gardner for their advice and helpful suggestions as internal assessors throughout my candidature. Finally, I am also grateful to my external examiners, David Lipson and Hannah Buckley, whose thoughtful feedback has helped to improve this work and its subsequent iterations.

I am incredibly indebted to the members of the FORE Lab Group. I would like to thank Amy Annells, Dr. Joel Brame, Christian Cando-Dumancela, Sofie Costin, Tarryn Davies, Nicole Fickling, Kate Matthews, Natalie Newman, Shawn Peddle, Dr. Jake Robinson, Heidi Rogers, Alex (Tay-Tay) Taylor, Declan Spoor, and Carl Watson for their help and camaraderie. There may not be friends in Carcassonne, but there are in FORE.

I would like to thank Victoria Drysdale, who has been, perhaps, the most tolerant, forgiving, and generous supporter of mine – both in the field and at home. This thesis would have been impossible without her unwavering help. To all my friends and family who have provided encouragement and understanding during the challenging times – and who may not realise the true impact of their support – I offer my heartfelt thanks. In particular, I am deeply grateful to Liam Waddill for those many cathartic walks, as well as to Johanna Moerkerk, Mara Schneiders, and Randal Hodgson for their support from afar. Their help has been a constant source of strength in my journeys through *PhD land*. Likewise, I would like to thank Evie, 'small bean', the love of my life, the ultimate WFH colleague, and the best cat in the world.

Finally, I would like to thank Sue Henderson who has been an inspiration throughout this journey, and someone whose friendship has been profoundly important to me.

I acknowledge the traditional custodians of the lands upon which I conducted my research. These include: the Kaurna Pangkarra, Peramangk, Ngarrindjeri, Narangga, and Nukunu Peoples.

Fundings and financial support

This research was made possible through financial support from the Australian Government Research Training Program Scholarship, the Holsworth Wildlife Research Endowment (Ecological Society of Australia), the Lirabenda Wildlife Research Fund (Field Naturalists Society of South Australia), and the Conservation Biology Grant (Biological Society of South Australia, Nature Conservation Society of South Australia). Finally, I would like to acknowledge the Flinders Accelerator for Microbiome Exploration (FAME) which facilitated key aspects of this research.

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Thesis abstract

In this thesis, I show that the pan-palaeotropical keystone grass, *Themeda triandra* forms important beneficial relationships with soil microbial communities across an aridity gradient. I also show that aridity alters microbial soil-to-endosphere colonisation dynamics. Plant-soil interactions are increasingly recognised as important to shaping ecosystem function and the health of host plants, particularly in grassland ecosystems. As such, understanding how these interactions influence the growth, fitness, and stress responses of ecologically significant species, such as, *T. triandra*, is key to advancing our knowledge of these processes. However, the mechanisms by which microbiota colonise different root compartments – across bulk soils, rhizospheres (microbiota around root surfaces), and root endospheres (microbiota within roots) – have been poorly characterised outside of model plant species. By investigating non-model species from wild populations under changing aridity conditions, and their microbiota, we can show how these microbial recruitment processes change across broad geographical distances. Furthermore, we can investigate changes in the functional properties of microbiomes from different plant compartments (i.e., soils, rhizospheres, and endospheres) to understand the extent to which plants can moderate the colonisation of microbiota into their roots.

Here, I address these knowledge gaps to produce a body of knowledge around a globally important grass. This research will contribute to our understanding of how host plants can respond to environmental stress, which represents essential knowledge for landscape management process faced with changing aridity due to the impending effects of climate change.

By using DNA-based approaches across natural field and greenhouse experiments, I make an original contribution to knowledge through exploring geographical patterns of *T. triandra* microbial community interactions over four research chapters. Specifically, I show that *T. triandra* enriches the abundance of key, host-associated, bacterial taxa with increasing aridity. I provide evidence that soil microbiota are progressively selected by *T. triandra* plants as they colonise host rhizospheres and endospheres, despite strong influences of local conditions within each population. Moreover, I provide evidence of *T. triandra*-microbial interactions by identifying changes in key functional gene profiles that promote growth and stress responses across *T. triandra* microbiomes. Furthermore, I enhance the current model of soil-to-endosphere colonisation, the 'two-step selection process', by showing that functional alpha diversity increases from bulk soils into rhizospheres, and endospheres – directly contrary to established theory on bacterial taxonomic diversity. Finally, I show how high and low aridity soil legacies significantly impact *T. triandra* growth under both stress and non-stress conditions. I identify that increasing bacterial diversity across soils, rhizospheres, and endospheres directly correlates with *T. triandra* growth, and that aridity is a key factor in determining the availability of soil microbiota for recruitment across broad geographic ranges.

Ultimately, my research makes new contributions to our understanding of the formation, distribution, and impacts of plant-microbe interactions in wild populations of a globally distributed keystone grass. These findings have important implications for conservation, ecosystem restoration, and agricultural practices related to *T. triandra* and other C4 grasses, and how these species might tolerate stress conditions across these different landscapes.

Declaration

I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university

2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and

3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Signed **REDACTED**..

Date 6/12/2024..

Details of co-authorship

Here I provide details of co-authorship contributions for each of my thesis data chapters.

Chapter two: Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C4 grass, *Themeda triandra*

Riley J. Hodgson*, Craig Liddicoat, Christian Cando-Dumancela, Nicole W. Fickling, Shawn D. Peddle, Sunita Ramesh & Martin F. Breed. This manuscript published in *Applied Soil Ecology* *https://doi.org/10.1016/j.apsoil.2024.105514*

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The candidate was the primary author of the manuscript. Project design: **RJH,** CL, MFB; Field work: **RJH**, MFB, CCD, SP, NWF; Lab work: **RJH**, CCD; Statistical analysis: **RJH**, CL; Writing manuscript: **RJH**; Revisions and editing: **RJH**, MFB, CL, NWF, SR, SP, CCD. All authors gave approvals before final submission.

Chapter three: Strong host modulation of rhizosphere-to-endosphere microbial colonisation in natural populations of the pan-palaeotropical keystone grass species, *Themeda triandra*

Riley J. Hodgson*, Christian Cando-Dumancela, Craig Liddicoat, Sunita A. Ramesh, Robert A. Edwards & Martin F. Breed. This article is in-press at *Ecology and Evolution* https://doi.org/10.1002/ece3.71595

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The candidate was the primary author of the manuscript. Project design: **RJH**, SR, MFB; Field work: **RJH**, CCD, MFB; Lab work: **RJH**, CCD; Statistical analysis: **RJH,** CL; Writing manuscript: **RJH**; Revisions and editing: **RJH**, CL, CCD, SR, MFB. All authors gave approvals before final submission.

Chapter four: Contrasting microbial taxonomic and functional colonisation patterns in wild populations of the pan-palaeotropical C_4 grass, *Themeda triandra*

Riley J. Hodgson***,** Christian Cando-Dumancela, Tarryn Davies, Elizabeth A. Dinsdale, Michael P. Doane, Robert A. Edwards, Craig Liddicoat, Shawn D. Peddle, Sunita A. Ramesh, Jake M. Robinson & Martin F. Breed
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The candidate was the primary author of the manuscript. Project design: **RJH**, SAR, MFB; Field work: **RJH**, CCD, TD, SDP, MFB; Lab work: **RJH**, CCD, MPD; Data analysis: **RJH**, RAE, CL; Manuscript writing: **RJH**; Revisions and editing: **RJH**, CCD, TD, EAD, MPD, CL, SDP, SAR, JMR, MFB. All authors gave approvals before final submission.

Chapter five: Arid soil bacteria legacies improve drought resilience of a keystone grass

Riley J. Hodgson***,** Christian Cando-Dumancela, Tarryn Davies, Victoria Drysdale, Nicole W. Fickling, Craig Liddicoat, Shawn D. Peddle, Sunita A. Ramesh, Declan Spoor, Alex Taylor, Martin F. Breed

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The candidate was the primary author of the manuscript. Project design: **RJH**, SAR, MFB; Field work: **RJH**, SDP, DS; Lab work: **RJH**, CCD, TD, VD, NF, AT; Statistical analysis: **RJH**; Writing manuscript: **RJH**; All authors revised and edited the manuscript and gave approvals before final submission.

Additional peer-reviewed publications

This is a list of peer-reviewed publications to which I contributed as a co-author, during my PhD candidature:

- Shawn D Peddle, **Riley J Hodgson**, Ryan J Borrett, Stella Brachmann, Tarryn C Davies, Todd E Erickson, Craig Liddicoat, Miriam Muñoz-Rojas, Jake M Robinson, Carl D Watson, Siegfried L Krauss, Martin F Breed 2024. Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions. *Biological Reviews*, **100**: 1-18. https://doi.org/10.1111/brv.13124 **(See Appendix A)**

- Jake M Robinson, **Riley J Hodgson**, Siegfried L Krauss, Craig Liddicoat, Ashish A Malik, Belinda C Martin, Jakki J Mohr, David Moreno-Mateos, Miriam Muñoz-Rojas, Shawn D Peddle, Martin F Breed 2023. Opportunities and challenges for microbiomics in ecosystem restoration. *Trends in Ecology and Evolution* **38** (12), 1189-1202 https://doi.org/10.1016/j.tree.2023.07.009 **(See Appendix B)**

- Christian Cando-Dumancela, Tarryn Davies, **Riley J Hodgson**, Craig Liddicoat, Shawn D Peddle, Carl D Watson, Martin F Breed 2023. A practical guide for restoration ecologists to manage microbial contamination risks before laboratory processes during microbiota restoration studies. *Restoration Ecology* **31** (1), e13687 https://doi.org/10.1111/rec.13687

Additional peer-reviewed publications

- Carl D Watson, Michael G Gardner, **Riley J Hodgson**, Craig Liddicoat, Shawn D Peddle, Martin F Breed 2022. Global meta-analysis shows progress towards recovery of soil microbiota following revegetation. *Biological Conservation* **272,** 109592 https://doi.org/10.1016/j.biocon.2022.109592

- **Riley J Hodgson**, Craig Liddicoat, Christian Cando-Dumancela, Colette Blyth, Carl D Watson, Martin F Breed 2023. Local and non-local soil microbiota impede germination of the endangered *Acacia whibleyana*. *Austral Ecology* **48** (2), 339-358 https://doi.org/10.1111/aec.13275 (Published manuscript based on Honours Thesis)

Chapter one: Thesis introduction

Note to examiners

This Chapter includes excerpts from the peer-reviewed publications Peddle et al. (2024) and Robinson et al. (2023). These are publications to which I made substantial contributions as a co-author. The excerpts have been integrated into this thesis introductory Chapter as block extracts. Full unmodified versions of these manuscripts can be found in Appendix A (Peddle et al. 2024) and Appendix B (Robinson et al. 2023). Citations referenced within the block extracts are found in the original text provided in the Appendices. At the beginning of each relevant section of this introductory Chapter, I provide a citation and justification for including these block extracts. As second-named author in Peddle et al. (2024), I draw more frequently from this text. Please refer to the signed Co-authorship Approvals for Higher Degree by Research Thesis for Examination*, submitted alongside this thesis in accordance with Clauses 5, 7, and 8 of the* HDR Thesis Rules.

1.1 Research aims and chapter outlines

In this thesis I explore the soil microbial-plant interactions of the pan-palaeotropical C4 grass species, *Themeda triandra*, across an aridity gradient. I focus on how environmental conditions shift host rhizosphere and endosphere recruitment.

My specific thesis aims are:

1. To characterise changes in the bacterial communities of *T. triandra* soils and roots, across a southern Australian aridity gradient
2. To analyse bacterial colonisation across *T. triandra* bulk soil, rhizosphere and endosphere communities
3. To explore the functional colonisation trends of microbiota across *T. triandra* soils and root compartments
4. To determine the effects of soil microbiota on the drought stress response of *T. triandra*

This thesis consists of an introductory chapter providing background of relevant scientific literature and setting the context of my research (*Chapter one*). This is then followed by four Data Chapters (*Chapters two* to *five*), and a thesis discussion chapter (*Chapter six).* The structure and content of each of these Chapters is detailed below. Across my data chapters, I use either research questions or hypotheses to frame my investigations, reflecting the stylistic preferences of the respective target peer-reviewed journals.

- *Chapter one: Thesis introduction*

In this chapter, I provide background for my thesis and introduce a select set of terms and concepts. I outline the importance of grassland ecosystems, emphasising why they are valuable and the urgent need for their conservation and restoration. I then review research on soil microbial communities, exploring the diversity across soil and plant microbiomes and their integration into ecological research. I discuss tools and techniques used in microbial ecology which have potential to enhance the research and practice of grassland restoration. Finally, I introduce *Themeda triandra*, the keystone grass species central to my research, covering its distribution, important eco-physiological traits, and significance within grassland ecosystems. Overall, this chapter establishes the rationale for my thesis, highlighting the need to better understand species like *T. triandra* and their microbial communities to support grassland restoration and conservation efforts.

- *Chapter two: Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C_4 grass,* Themeda triandra

This chapter explores how bacterial communities in the bulk soils and rhizospheres of *Themeda triandra* vary across an aridity gradient in southern Australia. I examine structural differences across these communities by looking at bacterial diversity, community composition and abundance, and evaluate the influence of climatic, edaphic, and ecological factors on bacterial community composition. This work contributes to my thesis by profiling these *T. triandra* bacterial communities and, importantly, evaluating the impact of aridity on their rhizospheres and associated bulk soils.

In this chapter, I characterised the core soil and rhizosphere bacterial microbiomes of *T. triandra* across a strong aridity gradient in southern Australia, which is representative of the global distribution of *T. triandra,* and investigated the following research questions: (1) how do *T. triandra*-associated soil and rhizosphere bacterial diversity and community composition change across a strong aridity gradient? and (2) what is the relative contribution of climatic, soil abiotic, ecological, and host related phenotypic traits on structuring the core *T. triandra*-associated microbiota?

- *Chapter three: Strong host modulation of rhizosphere-to-endosphere microbial colonisation in natural populations of the pan-palaeotropical keystone grass species,* Themeda triandra

This chapter examines the colonisation of bacterial communities from *T. triandra* rhizosphere into the endosphere, across the aridity gradient studied in *Chapter two*. I test whether they exhibit patterns predicted under the framework of the ‘two-step selection process’. I also consider the different deterministic and stochastic processes that drive the bacterial assembly within each of these compartments. This contributes to my thesis by exploring the specific recruitment dynamics of *T. triandra*, and whether they are moderated by local populations, or exhibit universal recruitment patterns. This builds a more detailed picture of how plant-soil-microbial interactions in this important grass are influenced by environmental conditions and regional variation.

In this chapter, I used neutral theory models and diversity-based analyses to explore the different processes driving selection and bacterial colonisation across different ecological populations. To do this I explored the following research questions: (1) Does *T. triandra* rhizosphere and root endosphere bacterial community composition and diversity align with the processes described in the two-step selection process, with reduced bacterial diversity in the endosphere compared to the rhizosphere? (2) Is there evidence of different deterministic or stochastic assembly processes influencing the assembly of rhizosphere and endosphere bacterial communities? And (3), are the bacterial communities in the *T. triandra* endosphere entirely constrained by the diversity of bacteria available in the rhizosphere, or are there other sources of bacterial recruitment?

- *Chapter four: Contrasting microbial taxonomic and functional colonisation patterns in wild populations of the pan-palaeotropical C4 grass,* Themeda triandra

This chapter examines the colonisation of microbial communities and their functional properties from *T. triandra* bulk soils into rhizospheres and then endospheres, across the aridity gradient analysed in *Chapter two* and *Chapter three*. I test whether the taxonomic and functional components in these compartments follow patterns predicted by the ‘two-step selection process’ and assess how aridity shapes their functionality. By investigating the diversity and composition of *T. triandra* microbial communities and the abundance of their functional genes, this chapter expands understanding of how host recruitment is functionally influenced by changing environmental conditions.

In this field-based study, I used a natural experimental design and shotgun metagenomics to investigate the colonisation patterns of microbiota, and their accompanying gene functions, in wild *T. triandra* populations across a globally-representative aridity gradient. Here, I hypothesised that (1) the microbial taxonomic and functional colonisation patterns in *T. triandra* would align with the two-step selection process (i.e., community and diversity filtering from bulk soil into roots); (2) there will be strong positive correlations between microbial taxonomic and functional colonisation patterns, where we expect that higher bacterial species diversity will be associated with higher functional diversity; and, (3) aridity will modulate both taxonomic and functional colonisation patterns, with higher aridity populations recruiting microbiota linked to water stress tolerance and drought resilience.

- *Chapter five: Arid soil bacteria legacies improve drought resilience of a keystone grass*

This chapter examines the effects of microbial communities from high and low aridity *T. triandra* bulk soils on the growth of *T. triandra* plants grown under water stress and no-stress conditions. I test whether both the taxonomic and functional components of the bulk soils, rhizospheres, and endospheres follow patterns predicted by the 'two-step selection process' framework under these stress impacts. This study builds on my findings from *Chapter two*, *Chapter three* and *Chapter four*, by experimentally testing the impacts on different microbiota on *T. triandra* growth and fitness under stress.

In my final data chapter, I conducted a glasshouse experiment, where I used 16S rRNA amplicon sequencing to characterise the diversity and composition *T. triandra*-associated bacterial communities of plants grown in high and low aridity soils under live versus sterilised, and water stress treatment conditions, plus the recruitment patterns of these microbiota from the bulk soils into *T. triandra* rhizospheres, and endospheres. I hypothesised that: (1) soil microbiota sourced from arid sites would enhance *T. triandra* growth under both stress and control conditions by providing mutualistic microbiota that support growth under arid conditions; (2) distinct microbial communities would be recruited into the rhizosphere and endosphere under each water treatment, reflecting shifts in host plant requirements; and (3) the presence of *T. triandra* plants would alter the bacterial community diversity and composition in soil due to a cumulative influence of bacteria-root interactions.

- *Chapter six: Thesis discussion*

The final chapter of my thesis serves as a comprehensive thesis discussion, summarising each of my data chapters in alignment with the overarching aims of the thesis. I begin by synthesising the key findings produced during my PhD. Following this, I relate these findings to the broader literature, highlighting new contributions and relevance to the field. I then propose directions for future research, focusing on overcoming project limitations and further consolidating scientific understanding of my research topics. Finally, I outline the broader implications of this work, not just for *T. triandra,* but similar plants and vegetation.

1.2 Grasslands

Grassland ecosystems are globally distributed, occurring in numerous varieties on every continent, except Antarctica. They cover approximately 30-40% of the earth's surface (Bardgett et al. 2021, Buisson et al. 2022), and support tremendous levels of biodiversity often with complex varieties of forb species (herbaceous, non-woody plants), invertebrates, animals and microbiota (Sloan et al. 2014, Hermann et al. 2016, Murphy et al. 2016). Furthermore, grasslands play a vital role in carbon storage and nutrient cycling, among other ecosystem services. Unfortunately, however, grasslands face severe levels of degradation and decline, highlighting the urgent need for effective conservation and restoration strategies to protect and sustain these ecosystems for future generations. Research into the ecology of grasslands is broad and, below, I outline our current understanding of the structure and functioning of grassland ecosystems. I highlight their ecological and cultural value via the ecosystem services they provide. I also examine the imminent and future threats to grasslands and summarise key strategies aimed at improving their global status.

1.2.1 Grasslands: classifications, diversity, and ecosystem services

At high trophic levels, grasslands are among the highest supporters of biodiversity (Wilson et al. 2012, Lyons et al. 2023). Their high ecological variability means that definitions for grasslands range widely, but despite this, they share many common features (Dixon et al. 2014). Historically, grasslands have been considered ephemeral ecosystems, mistakenly identified as part of early successional stages of other plant communities like forests (Harris 2009, Buisson et al. 2022). However, many are now well known to be ancient landscapes full of old perennial plants which have persisted ecologically for millennia, with complex belowground structures (Nerlekar and Veldman 2020, Buisson et al. 2022). Grasslands are typically dominated by graminoids, and they generally have low tree and shrub cover, often varying around 10% (but in some cases reaching 30% and greater in the tropics) (Dixon et al. 2014). Furthermore, high levels of competition in grasslands are thought to drive high species diversity through the many niches they facilitate, which leads to highly heterogeneous landscapes (Hodapp et al. 2018, Price et al. 2019, Eskelinen et al. 2022). The importance of heterogeneity in grasslands means that if they are degraded and lose their spatial variability, there is also a significant loss to their functional diversity (Hautier et al. 2018). Despite their low tree and shrub cover, belowground structures of grassland vegetation is estimated to reflect up to two thirds of all grassland biomass (Ma et al. 2021). As such, belowground functioning of grasslands is an important priority for future research (Peddle et al. 2024). To maintain grassland ecosystems, we need to develop an understanding of how they operate belowground.

The formation of grasslands is often determined by climatic and/or soil related factors, such as conditions that lead to trees and shrubs being unable to persist (Wakeling et al. 2012). Common examples include harsher climates like global drylands, or higher elevations, frequent fire, or shorter growing seasons (Wakeling et al. 2012, Linder et al. 2018). These factors often lead to low soil nutrient and/or moisture levels, high environmental exposure (i.e., harsh weather and growing conditions), and/or shorter growing seasons (Wakeling et al. 2012, Linder et al. 2018, Möhl et al. 2022). Other non-climatic factors include grazing pressures from herbivores, plus natural disturbances like fire (Wakeling et al. 2012, Linder et al. 2018). In these contexts, regular disturbance becomes important for maintaining grasslands. Therefore, grasslands represent a challenging ecosystem to manage.

Changing understandings of grassland communities

Grasslands are generally separated into: tropical/sub-tropical grasslands (i.e., savannas), and temperate grasslands (i.e., prairies, steppes, pampas, veldts, downs etc.) (Petermann and Buzhdygan 2021). Important historical distinctions also include natural and semi-natural grasslands (also known as cultural grasslands, or sometimes secondary grasslands). Natural grasslands refer to those historically untampered landscapes whereas, semi-natural grasslands often consider some form of low-intensity land use by people, usually in the context of historical land management (Petermann and Buzhdygan 2021). Examples of activities that maintain semi-natural grasslands include livestock grazing, burnings, and/or land clearing (Petermann and Buzhdygan 2021). Effective continuation of these management practises can be essential for persistence of grasslands, and the ecosystem services they provide (Shipley et al. 2024).

Recent work has challenged the traditional 'natural vs semi-natural' paradigm, however, with increasing recognition of land connections between Indigenous Peoples and their Ways of Being and ecosystem types (Bird et al. 2013, Bliege Bird et al. 2018, Hamilton et al. 2020, Montoya et al. 2020). Furthermore, while definitions of semi-natural grasslands share similarities with the novel ecosystems concept, and provide useful distinctions based on 20th century land use frameworks (Hobbs et al. 2006, Hobbs et al. 2009, Higgs 2017), the notion of natural grasslands has been criticised for overlooking the role of first nations peoples in shaping and managing these ecosystems – often over millennia (Fletcher et al. 2021). This critique is particularly relevant to contemporary debates in academia, where challenges to use of terms like 'wilderness' and 'naturalness' that previously ignored human influence in these ecosystems, are reshaping our understanding (Fletcher et al. 2021, Lemoine and Svenning 2022). Specifically, these human-environment connections are increasingly recognised as having developmental and even evolutionary impacts on grassland biodiversity and functioning through active ecological maintenance, and imposed disturbances (Fletcher et al. 2021, Lemoine and Svenning 2022).

Grassland ecosystem services

Globally, grasslands are estimated to directly impact the livelihood of more than a billion people (Bengtsson et al. 2019). Grasslands are highly productive ecosystems, and their biodiversity and functionality provide mechanisms of functional redundancy (Naeem et al. 1994, Isbell et al. 2015, Soliveres et al. 2016). Indeed, many grassland ecosystems support high levels of endemism, as habitat for many rare and endangered species, but vast areas of grasslands are threatened by human activities (see *1.2.2, Grassland degradation, threats and repair*) (Myers et al. 2000, Soliveres et al. 2016). As such, grassland ecosystems have high conservation value (Nerlekar and Veldman 2020), and can be very difficult to repair once they have been degraded (Hobbs et al. 2006, Nerlekar and Veldman 2020). Consequently, there are calls for greater representation of grasslands among biodiversity hotspots (Habel et al. 2013, Murphy et al. 2016). High biodiversity creates functional redundancy, making ecosystems more resilient to environmental stress (Louca et al. 2018, Biggs et al. 2020, Cheng et al. 2024) (see *1.3.1, The biodiversity of plant and soil microbiomes*), and these dynamics are especially important given our reliance on grasslands for their ecosystem services.

In Table 1, I outline ecosystem services provided by grasslands, including pollination and food production, climate regulation through water and carbon cycling, and cultural significance through traditional use, medicinal resources, and recreational activities. Additionally, grasslands support renewable energy generation, offering suitable landscapes for wind and solar infrastructure. These services highlight the anthropocentric value of grasslands, strengthening the case for investing resources into their conservation and protection.

The provision of these ecosystem services differs across grassland ecosystems (Malinga et al. 2015), and distinct grasslands may provide one or many combinations of these services, varying over years and seasons (Ojima et al. 1993). Furthermore, grasslands, like all ecosystems, are continuous and heterogeneous environments, with functionality that exists in varying states across the landscape (Xia et al. 2023). This variation includes functional differences, for example, between temperate and tropical grasslands as a result of differing species assemblages and climatic characteristics (Xia et al. 2023). Additionally, the management practices required to maintain these services differ regionally (Bengtsson et al. 2019, Xia et al. 2023).

The highly synergistic relationship between each of these services means that many of these services are inextricably linked and dependent on shared functional properties (Zhao et al. 2020). For example, the loss of species and multifunctionality can leave grasslands vulnerable to disturbance (e.g., overgrazing, invasive species) and environmental stress (e.g., climate change) (Lewis et al. 2010, Hoover et al. 2014, Volaire et al. 2019). Furthermore, the severity of grassland degradation directly affects the provision of all ecosystem services described in Table 1 (Hönigová et al. 2012).

Table 1.1. Overview of grassland ecosystem services

Ecosystem services	Importance	Benefits	References/Further reading
Agricultural services: pollination	• Enhances biodiversity and ecosystem health • Vital for supporting crop and ecosystem resilience	• Provides diverse habitats for pollinators, such as bees, butterflies, and beetles • Provides nesting sites via soil, grass tussocks, and dead plant materials • Pollinator diversity enhances crop yields and supports adjacent agriculture and ecosystems	• Bengtsson et al. 2019 • Hederström et al. 2024 • Willmer and Stone 2004 • Vujanović et al. 2023
Agricultural services: food production	• Supports agricultural productivity and livestock industries • Provides food security	• Supports livestock grazing, yielding resources like meat and dairy • Sustains livestock through abundant biomass and forage resources • Honey production through insect biodiversity, and supporting commercial bee populations	• O'Mara 2012 • Bengtsson et al. 2019
Climate services: water regulation	• Manages water flow, reducing flooding and erosion • Maintains water quality	• Directs water into streams and acts as natural filters in catchment areas • Traps sediments and pollutants, enhancing water quality • Stable soil reduces runoff and erosion • Adjacent wetlands benefit from water and pollutant regulation, maintaining ecosystem functions	• Bengtsson et al. 2019 • Zhao et al. 2020 • Kretz et al. 2021 • Sand-jensen 1998 • Borin et al. 2005 • Hönigová et al. 2012

Climate services: carbon cycling	• Essential for carbon storage, mitigating climate change • Balances carbon emissions	• Sequesters 0.3-0.5 billion tonnes of carbon annually in soil and biomass • Converting croplands to grasslands, increases soil carbon by up to 30% • Restoration and conservation improve long-term carbon storage • High conservation value due to 'irrecoverable' carbon critical for climate regulation (low sequestration rate carbon)	• Ojima et al. 1993 • Lyons et al. 2023 • Bardgett et al. 2021 • Farley et al. 2013 • Burrascano et al. 2016 • Hönigová et al. 2012 • Zhao et al. 2020 • Deng et al. 2014 • Goldstein et al. 2020
Cultural services: traditional practices	• Provides resources for cultural, construction, and medicinal purposes • Essential for indigenous knowledge and tradition	• Offers materials for medicinal and traditional use • Contributes to cultural heritage, especially for indigenous communities/traditional owners. • Supports practices that utilise grassland materials for community-specific needs	• Pascoe 2018 • Gebashe et al. 2019 • UN General Assembly 2007
Cultural services: recreation	• Promotes eco-tourism and aesthetic enjoyment • Supports recreational engagement with nature	• Attracts tourists for aesthetic/scenic beauty, bird-watching, and wildlife viewing • Provides space for activities like hiking, biking, and off-roading • Educates visitors on ecosystem importance and cultural appreciation	• Kelly et al. 2003 • Wang et al. 2024 • Liddle 1991
Cultural services: medicine	• Supplies natural medicinal resources • Cultural importance for traditional medicine	• Supports medicinal plant species used in various cultures.	• Pascoe 2018 • Gebashe et al. 2019

		• Provided appropriate ethical conduct, can provide reservoir of natural resources for medicinal research	
Energy services: wind and solar energy*	• Potential renewable energy source • Reduces reliance on fossil fuels	• Offers open landscapes suitable for wind and solar energy infrastructure • Minimal tree cover reduces interference with wind and sunlight, optimising energy production.	• Ott et al. 2021 • Bai et al. 2022
Energy services: bioenergy*	• Potential renewable energy source • Reduces reliance on fossil fuels • Alternative use when animal husbandry/grazing is not needed/land abandonment	• Use of 'excess' grasses which would be otherwise grazed, for anaerobic digestion for biogas to generate electricity and heat • Potential source of biomethane fuel	• Ketzer et al. 2017 • Donnison and Fraser 2016

* Poor implementation can undermine grassland biodiversity, functioning, and provision of other ecosystem services

1.2.2 Grassland degradation, threats and repair

Ecosystem degradation is generally defined as a loss of ecosystem productivity, biodiversity, or ecosystem services, usually when human-caused processes have led to persistent decreases in these metrics from a historical baseline (Bardgett et al. 2021). Grasslands do not usually receive the level of protection that other ecosystems receive (e.g., temperate and tropical forests, coral reefs), making them particularly vulnerable (Dixon et al. 2014). Indeed, temperate grasslands are the least protected biome in the world with only 4% under protection status (Petermann and Buzhdygan 2021). Furthermore, in Australia, grasslands contribute 40% of value to agricultural production (Bell et al. 2014). Approximately 70% of Australia's grasslands have been partially or completely destroyed (ACT Government 1997), and in some areas such as Australia's south-east 99.5% of original low land grasslands have been lost (Williams et al. 2005).

In Table 1.2, I outline different threats and degradation processes impacting grassland ecosystems. These processes reflect the different causes of global grassland decline, and break down each of the contributing factors, which are a product of direct and indirect anthropocentric influence.

Table 1.2. Overview of threats and stressors to grassland ecosystems

Threat/degradation process	Impact	Contributing factors	References/Further reading
Urbanisation	• Reduces biodiversity, creating species-poor grassland patches • Fragments habitats, impacting ecosystem stability	• Land clearing for infrastructure • Soil compaction and nutrient-rich topsoil addition • Regular mowing, pesticide use, and altered fire regimes near urban areas	• Li et al. 2022 • Williams et al. 2005 • Fekete et al. 2024
Agriculture: mechanised farming	• Causes soil compaction and erosion, and disruption of soil fungal networks • Reduces habitat quality for native species • Fragmentation of grasslands	• Conversion of grasslands to cropland for row crops • Use of heavy machinery that impacts soil health • Habitat fragmentation from field boundaries and fencing	• Dixon et al. 2014 • Wright and Wimberly 2013 • Ramankutty et al. 2008
Agriculture: livestock grazing	• Reduces native plant cover • Degrades soil • Increases vulnerability to erosion and runoff	• Overgrazing by livestock, particularly hard-hoofed animals like cattle and sheep • Trampling damages native plant roots and compacts soil, especially in ecosystems unadapted to livestock	• Bardgett et al. 2021 • Williams et al. 2015
Agriculture: abandonment	• Causes soil erosion and encourages invasive species encroachment • Alters carbon and water cycling dynamics	• Economic downturns, drought, or loss of economic viability • Political instability or conflicts • Can lead to degradation through natural succession or invasion by woody species	• Öckinger et al. 2006 • Valkó et al. 2016 • Potapov et al. 2022 • Subedi et al. 2022

Agriculture: pesticide use	• Harms non-target species, reducing invertebrate and pollinator populations • Contributes to soil and water pollution	• Application of pesticides to control crop pests but affects native grassland species • Leads to bioaccumulation and potential toxicity in surrounding ecosystems	• Ruuskanen et al. 2023
Invasions	• Alters ecosystem dynamics, leading to hybrid ecosystems • Reduces biodiversity and ecosystem productivity	• Invasive plants outcompeting natives • Grazing pressures from feral animals (e.g., horses, goats, pigs) • Soil instability and reduced seed bank due to exotic rodents	• Zhang et al. 2024 • Humphries et al. 2022
Climate change	• Alters ecosystem state (e.g., desertification, aridity change) • Increases vulnerability to extreme weather events • Shifts species distributions and reduces biodiversity	• Changes in temperature, precipitation patterns, and frequency of fires and floods • Climate-driven increase in extreme weather events, impacting grassland stability and resilience	• Zhao et al. 2023 • Joyce et al. 2016 • Zhu et al. 2024
Ecosystem change: woody encroachment	• Reduces grassland area and plant diversity • Alters carbon and nutrient cycling • Disrupts habitat for grassland species	• Encroachment of woody plants and trees into grasslands due to fire suppression, climate change, or mismanagement. • Particularly severe in semi-arid grasslands and areas like Australia and Africa	• Zhang et al. 2024 • Öckinger et al. 2006 • Valkó et al. 2016
Ecosystem change: afforestation	• Displaces grassland ecosystems, reducing biodiversity • Undermines grassland carbon dynamics and soil stability	• Tree planting initiatives aimed at carbon storage that overlook grassland ecosystem roles • Major afforestation projects planned for grassy biomes, especially in Africa, posing risks to native grasslands	• Buisson et al. 2022 • Hermann et al. 2016 • Williams et al. 2015

Prioritising grassland restoration

There is an urgent need to conserve and restore grassland ecosystems, but there are many different facets to restoring grasslands that need to be considered. The international Society of Ecosystem Restoration (SER) defines ecological restoration as aiding the recovery of ecosystem which has been degraded, damaged, or destroyed (Gann et al. 2019). Restoration activities, while complex, consider many different actions and stages, existing across a restorative continuum that considers, (1) reducing degradation impacts, (2) remediation, (3) rehabilitation, and (4) ecological restoration (Gann et al. 2019). While returning lost species is the most intuitive component of restoration activities across different ecosystems, like grasslands, many restoration ecologists also consider the importance of returning ecosystem functionality, over the return of ecosystems to a prior historical state (Hobbs et al. 2006, Hobbs et al. 2009, Hobbs et al. 2014). This view gives greater weight to irreversible change, such as climate change or abiotic barriers that inhibit the return of local, native species (e.g., altered soil structure, changed nutrient profiles).

In *1.3.3, Applications of microbial diversity for ecosystem interventions*, below, I expand on the potential of soil microbiota for their applications to restoration ecology which extend to applications in grassland ecosystems. However, a description of current and emerging interventions that are considered for the recovery of grassland biodiversity and function can be found in Table 1.3.

Table 1.3. Comparison of common and emerging intervention strategies for grassland recovery

Strategy	Description	Limitations	Restoration uptake	References/Further reading
Seasonal burning	• Applying fire to stimulate recruitment from seedbank and open canopy/soil for the establishment of seedlings • Can facilitate return of traditional land practises	• Often not applied during seasonably appropriate times	Established	• Lunt and Morgan 1999 • Lewis et al. 2010 • Bird et al. 2013 • Bliege Bird et al. 2018
Carbon addition	• Additions of carbon through sugar and sawdust to stimulate microbial activity and facilitate the immobilisation of microbial nutrients	• Varied results	Established	• Blumenthal et al. 2003
Herbicides	• Reduce survival of invasive species using herbicides such as glyphosate.	• Unknown influence on soil microbial communities, may promote germination of some weedy species • Can eliminate native plants	Established	• Weidlich et al. 2020
Scraping	• Removal of topsoil to eliminate previous land use soil legacies such as: nutrient loads, invasive seedbank, or soil microbiota	• Expensive, and difficult to apply to lands that are rocky, not flat, our outside agricultural or mining contexts	Established	• Smith et al. 2021

Soil inoculations/ amendments	• Transfer of soils and/or their microbial communities with to benefit ecosystem functionality • Sometimes targeted around specific species, or reference communities • Inoculations can occur using microbial suspensions, dispersed via manufactured pellet or by direct soil transfers, or cultivated with tube stock • Soil amendments, such as biochar can potentially shift the microbial environment to be more suitable for target plants	• Difficult to control, highly ecosystem/species dependent	Emerging	• Robinson et al. 2023 • Peddle et al. 2024
Solarisation	• Layering clear plastic sheets during warm seasons to bake soil and eliminate viable naïve seeds and soil legacies of invasive species	• Long-timeframe for effect	Emerging	• McQuillan et al. 2024
Slashing/Strategic mowing	• To reduce weed loads and remove biomass from restoration plots to give native plants better opportunities. • Mowing can target weedy area, giving natives more growing space • Alternatively mowing in corridors can support restoration objectives	• Can be destructive even to desirable plant species • Does not address seedbank or soil legacies	Established	• Smith et al. 2018 • Facelli and Facelli 2022
Ecological phage therapy	• Ecological phage therapy involves using bacteriophages to target specific bacteria in degraded soils, potentially accelerating microbiota recovery and ecosystem restoration	• Unknown ecological consequences • Limited specificity • Novel approach	Exploratory	• Davies et al. 2024
Eco-acoustic stimulation	• Eco-acoustic stimulation shows potential in promoting growth in plant-supportive fungi, but its beneficial effects may be sensitive to specific species	• Unknown ecological consequences • Limited specificity • Novel approach	Exploratory	• Robinson et al. 2024

1.3 Applications of microbiomes to restoring ecosystems

Soils form the foundation of all terrestrial ecosystems, housing approximately 59% of all species (Anthony et al. 2023). While soils are well known to provide homes for plants and animals, they also sustain rich biodiversity of microorganisms such as bacteria, archaea, and fungi (Anthony et al. 2023). These organisms are fundamental to geochemical processes, like the carbon and nutrient cycles which have fundamental roles on Earth (Kardol et al. 2013). As such, soil microbiota have serious consequences for ecosystem functioning (Kardol et al. 2013). They also influence the lifecycles of individual plants within the context of a larger ecological community (Bever et al. 2010, Herzberger et al. 2015), and there is a complex ecological network of plant-microbial interactions which range from symbiotic to pathogenic interactions (Bulgarelli et al. 2013, Hardoim et al. 2015, Vandenkoornhuyse et al. 2015, Compant et al. 2021). Our understanding of plant ecology and our capacity to shape or alter plant communities, therefore, is partly impacted by soil microbial diversity, and how they form functional relationships with plant species.

Here, I consider current and emerging perspectives on the relationships between plants and their associated soil microbiota. I discuss microbial ecological perspectives on soil and plant microbiome functioning, and their symbiotic relationships with host plants. I also describe current techniques used to understand the different characteristics of microbiomes (such as diversity and functional potential), and approaches used to make inferences in microbial ecology and plant eco-physiology. Finally, I consider specific ecological applications of soil microbiota that can inform environmental interventions for conservation of ecological restoration.

1.3.1 The biodiversity of plant and soil microbiomes

This section includes excerpts from Peddle et al. (2024) and Robinson et al. (2023), which I identify by boxed formatting and dark blue text. These are peer-reviewed publications to which I have made substantial contributions to as a co-author. Full unmodified versions of these manuscripts can be found in Appendix A (Peddle et al. 2024) and Appendix B (Robinson et al. 2023). Citations referenced within the extracted sections are found in the original text provided in the Appendices. Please refer to the signed Co-authorship Approvals for Higher Degree by Research Thesis for Examination*, submitted alongside this thesis in accordance with Clauses 5, 7, and 8 of the* HDR Thesis Rules.

Below is an extract from Peddle et al. (2024) (see Appendix A for the full text of this paper), which provides detail on diversity patterns across soil microbiota and their interactions with plant communities. The following work was written in a restoration ecology context, which represents important applications of microbial ecology tools, particularly as we consider the need to respond to degradation of important ecosystems like grasslands (see, *1.2.2 Grassland degradation, threats and repair,* above*)*:

> "Over the last 15 years … increased attention has been given to soil microbiota – the bacteria, archaea, fungi, viruses and protists within soil – and their interactions in the soil system and with aboveground biota due to their essential functional roles (Harris, 2009; McKinley, 2019; Eisenhauer *et al*., 2017). Soil microbiota are among the most biodiverse and functionally important ecosystem components and are essential to many biogeochemical processes. For example, biological nitrogen fixation by diazotrophs, nitrogen-fixing bacteria and archaea forms the foundation of Earth's terrestrial productivity (Zhu *et al*., 2022; Vitousek *et al*., 2013) and cyanobacteria (carbon and nitrogen fixers) combine with fungi, bacteria, lichens, and other organisms to form biological soil crusts ('biocrusts') which can stabilise soil landscapes and enhance water availability (Weber *et al*., 2022; Yan-Gui *et al*., 2013). Furthermore, soils are home to over half of Earth's biodiversity (Anthony, Bender & van der Heijden, 2023) and belowground microbial biomass is often comparable in scale to aboveground plant or animal biomass (Fierer, 2017). Soil microbiota also interact with aboveground ecosystem components and are intimately involved in plant and animal health, and *vice versa*. For example, the relationship between plants and arbuscular mycorrhizal fungi is one of the oldest terrestrial symbiotic interactions (Field & Pressel, 2018; Tisserant *et al*., 2013) where plants depend on fungi to gather essential nutrients in exchange for carbohydrates. Consequently, we can expect reciprocal shifts in above- and belowground ecosystem components (Kardol & Wardle, 2010; Prober *et al*., 2015). Therefore, improving the

integration of soil microbiota and associated microbial ecology into ecosystem restoration will have considerable benefits across restoration planning, intervention, and monitoring phases [Figure 1.1].

Historically, scientists faced technological challenges in quantifying and grasping the diversity and composition of soil microbiota, as traditional culture-dependent methods were only able to grow <1% of microbial taxa (Alivisatos *et al.*, 2015; Vartoukian, Palmer & Wade, 2010). However, modern sequencing technologies enable a detailed taxonomic and functional understanding of soil microbiota. For example, the now routine high-throughput amplicon sequencing of DNA extracted from soil samples can provide a detailed taxonomic view of the microbiota within a given sample (Berg *et al.*, 2020; Fierer, 2017). These amplicon data sets can then be associated with spatial, land-use, environmental condition and/or restoration-intervention data to answer ecological questions (Tedersoo *et al.*, 2019; Thomsen & Willerslev, 2015; Breed *et al.*, 2019)."

Peddle et al. (2024), pages: 2–3

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Available online at https://doi.org/10.1111/brv.13124

(Peddle et al. 2024)

Figure 1.1. The Society for Ecological Restoration (SER) recovery wheel (Gann et al. 2019), adapted from Peddle et al. (2024), see Appendix A. Soil microbiota can be better integrated into the planning, intervention, and monitoring phases of ecosystem restoration projects. Integrations of these kinds could contribute to each of the six SER recovery outcome themes (species composition, structural diversity, ecosystem functioning, external exchanges, absence of threats, and physical condition).

Peddle et al. (2024), page: 3

Soil microbial communities also have several complex functional roles. The following excerpt from Appendix B (Robinson et al. 2023) describes how microbiota directly and indirectly influence ecosystem processes, including how microbial community dynamics intersect with plant and animal health, and ecosystem services. As such, microbiota across both terrestrial and aquatic ecosystems support nutrient cycling, decomposition of organic matter, and promoting soil structure stability (Robinson et al. 2023).

> "Microbiota directly and indirectly affect many ecosystem processes [Figure 1.2]. Research to understand the mechanistic basis of these effects is increasingly popular. Some of these effects include plant and animal health (e.g., inducing immune responses, outcompeting incoming opportunistic pathogens, producing antibiotic compounds) [10], nutrient cycling (e.g., controlling the fate of belowground carbon by decomposing organic matter or stabilizing it in the soil mineral matrix) [11], drought stress tolerance (e.g., plant growth-promoting microorganisms can biochemically induce systemic tolerance) [12], intra- and inter-kingdom communication (e.g., via quorum sensing and biochemical lures, respectively) [13], hormone production in plants and animals (e.g., microbe-derived auxin as a signalling molecule in plant development) [14], climate regulation (e.g., by producing and consuming CO_2, CH_4, and N_2O) [15], and pollination (e.g., by attracting pollinators and inducing pollen bursting) [16]. Researchers can use microbiomics to ask important questions and understand the connections between aboveground plant and animal communities (in terrestrial and aquatic systems) and belowground microbiota."
>
> **Robinson et al. (2023), page: 1190**

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Available online at https://doi.org/10.1016/j.tree.2023.07.009

(Robinson et al. 2023)

Figure 1.2. The importance of microbial communities to ecosystem functionality, adapted from Robinson et al. (2023), see Appendix B. Microbiota play functional roles in terrestrial and aquatic ecosystems, including but not limited to: nutrient cycling, climate regulation, substrate formation, animal and plant health.

Robinson et al. (2023), page: 1192

These functions are important for maintaining ecosystem resilience, particularly under stress conditions like drought (Buisson et al. 2019). Additionally, microbial interactions with plants can enhance growth and resistance to diseases, further influencing biodiversity and productivity (Wagg et al. 2014, Ngumbi and Kloepper 2016, Berendsen et al. 2018, Neuenkamp et al. 2019). Thus, microbiota serve as foundational drivers of ecosystem health, supporting diverse biological communities and contributing to the sustainability of natural and managed environments.

Plant-soil interactions and soil legacies.

Plant-soil interactions are extremely important and well-studied processes across a range of ecosystems (Bever et al. 2010). Exploring plant-soil dynamics can identify the growth promoting (or detracting) components of soil environments for plants, often shaping plant-plant interactions and structure of the aboveground vegetation (Bever et al. 2010). The characteristics of soil microbiota are influenced by a mix of biotic and abiotic conditions produced by past plant-soil legacies, and host or neighbouring species (Brinkman et al. 2017, Wubs et al. 2019, Kuťáková et al. 2023, Jiang et al. 2024).

Ecological soil legacies can include positive plant-soil feedbacks, where plants grow better in soils previously occupied by members of the same species. This dynamic typically results in mono-dominance of species that respond well to these habituated soil microbial and soil conditions (Bever et al. 1997, Bever et al. 2010). Positive plant-soil feedbacks are often observed in grass species, where hosts have been found to output mutualistic, growth promoting microbiota (Bever et al. 1997, Bever et al. 2010). Negative plant-soil feedbacks, however, create barriers that inhibit plant growth in a second generation. They describe the growth reducing effects of soil microbial and edaphic conditions, facilitated by a generation of plants (Bever et al. 1997, Bever et al. 2010). These dynamics include allelopathy – the release of compounds that alter growth and germination of competitors, and can be produced by the encouragement of pathogenic microbiota (Callaway and Ridenour 2004). Negative feedbacks have a diversity promoting effect on an ecological community (Wang et al. 2019, Beals et al. 2020). By disrupting the dominance of some species, they encourage the coexistence of other plants, maintaining a highly competitive environment (Wardle et al. 2004, Reinhart 2012, van der Putten et al. 2016, Wang et al. 2019).

Plant-soil relationships reflect mutual investment and reward dynamics (Bever et al. 2010). Both the microbiota and the plants themselves interact with each other on economic terms, via provision of services in exchange for rewards, and they can even mimic beneficial microbes to steal resources (i.e., ‘cheater organisms’) (Kiers et al. 2002, Kiers et al. 2011, Verbruggen et al. 2013, González et al. 2018). Furthermore, whether microbiota act as facilitators or followers of different plant communities – that is, whether microbiota are the principal architects of plant community diversity patterns, or a product of it – is an important consideration for utilising microbiota in restoration interventions (Harris 2009). It is likely that plant-soil relationships are more complex than described above, and patterns of plant/microbial behaviours will change, not only based on plant species, but their entire ecological communities with competition dynamics and disturbance/successional shifts (Kardol et al. 2006, Cline and Zak 2015, Wang et al. 2019, Gao et al. 2022).

Ecological frameworks for plant-soil dynamics

Our capacity to predict ecosystem responses to change or management interventions, hinges on our understandings of how plant and microbial community interactions play out across different ecological contexts. In *1.2.1 Grasslands: classifications, diversity, and ecosystem services,* above, I raise functional redundancy as a mechanism for instilling ecosystem stability. Here, functional redundancy is produced via high biodiversity – the presence of multiple species performing similar roles, often through different mechanisms. This redundancy allows ecosystems to maintain functioning even if some species are vulnerable to environmental stress or disturbance, as other species can assume their roles via alternative functional pathways (Louca et al. 2018, Biggs et al. 2020). Furthermore, global meta-analyses have found grassland biodiversity contributes to resistance against invasive plants ('the biotic resistance hypothesis') (Cheng et al. 2024), and supports ecosystem multifunctionality – with rare taxa occupying a position of particular important in these communities. Rare microbiota in one ecological context may increase in abundance following disturbances (West and Whitman 2022), enhancing community resilience and phylogenetic plasticity (Jousset et al. 2017, Jia et al. 2018). As such, functional redundancy is an important mechanism for supporting ecosystem resilience and resistance across different macro and micro ecosystem scales (Louca et al. 2018, Biggs et al. 2020). Consequently, rare species are crucial for biodiversity and functional stability.

There are several additional frameworks for theorising how plants successfully colonise new environments or resist invasion, via their plant-soil dynamics (Bever et al. 2010, Debray et al. 2022, Liu and Salles 2024). For instance:

- The ‘novel weapons hypothesis’: Invasive plants may allocate resources or compounds to soils that harm native plants, allowing them to outcompete local species by deploying pathogenic microbiota against neighbouring plants which will not have been exposed to these ‘novel weapons’ (Callaway and Ridenour 2004).
- The ‘enemy release hypothesis’: Invasive plants may succeed in new environments due to the absence of long-term, co-evolved pathogens. As such, the absence of natural predators or pathogens can facilitate a competitive advantage for these plants over native species (Agrawal et al. 2005).
- The ‘cry for help hypothesis’: Plants may recruit also established microbial symbioses during times of stress to overcome adverse conditions (Rolfe et al. 2019). When antagonistic conditions arise, plant species may change their conduct to adjust to new threats.

Ultimately, across different ecosystems, biodiversity is a factor that is consistently found to impact the success or failure of invasive species, and different theoretical frameworks can help to predict and justify change to future inventions.

Microbial colonisation of plant endospheres and rhizospheres

The ways plant species recruit microbiota around its roosts reflects a complex economic exchange of services (Kiers et al. 2002, Kiers et al. 2011, Gonzalez et al. 2018). The symbiotic interactions, not only extend to the microbiota in the soil, directly in contact with plant roots (rhizosphere microbiota) – they also colonise the internal structures of plant roots (endosphere microbiota) (Figure 1.3) (Bulgarelli et al. 2013). The processes by which microbiota move across these different compartments – from bulk soils into rhizospheres, and then into endospheres – is characterised by the two-step selection process (Bulgarelli et al. 2013, Ling et al. 2022).

Figure 1.3. Microbial communities occupy the bulk soil, rhizosphere and endosphere of host plants. The mechanisms by which microbiota colonise each of these compartments s is complex, but described through the 'two-step selection process' theoretical framework. Here, microbiota move, first, from (a) the bulk soil into (b) the rhizosphere, then, secondly, from the rhizosphere into (c) the endosphere.

Plants can recruit microbial communities around their roots by exuding metabolic resources, like organic acids, and facilitating habitat for desirable species (step 1) (Bulgarelli et al. 2012, Bulgarelli et al. 2013). In return microbiota can perform services for the plant, aiding nutrient acquisition or protection from pathogens (Bulgarelli et al. 2012, Bulgarelli et al. 2013). Rhizosphere microbiota can then colonise plant endospheres through this process which is regulated by the immune systems of host plants via controlled filtering of beneficial taxa (Bulgarelli et al. 2012, Bulgarelli et al. 2013). These microbial communities can improve plant growth via release of growth compounds like phytohormones, but can also improve host plant physiological responses to environmental stress by influencing plant metabolic processes (Vetterlein et al. 2020, Adeleke et al. 2021, Lyu et al. 2021, Santoyo 2022). For a full review of these plant colonisation dynamics and important functional roles of endosphere and rhizosphere microbiota, see Bulgarelli et al. (2013).

The source of colonising microbiota into plant root endospheres are also varied (e.g., soils, pollinators, parent plants) (Barajas et al. 2020, Vetterlein et al. 2020, He et al. 2024). Moreover, we have little understanding of the moderating factors that contribute to their success in colonising plant tissues (e.g., host plant filtering via immune regulation, and microbial fitness traits) (Bulgarelli et al. 2013, Urbina et al. 2018). As such, how natural ecological processes influence and/or disrupt these symbioses is an key consideration for predicting the consequences of environmental stress on natural plant communities (Grady et al. 2019, Moroenyane et al. 2020, Choi et al. 2021, Debray et al. 2022, Lin et al. 2022, Guo et al. 2024).

In the introductions and discussions of *Chapter two, three, four* and *five*, I provide a detailed discussion of current literature on microbial colonisation dynamics and the associated functional processes occurring across different soil and plant compartments – bulk soils, rhizospheres, and endospheres of host plants. Accordingly, I defer to these sections for a more detailed and focussed discussion of this content.

1.3.2 Tools and techniques for microbial community profiling

This section includes extracts from Peddle et al. (2024) and Robinson et al. (2023), which I identify by boxed formatting and dark blue text. These are peer-reviewed publications to which I have made substantial contributions as a co-author, although I was not the lead author. Full versions of these texts can be found in Appendix A (Peddle et al. 2024) and Appendix B (Robinson et al. 2023). Citations referenced within the extracted sections are found in the original text provided in the Appendices. Please refer to the signed Co-authorship Approvals for Higher Degree by Research Thesis for Examination*, submitted alongside this thesis in accordance with Clauses 5, 7, and 8 of the* HDR Thesis Rules.

High throughput DNA sequencing tools enable detailed profiling microbial communities across different environments (e.g., from bulk soils, or plant compartments, like rhizospheres and endospheres), and provide large complex datasets that can describe whole community taxonomic, and functional based identification and abundance. Taxonomic microbial community profiling is often conducted using high-throughput sequencing. These methods use PCR amplifications of taxonomic marker genes. These genes are then annotated with taxonomic information based on a curated database. From these data, we can get an indication of the taxonomy and abundance of organisms that can be identified within the target gene region (although, biological abundance may differ substantially from sequence abundance) (Breed et al. 2019). Common applications of the amplicon sequencing in microbial based studies include bacteria via 16S rRNA gene based on amplification of V1-V5 regions. Additionally, profiling of archaeal communities utilises the 18S gene region, while fungal communities are identified using the ITS (internal transcribed spacer) regions (Abdelfattah et al. 2018, Robinson et al. 2023). There is incredibly high uptake of these methods across many domains of microbial ecology in both human and ecological health domains (Mohr et al. 2021, Robinson et al. 2023). Amplicon sequencing is the most widespread DNA based method for profiling microbial communities, for its high accuracy (genus level for bacteria via the 16S rRNA gene), and high affordability, relative to other methods (Robinson et al. 2023, Peddle et al. 2024).

The following sections contain excerpts from Peddle et al. (2024), Appendix A, and Robinson et al. (2023), Appendix B, on other DNA based microbial profiling techniques which provide important insights into the taxonomic and functional characteristics of microbiota, in addition to amplicon sequencing approaches:

> “Growing opportunities from shotgun metagenomic and/or metatranscriptomic data sets are now available to provide high-quality insights into microbial functions in a restoration context (Breed *et al.*, 2019; Sun *et al.*, 2020). Shotgun metagenomics is similar to amplicon sequencing, but instead of amplifying a targeted gene region, it involves random sequencing of all DNA from within a sample. These random fragments of the metagenome within a sample can be aligned to functional and taxonomic databases and/or assembled into metagenome-assembled genomes (MAGs). These approaches can provide functional gene abundance data directly instead of just taxonomic data or inferred functions from amplicon data. Restoration scientists can then interrogate these functional gene abundance data for functions of interest – such as genes associated with nitrogen fixation or primary productivity – and compare these before and after restoration to assess changes to key ecological functions and processes (Sun & Badgley, 2019). Importantly though, metagenomics involves

> a much higher sequencing cost, and the complexity of data processing and analysis requires expertise that could place a disproportionate burden on restoration projects. Furthermore, both amplicon and shotgun metagenomics do not discern between active and inactive organisms as relic DNA in the sample is also sequenced (Li *et al.*, 2017; Sun & Ge, 2023).
>
> An additional layer of functional information can be obtained by collecting, isolating, and sequencing RNA (as opposed to DNA used in amplicon and metagenomic approaches) from a soil sample with metatranscriptomics. This technique can be a powerful asset in studying soil ecosystem services carried out by microbiota but is not yet widely used in ecological contexts (Breed *et al.*, 2019). Analysing total community RNA transcripts can potentially reveal a microbiome's gene expression under specific conditions, known as the active functional profile. This approach provides an opportunity to study direct alterations of the (meta-)transcriptome in response to different environmental conditions. High functional redundancy is common in soil microbiomes (Louca *et al.*, 2018; Prosser, 2020) and identifying relationships between microbial community structure and function remains challenging because observed community functions are often difficult to link to specific taxonomic groups. Furthermore, RNA-based methods are generally more expensive and time-consuming than DNA-based methods (Cordier *et al.*, 2019), and the unstable nature of RNA molecules presents a technical challenge. Because transcriptional profiles can vary considerably over time, any information gained *via* metatranscriptomics should be interpreted as a 'snapshot' in time. Nonetheless, metatranscriptomics can be a powerful asset in trying to shed light on the dynamics of ecosystem functions carried out by microbiota and warrants consideration as part of a multi-omics approach (Aguiar-Pulido *et al.*, 2016)."
>
> **Peddle et al. (2024), page: 13**

Non-DNA based approaches include tools like characterisation of phospholipid fatty acid analysis. This is detailed in the excerpt from Peddle et al. (2024), Appendix A, below:

> "High-throughput amplicon sequencing is an increasingly common method used to characterise microbial diversity and community composition in a sample. Amplicon-based data can be used to assess differences in microbial communities across restoration treatments, controls, or ages… Since amplicon sequencing is increasingly accessible and affordable, there has been rapid, recent growth in restoration studies using this approach (Mohr *et al.*, 2022). This method presents a detailed picture of microbial diversity and community composition, which is not provided by culture-dependent methods or phospholipid fatty acid analysis (PLFA) approaches. Since phospholipids are only collected from live microbes during sampling, PLFA provides a snapshot of live microbial biomass. As such, PLFA has an advantage over DNA sequence-based approaches where DNA is sampled from both live and dead microbes and living biomass cannot be estimated (Seymour, 2019). However, unlike

> sequence-based approaches, PLFA cannot provide detailed taxonomic insights into microbial diversity or composition. Therefore, it has been recommended that combining PLFA and sequence-based approaches can provide an accurate assessment of both live microbial biomass and community composition (Nkongolo & Narendrula-Kotha, 2020)."
>
> **Peddle et al. (2024), pages: 11–13**

Successful implementation of microbial profiling techniques also depends on accessible bioinformatic workflows which can be used to make ecological meaning out of raw meta-sequencing data. The processing of DNA sequence data can require expertise in a range of different microbiome analysis techniques, and also specialist knowledge of metabolic processes. Robinson et al. (2023), contains a discussion of pipelines, workflows, and techniques that are commonly used by microbial ecologists to generate a variety of important taxonomic, or functional datasets:

> "A range of bioinformatic workflows can be used to generate insights into microbiota in a restoration context. For instance, QIIME2, DADA2, and Phyloseq can be used for amplicon processing, MetaPhlAn3 and HUMAnN4 for metagenomics and metatranscriptomics, MelonnPan for metabolomics, and MetaLab for metaproteomics [Figure 1.4], with varying levels of detail [58]. For instance, shotgun metagenomics provides the opportunity to bioinformatically examine changes in functional genes during the recovery of ecosystem (and microbial) function. Sun and Badgley analyzed soil metagenomes from mine soils spanning 6–31 years since reforestation, and used the MG-RAST pipeline to analyze functional genes [59]. They found that the N-cycling groups, ammonia- and nitrite-oxidizing bacteria, increased significantly with time since restoration. The authors suggest that their work helps to identify possible mechanisms (e.g., biochemical) linking the soil microbiome to ecosystem recovery.
>
> … Metabolomic pipelines [Figure 1.4] can be used to understand microbial metabolic processes and products, thus providing a detailed assessment of active functional roles and interactions between and within organisms. This can enhance monitoring capabilities [61]. For example, organisms of restoration interest can be affected by stressors such as xenobiotic (not naturally produced) pollutants– a key cause of ecosystem degradation [62]. Metabolomics can facilitate a better understanding of the effects of these perturbations on plant and animal communities by providing phenotypic and biological information that is vital for effective monitoring [61]. metabolites can be produced on a timescale of seconds to hours, the application of metabolomics provides an assessment of rapid responses to stress. This can be especially useful in restoration by enabling adaptive monitoring. Finally, metaproteomics can be used to study microbial proteins, thereby providing

insight into the phenotypes of microbes at the molecular level [62]. Both metabolomics and metaproteomics can provide direct assessments of the putative functions ascribed using sequencing approaches. This can aid ecological understanding of the functional response of recovering microbiota at an ecosystem scale, and potentially allow more targeted restoration interventions and detailed monitoring schemes. For instance, researchers have used meta-proteomics to [analyse] the structure and function of microbial communities, particularly in soils, which provides information on their contribution to ecosystem services [63]. Soil proteins can provide information about the biogeochemical potential of soils and pollutant degradation [64]. Microbes and their protein metabolites can also act as bioindicators of soil quality– a potentially important attribute in restoration monitoring schemes."

Robinson et al. (2023), pages: 1198–1199

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Available online at https://doi.org/10.1016/j.tree.2023.07.009

(Robinson et al. 2023)

Figure 1.4. Differences in microbial DNA-based profiling approaches, adapted from Robinson et al. (2023), see Appendix B. These approaches consider both taxonomic and functional microbial profiles, and vary across a gradient of potential to realised functional annotations.

Robinson et al. (2023), page: 1194

These techniques provide an important cornerstone for microbial ecology studies. Their uptake is limited by specialised knowledge of complex laboratory and computational procedures. However, applications of these tools can produce important insights into how microbial communities both change and function across many biological systems.

1.3.3 Applications of whole-soil translocations of microbiota for ecosystem interventions

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This section includes extracts from Peddle et al. (2024) which I identify by boxed formatting and dark blue text. This is a peer-reviewed publications to which I have made substantial contributions as second-named author. Full versions of this text can be found in Appendix A (Peddle et al. 2024). Citations referenced within the extracted sections are found in the original text provided in the Appendices. Please refer to the signed Co-authorship Approvals for Higher Degree by Research Thesis for Examination*, submitted alongside this thesis in accordance with Clauses 5, 7, and 8 of the* HDR Thesis Rules.

There are many diverse applications for plant-soil feedbacks and soil legacies for the management of important plant species or ecosystems. This is especially pertinent where there is need for large scale interventions to aid in conservation or restoration efforts. Peddle et al. (2024) also describes several practical and theoretical considerations for implementing whole-soil translocations for ecosystem restoration (see Appendix A). The excerpt below discusses the potential applications of translocating microbial communities in soils, it describes key research questions, substantial knowledge gaps, and ecological considerations that limit uptake of these approaches:

> "Translocating whole soil communities – whether in the form of intact turfs or homogenised bulk soil – is one way of inoculating soil microbiota into degraded ecosystems to shift the microbial community towards one that is more representative of a target ecosystem. This essentially involves collecting soil from a reference ecosystem and translocating it directly into a restoration site (Koziol *et al.*, 2018; Wubs *et al.*, 2016; Carbajo *et al.*, 2011). Inoculating degraded sites with reference ecosystem soil and associated biota has been shown to improve the growth and establishment of desirable native plants and exclude weeds in both greenhouse and field conditions (Koziol *et al.*, 2018; Wubs *et al.*, 2016; Fahey & Flory, 2022). For example, Wubs *et al.* (2019*a*) showed that soil inoculations can have ecosystem legacy effects that steer successional changes and can last for at least two decades. Importantly, however, Gerrits *et al.* (2023) highlight how the directionality of this legacy effect depends on the suitability or fit of translocated soil to the recipient site, with mismatches steering communities in the wrong direction. Similar interventions can also shift the direction of the development of vegetation communities (Wubs *et al.*, 2016) and improve prospects for native vegetation success (Wubs *et al.*, 2019*b*). However, while research has shown a benefit for the restoration of vegetation, few studies have focussed on the efficacy of soil translocations to shifting whole microbial communities themselves.

Substantial knowledge gaps remain on the effectiveness of soil translocations, including: what methods are most effective (e.g. bulk soil, intact turfs, volumes required), to what extent do soil physical and chemical properties in recipient sites impact establishment, how do priority effects impact on microbial community recovery (i.e. establishment may be dependent on the order of arrival of specific taxa), and, how does the coalescence of distinctly different soil communities impact successful establishment? As such, further research on whole-soil translocations and inoculations should focus on addressing these knowledge gaps *via* embedded experiments to understand better how soil volume, translocation method, and community coalescence dynamics affect microbial community assembly across varied ecosystems and soil types. Addressing these knowledge gaps will then enable the research community to develop decision-support frameworks to help determine when whole-soil translocations will provide restoration benefits that are commensurate with cost.

Another critical open question relating to soil translocation is: how can we minimise the impacts soil translocations have on donor ecosystems? While soil translocations may be effective, soil collection can impact remnant habitats and consideration is needed to limit impacts to remnant sites while providing a benefit to degraded sites. Solutions are needed to scale up soil translocations outside situations where soil can be harvested because existing remnant habitat is already being cleared. As such, decisions on interventions impacting remnant habitat will need to weigh factors such as the contribution of remnant habitat to support the integrity and viability of restoration or conservation efforts (Tulloch *et al.*, 2016; Wintle *et al.*, 2019), or if a degree of destructive harvesting of soil resources from remnant sites can provide restoration benefits that outweigh impacts to remnant habitat. To address the need for reliable seed sourcing in restoration or revegetation, seed-production areas are being established instead of relying on sourcing seeds from remnant habitats (i.e. target plants are grown *ex-situ* '*en masse*' to produce seed stock) (Zinnen *et al.*, 2021). This concept could potentially be applied to soil microbiota with soil microbiota production areas, although various open questions (i.e. how do we cultivate whole target microbial communities, can we subset communities to focus on particular taxa, and what is the 'ideal' composition of these communities) need to be addressed before soil microbiota production areas can be effectively implemented at scale.

Despite these knowledge gaps, whole-soil translocations are increasingly used in large-scale restoration projects where topsoil is salvaged as part of the initial disturbance (e.g. surface strip mining) and then reinstated during restoration (Tibbett, 2010; Schmid *et al.*, 2020; Liddicoat *et al.*, 2022). The objective of topsoil transfer is to preserve the soil-stored seedbank rather than the soil microbiota *per se*. Still, benefits from the reservoir of microbiota contained in these topsoils present an opportunity to improve restoration outcomes. Limiting the amount of time for which soils are stockpiled before translocation is crucial as stockpiling can disrupt biological integrity and impact microbial diversity and composition (Hernandez *et al.*, 2024; Valliere *et al.*, 2022). In best-practice cases, the direct return of harvested topsoil to nearby restoration sites will limit the physical and

> biological degradation of soil from long-term stockpiling (Rokich *et al.*, 2000; Peddle *et al.*, 2022). However, the impact of the collection and homogenisation of vertical soil profiles during the transfer process on soil microbiota is likely detrimental but still poorly understood."
>
> **Peddle et al. (2024), pages: 8-9**

As described above, studies on the effective translocation or inoculation of soil microbial communities within or across ecological communities represent a key consideration for ecosystem interventions. With the growing recognition of the key role soils and their biota have in restoring biodiversity and ecosystem functioning, it is essential for ecologists and environmental practitioners to develop innovative approaches that integrate these functional roles into ecosystem management.

Targeting specific microbiota

Microbial-based soil interventions will inevitably involve complex networks of species interactions, making them challenging to control. Approaches that focus on key microbial species or on restoring ecosystem functions will need to prioritise beneficial microbiota while actively managing against pathogens or other inhibitors to success. Below, Peddle et al. (2024) considers the limitations and future directions for targeting specific beneficial microbiota during interventions (Appendix A):

> "Specific microbial taxa can be lacking in an ecosystem, disproportionally impacting plant fitness (Thrall *et al.*, 2001). For example, obligate symbionts (e.g. rhizobia) often fail to persist in degraded soils since their survival relies on the presence and persistence of their host plant species (Thrall *et al.*, 2001; Berruti *et al.*, 2016) or other microbes within a whole community. For bulk soil inoculations, microbiota specificity is low as this approach relies on a whole-of-community transfer. Therefore, varying degrees of specificity are relied upon for microbial cultures and suspensions [i.e., microbiota in liquid media] when targeted for use as an additive in direct soil applications or *via* priming, coating and extruded pelleting approaches [i.e., inoculating seeds with microbial assortments through liquid application, soil integration, or physical encasement]. Because of this variable specificity, the required level of microbe–host matching is an important factor to consider when developing soil microbiota interventions (e.g. does an inoculum need to land precisely within the root zone of a target plant to succeed?). Furthermore, reliance on expensive and highly technical approaches could be a liability for restoration practice where patents and corporate control of technology could limit affordable uptake and equitable use of tools needed to improve restoration outcomes (Osborne *et al.*, 2021).

> A targeted consortium of microbes (e.g. multiple taxa of cyanobacteria) may be preferable over individual strains (Chua *et al.*, 2019; Dadzie *et al.*, 2022), especially since a diverse community should result in more resilient microbiota (Chua *et al.*, 2019; Rodriguez & Durán, 2020; Berendsen *et al.*, 2018). Culturing diverse microbial consortia can be challenging however, as varying capture and growth rates across taxa are likely (Kaminsky *et al.*, 2019). A further roadblock is selecting the appropriate techniques to capture, extract and transfer the targeted microbiota or strains. This will be particularly challenging for obligate symbionts, which can be particularly hard to isolate and culture (Berruti *et al.*, 2016).
>
> The use of plant hosts has been proposed as a way to culture a targeted microbiota. Trap cultures in soil, for example, involve collecting soil samples containing target microbial communities – such as arbuscular mycorrhizal fungi – from whole soil in a reference ecosystem, which is then propagated with host plants *ex-situ* for later inoculation (Koziol *et al.*, 2018). Techniques like this could be scaled up in soil microbiota production areas which could reduce impacts on remnant ecosystems comparatively to the direct transfer of topsoil. However, these soil-culturing systems require substantial time and technical investments to establish them. Once operational, communities may shift away from their 'wild type' or desired community. Evidence suggests that these communities can change to undesirable states over time, due to the build-up of soil pathogens (Bauer, Mack & Bever, 2015) or reduced diversity within the microbial communities, which could harm host plants (Trejo-Aguilar *et al.*, 2013) undermining the effectiveness of microbial products. Alternatively, harnessing the positive soil legacies of plants and host-mediated microbiome engineering have been proposed as methods of selecting for specific functional outcomes in microbial communities by subjecting plants to specific selective pressures (e.g. inducing drought tolerance in a host-plant's microbiota *via* instigating water stress) (Mueller & Sachs, 2015; Pineda, Kaplan & Bezemer, 2017; Gopal & Gupta, 2016). However, our ability to introduce targeted microbiota or a select microbial strain depends on our capacity to identify specific taxa of interest and extract, propagate, and successfully re-introduce them effectively and in a replicable way."
>
> **Peddle et al. (2024), pages: 10-11**

The different approaches we use to make beneficial microbial interventions across ecosystems, like grasslands, will need to address specific restoration barriers and targets (e.g., species specific or community level goals). As such, they will need to be evaluated in terms of their cost, feasibility, and impact on a case-by-case basis.

Promoting positive soil legacies

Finally, the following section contains excerpts from Peddle et al. (2024), Appendix A, and discusses how positive soil legacies can be used to improve the fitness of species in response to stress, by leveraging beneficial soil legacies, which could enhance the success of species used in revegetation (Koziol et al. 2018, Chua et al. 2019). These positive soil legacies arise when a preparatory generation of plants recruits and nurtures beneficial soil microbiota, thereby conditioning the soil to support the fitness of future plant generations (Gopal and Gupta 2016, Pineda et al. 2017). The following excerpt from Peddle et al. (2024) explores the potential applications of this approach, and considers proof-of-concepts across other ecological systems:

> "The potential for creating positive soil legacies through priming the soil with specific plants has been demonstrated with the wildflower *Senecio jacobaea* (Pineda *et al.*, 2017). When exposed to insect pests, this plant generated a feedback mechanism where sugars and organic acids exuded from its roots maintained a distinct soil fungal community that affected the regulation of amino acids in the host plant's phloem sap, providing the plant with reduced herbivore populations (Kos *et al.*, 2015). Also, Buchenau, van Kleunen & Wilschut (2022) observed some European grasses could see improved growth in the second generation of plants grown in drought-exposed and nutrient-limited soils due to a positive legacy of the soil microbiota. The next step for utilising positive soil legacies better is to improve understanding of the generality of this effect as it is not present for all plant species (Kaisermann *et al.*, 2017).
>
> Understanding microbial-mediated stress responses in plants and how plant–microbial interactions can be applied to improve plant stress tolerance presents promising restoration opportunities (Larson, Venette & Larson, 2022; Petipas, Geber & Lau, 2021; Valliere *et al.*, 2020). The transfer of soil microbiota from non-local soils or across environmental gradients (e.g. temperature, aridity, nutrient) into revegetation sites could instil stress-ameliorating interactions between plants and the relocated microbiota. This could build resilience to developing stress and disturbance expected under climate change or site-specific legacies of previous land use – provided we improve our understanding of patterns of host-plant-specific *versus* general adapted microbial functions (Petipas *et al.*, 2021)."
>
> **Peddle et al. (2024), page: 11**

Positive soil legacies hold strong potential for integration into ecological systems facing environmental stress (Peddle et al. 2024). It remains unclear, however, how effectively they can be utilised during ecological interventions, such as the through soil translocation of microbiota into novel soil conditions. Expanding the broad applicability of these approaches should be a priority for future research, particularly in studies manipulating plants and ecological communities in need of conservation or restoration interventions (Peddle et al. 2024).

Microbiota have key roles in sustaining ecological and geochemical processes within soils. While there are many knowledge gaps regarding how environmental variations influence plant-soil relationships among key species, and how these dynamics are shaped by deterministic versus stochastic processes, there is substantial potential to incorporate microbiota into ecological research and practice. The success of soil interventions is likely to vary across distinct environmental contexts; thus, the pathway to application should thoroughly consider relevant conditions. Initial steps could involve evaluating the natural dependencies or competitive advantages between specific plants and local soil-microbial conditions.

1.4 Study species: *Themeda triandra*

Themeda triandra (Forssk.) is a perennial C4 grass species with a pan-palaeotropical distribution (Figure 1.5a). It is considered a keystone species, forms tussocks, and typically reaches heights of around 1 m. *T. triandra* ecology and physiology has been well studied, but there is little known about its interactions with soil microbial communities (discussed below). Here, I examine the current research on ecological traits of *T. triandra* that contribute to its global distribution, and its role in shaping grassland ecosystems. I discuss known physiological traits and adaptations that enhance its performance across these environments – such as, seed dormancy and germination, disturbance and successional dynamics, polyploidy, adaptations to drought, and soil microbial interactions. As a keystone grass, dominant across grasslands globally, *T. triandra* has important ecological roles (Snyman et al. 2013). As such, there is a need to understand the soil dynamics and microbial ecology of this plant to aid restoration of this species across degraded grasslands (Cole and Lunt 2005, Williams et al. 2015).

1.4.1 Distribution

There are as many as 27 recognized species in the *Themeda* genus, occupying various niches as annual or perennial plants, with diversification beginning approximately 5 million years ago (Alpers et al. 2016). *T. triandra* is thought to have evolved in South Asia around 1.5 million years ago, before spreading to Australia 1.3 million years ago, and Africa 500 thousand years ago, resulting in a pan-palaeotropical distribution (Figure 1.5b) (Dunning et al. 2017).

Figure 1.5. The keystone grass species, *Themeda triandra*, and map of its pan-palaeotropical distribution. (a) Photographs of *T. triandra* plants (left), showing its distinctive seed head (right) (photographs were supplied from the personal collection of Riley Hodgson). (b) Pan-palaeotropical distribution of *T. triandra* based on observations (points) from the Global Biodiversity Information Facility between years 2000-2023 (GBIF.org 2023). Points are likely underrepresented geographically across its distribution due to different practises of obtaining reliable records of occurrence. The colour gradient represents mean annual aridity index from Version 3 of the Global Aridity Index and Potential Evapotranspiration Database (Zomer et al. 2022). Aridity index is a measure of annual precipitation/annual potential evaporation, and low values correspond to more arid conditions (i.e.,

hotter/drier conditions), whereas high values correspond to less arid conditions (i.e., cooler/wetter conditions). (c) The global distributions of *T. triandra* plants exist across a wide-spanning aridity gradient, as indicated by the density of GBIF observations (GBIF.org 2023), mapped to the Global Aridity Index and Potential Evapotranspiration Database (Zomer et al. 2022) and the aridity classifications from the UNEP World Atlas of Desertification (UNEP 1992).

Aridity is a key factor shaping the distribution of *T. triandra* (Figure 1.5b) in addition to the plant communities and edaphic conditions of the ecosystems that this grass inhabits (Zomer et al. 2022, Shi et al. 2024). This species occupies a wide range of aridity zones, including humid and semi-arid regions (Table 1.4; Figure 1.5c). The aridity index – defined as the ratio of precipitation to evapotranspiration – serves as a useful metric for assessing climatic water availability in regions where *T. triandra* thrives (UNEP 1992, Zomer et al. 2022). *T. triandra*’s distribution across arid and semi-arid conditions can be attributed to a range of morphological, biochemical, and physiological adaptations that enable this species to avoid water and nutrient stress (discussed below*)*.

Table 1.4. Aridity classification guide according to different aridity index thresholds adapted from UNEP World Atlas of Desertification (UNEP 1992)

Classification	Aridity Index
Hyper-arid	AI < 0.03
Arid	0.03 ≤ AI < 0.20
Semi-arid	0.20 ≤ AI < 0.50
Dry sub-humid	0.50 ≤ AI < 0.65
Humid	0.65 ≤ AI

1.4.2 Ecology and physiology

The name *triandra* derives from "tri-" and "-andrus" indicating the presence of three stamens. It was formerly known known as *Themeda australis* in some regions, though it is now considered synonymous to *T. triandra*. Growing in a tall dense tuft, *T. triandra* forms tussocks and generally reaches heights of 1 m (Snyman et al. 2013). It is a reproductively flexible species, with pathways including sexual and asexual (specifically, apomixis) reproduction (Ahrens et al. 2020). *T. triandra* has distinct genetic populations, resulting in regional ecotypes across its range (Dunning et al. 2017, Godfree et al. 2017, Ahrens et al. 2020).

Seed dormancy and germination

Seed viability and germination rates for *T. triandra* vary widely across different regions, and numerous seed treatments have been tested on *T. triandra* with mixed results (Durnin et al. 2024). High temperatures, smoke water, and gibberellic acid (among others) can enhance germination rates (Snyman et al. 2013, Durnin et al. 2024), but soil moisture remains the best cue for germination after dormancy is broken (Snyman et al. 2013). In practice, seed smoke treatments are difficult to standardise and apply, unlike treatments with gibberellic acid which are regularly employed in labs (Durnin et al. 2024). Furthermore, the time to overcome dormancy for *T. triandra* is highly variable across different populations (Durnin et al. 2024). Prolonged dormancy in *T. triandra* seeds from some regions may be an adaptation to ensure germination aligns with the start of spring, allowing for extended growth during summer. Furthermore, the optimal dormancy times, temperatures, and seed characteristics for the best germination outcomes across different populations are thought to depend on an unknown combination of local environmental conditions and genetic traits (Saleem et al. 2009, Durnin et al. 2024). Variable *T. triandra* seed quality and low seed fill rates across mature florets presents a challenge for establishing seed production areas, posing a significant limitation for both agricultural and ecological restoration efforts (Durnin et al. 2024).

Fire ecology and succession

T. triandra demonstrates a preference for regular disturbance such as periodic fires, grazing, or mowing (Morgan and Lunt 1999, Snyman et al. 2013, Price et al. 2019), but it is also sensitive to overgrazing or extreme disturbance (McNaughton 1985, Allsopp 1998, Lunt and Morgan 1999, Gonzalez et al. 2018). As such, *T. triandra* is considered a ‘decreaser species’, and can provide a good indication of ecosystem health based on its prevalence across an ecological community (Theunissen 1992).

Fire is a regular disturbance process across many grasslands due to climatic, evolutionary and cultural processes that facilitate the accumulation of flammable biomass and the presence of fire triggers (Morgan 1999). In fire-adapted ecosystems, like *T. triandra* grasslands, burning promotes plant diversity by providing new niches into which seedlings can recruit, making fire an important functional process (Lunt and Morgan 1999, Morgan and Lunt 1999, Price et al. 2019). Furthermore, fire is a key factor that drives *T. triandra* persistence (Snyman et al. 2013, Simpson et al. 2016, Smith et al. 2019). The litter that *T. triandra* plants deposit is highly flammable and, as a hemicryptophytic plant, *T. triandra* has renewal buds located at ground level, from which it can resprout (Snyman et al. 2013). The fire-adapted traits of *T. triandra* grasses, therefore, provide this species an early advantage during recovery after burns, helps them to dominate over other species during secondary successional processes.

The seeds of mature *T. triandra* plants have long awns that twist deeper into the soil as they wet and dry, and as such, are usually not disturbed by mild burns (Snyman et al. 2013, Durnin et al. 2024). However, *T. triandra* seeds generally have a short lifespan within the seedbank – persisting for approximately 1-2 years (Snyman et al. 2013, Durnin et al. 2024). For *T. triandra* to dominate grasslands, they rely on the persistence of established stands with regular seed production. Ultimately, if lost (e.g., due to clearing or severe degradation), *T. triandra* populations are unlikely to naturally return from the soil after extended time periods (Snyman et al. 2013).

Adaptations to water stress

T. triandra plants have several adaptations that improve their performance in arid conditions or under water stress. *T. triandra* uses the C4 pathway for photosynthesis, and as such it is a summer-growing species and has traits that reduce its risk of dehydration in warmer climates (Ehleringer et al. 1997). The C4 process works by fixing CO_2 into a four-carbon compound in mesophyll cells, which is then transported to bundle sheath cells to concentrate CO_2. This reduces photorespiration and makes C4 plants more efficient in warm climates. As such, C4 grasses tend to segregate from typical grasses that use the C3 pathway (winter growing grasses), along temperature gradients (Griffith et al. 2015). During hot, drier months, the C_4 photosynthetic pathways also allows plants, including *T. triandra*, to rapidly accumulate biomass between fire events, which feed into secondary successional dynamics discussed above, contributing to their dominance across grassland ecosystems (Snyman et al. 2013, Griffith et al. 2015).

In Table 1.5, I outline additional anatomical and physiological traits of *T. triandra* plants that improve its tolerance to water stress conditions (including further descriptions of those mentioned above). These processes reflect the different competitive advantages sustained by *T. triandra* plants, contributing to its success across dry lands and under high aridity conditions.

Soil microbial interactions

T. triandra is known to strongly associate with its soil microbiota, which can aid its growth and fitness (Hassen and Labuschagne 2010, Petipas et al. 2017). Previous studies have shown how arbuscular mycorrhizal fungi improve *T. triandra* growth and tolerance to low water conditions and simulated herbivory (Petipas et al. 2017, Gonzalez et al. 2018, Petipas et al. 2021). Although *T. triandra* requires aboveground disturbance to maintain healthy populations, it is sensitive to overgrazing and studies have shown that when grazed it loses root colonisation by arbuscular mycorrhizal fungi (Allsopp 1998).

Table 1.5. Adaptive physiological and anatomical traits that improve *T. triandra* fitness under low water conditions (i.e., drought)

Adaptive Trait	**Description**	**Benefit**	**Reference/further reading**
Narrow leaves	• Can curl up under stress	• Reduce exposure of leaf surfaces that can undergo water loss	• (Snyman et al. 1997)
Stomatal density and leaf hairs	• High density of stomata on underside of leaves and leaf hairs • Increase the plants boundary layer (thin layer of still air surrounding leaf)	• Improves the water holding capacity in the leaf surfaces • Reduces evaporation rate	• (Snyman et al. 1997)
Thick, long taproot	• Long roots that penetrate deep into the soil profile, and have robust thick structures	• Access water deeper in the soil during low water conditions	• (Snyman et al. 1997)
Plant dormancy	• At low leaf water potential, *T. triandra* can slow transpiration processes • Can also close stomata	• Reduces water loss due to transpiration during dry weather conditions • Allows for swift recovery after drought	• (Snyman et al. 1997)
C4 photosynthesis	• C4 plants first fix CO_2 into a four-carbon compound transported into bundle sheath cells	• More efficient photorespiration during low water conditions • Better in hotter, dry conditions, compared to C3 photosynthetic pathway	• (Snyman et al. 1997) • (Ehleringer et al. 1997) • (Griffith et al. 2015)
Polyploidy	• Multiple sets of chromosomes in *T. triandra* genomes • Varies across populations	• Increases genetic diversity, offering greater potential for adaptation to environmental stresses (i.e., drought)	• (Ahrens et al. 2020) • (Hayman 1960) • (Godfree et al. 2017) • (Snyman et al. 1997)

Little attention has been paid to the bacterial communities present in the roots of *T. triandra*, and most research has focussed on arbuscular mycorrhizal fungi. Although *T. triandra*-associated bacteria have been identified in prior studies, it is unclear what benefits or functional roles they provide in their native communities (Idris et al. 2009, Hassen and Labuschagne 2010). *T. triandra*-associated soil bacterial communities can be susceptible to climate change impacts. Studies exposing these soil communities to experimental warming, elevated CO2 levels, and under desertification processes have demonstrated that their bacterial and fungal communities are prone to compositional changes (Hayden et al. 2012, Tang et al. 2021). The microbial communities in *T. triandra* roots and soils are also thought to be influenced by belowground traits associated with C4 grasses, such as root tissue density and specific root area (Egidi et al. 2024), but species-specific details are lacking. Further understanding the composition of the microbial communities that directly interact with its root structures could be useful for identifying conditions that support plant fitness across diverse climatic and soil conditions (Hayden et al. 2012, Snyman et al. 2013, Gonzalez et al. 2018, Tang et al. 2021).

1.4.3 Cultural significance

Traditional human uses of *T. triandra* vary as greatly as the Peoples and societies with whom it shares a cultural history. Australian Aboriginal Peoples, for instance, use *T. triandra* for making ropes for nets, for food, and often utilised the *T. triandra* grasslands during hunting (Pascoe 2018). *T. triandra* grasslands were also maintained by different Australian Aboriginal Peoples using fire in strategic burns which created opportunities for hunting and cleared land (Pascoe 2018). *T. triandra* is a valuable fodder crop for livestock, such as cattle and sheep, and has been harvested for grain (Male et al. 2022). *T. triandra* has also been used by First Nations Peoples of Africa and Asia for these purposes (Snyman et al. 2013), and there is an interest in the potential of domesticating this grass as a traditional owner-led initiative. Through the adoption of the *United Nations Declaration on the Rights of Indigenous Peoples* by countries including Australia, traditional uses of plants like *T. triandra* are recognised and protected under intellectual rights to self-determination (UN General

Assembly 2007, Male et al. 2022). Ultimately, agriculturalisation of plants like *T. triandra* could lead to ecological and cultural benefits through diversified and drought resistant agriculture, with permanent perennial cropping.

References

Abdelfattah, A., A. Malacrinò, M. Wisniewski, S. O. Cacciola, and L. Schena. 2018. Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. Biological Control **120**:1-10.

ACT-Government. 1997. Natural temperate grassland: an endangered ecological community. Action Plan. 1. ACT Government, Canberra.

Adeleke, B. S., O. O. Babalola, and B. R. Glick. 2021. Plant growth-promoting root-colonizing bacterial endophytes. Rhizosphere **20**:100433.

Agrawal, A. A., P. M. Kotanen, C. E. Mitchell, A. G. Power, W. Godsoe, and J. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology **86**:2979-2989.

Ahrens, C. W., E. A. James, A. D. Miller, F. Scott, N. C. Aitken, A. W. Jones, P. Lu-Irving, J. O. Borevitz, D. J. Cantrill, and P. D. Rymer. 2020. Spatial, climate and ploidy factors drive genomic diversity and resilience in the widespread grass *Themeda triandra*. Molecular Ecology **29**:3872-3888.

Allsopp, N. 1998. Effect of defoliation on the arbuscular mycorrhizas of three perennial pasture and rangeland grasses. Plant and Soil **202**:117-124.

Alpers, D. L., F. M. Walker, A. C. Taylor, P. Sunnucks, S. Bellman, B. D. Hansen, and W. B. Sherwin. 2016. Evidence of Subdivisions on Evolutionary Timescales in a Large, Declining Marsupial Distributed across a Phylogeographic Barrier. Plos One **11**:22.

Anthony, M. A., S. F. Bender, and M. G. A. van der Heijden. 2023. Enumerating soil biodiversity. Proceedings of the National Academy of Sciences **120**:e2304663120.

Bai, Z., A. Jia, Z. Bai, S. Qu, M. Zhang, L. Kong, R. Sun, and M. Wang. 2022. Photovoltaic panels have altered grassland plant biodiversity and soil microbial diversity. Frontiers in Microbiology **13**.

Barajas, H. R., S. Martínez-Sánchez, M. F. Romero, C. H. Álvarez, L. Servín-González, M. Peimbert, R. Cruz-Ortega, F. García-Oliva, and L. D. Alcaraz. 2020. Testing the Two-Step Model of Plant Root Microbiome Acquisition Under Multiple Plant Species and Soil Sources. Frontiers in Microbiology **11**.

Bardgett, R. D., J. M. Bullock, S. Lavorel, P. Manning, U. Schaffner, N. Ostle, M. Chomel, G. Durigan, E. L. Fry, D. Johnson, J. M. Lavallee, G. Le Provost, S. Luo, K. Png, M. Sankaran, X. Hou, H. Zhou, L. Ma, W. Ren, X. Li, Y. Ding, Y. Li, and H. Shi. 2021. Combatting global grassland degradation. Nature Reviews Earth & Environment **2**:720-735.

Beals, K. K., J. A. Moore, S. N. Kivlin, S. L. J. Bayliss, C. Y. Lumibao, L. C. Moorhead, M. Patel, J. L. Summers, I. M. Ware, J. K. Bailey, and J. A. Schweitzer. 2020. Predicting Plant-Soil Feedback in the Field: Meta-Analysis Reveals That Competition and Environmental Stress Differentially Influence PSF. Frontiers in Ecology and Evolution **8**.

Bell, L. W., R. C. Hayes, K. G. Pembleton, and C. M. Waters. 2014. Opportunities and challenges in Australian grasslands: pathways to achieve future sustainability and productivity imperatives. Crop and Pasture Science **65**:489-507.

Bengtsson, J., J. M. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. J. O'Farrell, H. G. Smith, and R. Lindborg. 2019. Grasslands—more important for ecosystem services than you might think. Ecosphere **10**:e02582.

Berendsen, R. L., G. Vismans, K. Yu, Y. Song, R. de Jonge, W. P. Burgman, M. Burmølle, J. Herschend, P. A. H. M. Bakker, and C. M. J. Pieterse. 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. The ISME Journal **12**:1496-1507.

Bever, J., I. Dickie, E. Facelli, J. Facelli, J. Klironomos, M. Moora, M. Rillig, W. Stock, M. Tibbett, and M. Zobel. 2010. Rooting Theories of Plant Community Ecology in Microbial Interactions. Trends in Ecology & Evolution **25**:468-478.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561-573.

Biggs, C. R., L. A. Yeager, D. G. Bolser, C. Bonsell, A. M. Dichiera, Z. Hou, S. R. Keyser, A. J. Khursigara, K. Lu, A. F. Muth, B. Negrete Jr., and B. E. Erisman. 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. Ecosphere **11**:e03184.

Bird, R. B., N. Tayor, B. F. Codding, and D. W. Bird. 2013. Niche construction and Dreaming logic: aboriginal patch mosaic burning and varanid lizards (*Varanus gouldii*) in Australia. Proceedings of the Royal Society B: Biological Sciences **280**:20132297.

Bliege Bird, R., D. W. Bird, L. E. Fernandez, N. Taylor, W. Taylor, and D. Nimmo. 2018. Aboriginal burning promotes fine-scale pyrodiversity and native predators in Australia's Western Desert. Biological Conservation **219**:110-118.

Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prarie restoration. Ecological Applications **13**:605-615.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S.

Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology **37**:852-857.

Borin, M., M. Vianello, F. Morari, and G. Zanin. 2005. Effectiveness of buffer strips in removing pollutants in runoff from a cultivated field in North-East Italy. Agriculture, Ecosystems & Environment **105**:101-114.

Breed, M. F., P. A. Harrison, C. Blyth, M. Byrne, V. Gaget, N. J. C. Gellie, S. V. C. Groom, R. Hodgson, J. G. Mills, T. A. A. Prowse, D. A. Steane, and J. J. Mohr. 2019. The potential of genomics for restoring ecosystems and biodiversity. Nature Reviews Genetics **20**:615-628.

Brinkman, E. P., C. E. Raaijmakers, W. de Boer, and W. H. van der Putten. 2017. Changing soil legacies to direct restoration of plant communities. AoB PLANTS **9**.

Buisson, E., S. Archibald, A. Fidelis, and K. N. Suding. 2022. Ancient grasslands guide ambitious goals in grassland restoration. Science **377**:594-598.

Buisson, E., S. Le Stradic, F. A. O. Silveira, G. Durigan, G. E. Overbeck, A. Fidelis, G. W. Fernandes, W. J. Bond, J.-M. Hermann, G. Mahy, S. T. Alvarado, N. P. Zaloumis, and J. W. Veldman. 2019. Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. Biological Reviews **94**:590-609.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature **488**:91-95.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. v. Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology **64**:807-838.

Burrascano, S., M. Chytrý, T. Kuemmerle, E. Giarrizzo, S. Luyssaert, F. M. Sabatini, and C. Blasi. 2016. Current European policies are unlikely to jointly foster carbon sequestration and protect biodiversity. Biological Conservation **201**:370-376.

Butler, F. E. B., M. K. Good, J. W. Morgan, and N. L. Schultz. 2023. Relative contribution of photodegradation to litter breakdown in Australian grasslands. Ecology and Evolution **13**:e10710.

Callaway, R. M., and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment **2**:436-443.

Cheng, C., Z. Liu, W. Song, X. Chen, Z. Zhang, B. Li, M. van Kleunen, and J. Wu. 2024. Biodiversity increases resistance of grasslands against plant invasions under multiple environmental changes. Nature Communications **15**:4506.

Choi, K., R. Khan, and S. W. Lee. 2021. Dissection of plant microbiota and plant-microbiome interactions. Journal of Microbiology **59**:281-291.

Chu, Y., A. K. S. Wee, R. S. Lapuz, and K. W. Tomlinson. 2021. Phylogeography of two widespread C4 grass species suggest that tableland and valley grassy biome in southwestern China pre-date human modification. Global Ecology and Conservation **31**:e01835.

Chua, M., T. E. Erickson, D. J. Merritt, A. M. Chilton, M. K. J. Ooi, and M. Muñoz-Rojas. 2019. Bio-priming seeds with cyanobacteria: effects on native plant growth and soil properties. Restoration Ecology **28**:168-167.

Cline, L. C., and D. R. Zak. 2015. Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession. Ecology **96**:3374-3385.

Cole, B. I., and I. D. Lunt. 2005. Restoring Kangaroo Grass (*Themeda triandra*) to grassland and woodland understoreys: a review of establishment requirements and restoration exercises in south-east Australia. Ecological Management & Restoration **6**:28-33.

Compant, S., M. C. Cambon, C. Vacher, B. Mitter, A. Samad, and A. Sessitsch. 2021. The plant endosphere world – bacterial life within plants. Environmental Microbiology **23**:1812-1829.

Davies, T., C. Cando-Dumancela, C. Liddicoat, R. Dresken, R. H. Damen, R. A. Edwards, S. A. Ramesh, and M. F. Breed. 2024. Ecological phage therapy: Can bacteriophages help rapidly restore the soil microbiome? Ecology and Evolution **14**:e70185.

Debray, R., R. A. Herbert, A. L. Jaffe, A. Crits-Christoph, M. E. Power, and B. Koskella. 2022. Priority effects in microbiome assembly. Nature Reviews Microbiology **20**:109-121.

Deng, L., Z.-p. Shangguan, and S. Sweeney. 2014. "Grain for Green" driven land use change and carbon sequestration on the Loess Plateau, China. Scientific Reports **4**:7039.

Diaz-Colunga, J., N. Lu, A. Sanchez-Gorostiaga, C.-Y. Chang, H. S. Cai, J. E. Goldford, M. Tikhonov, and Á. Sánchez. 2022. Top-down and bottom-up cohesiveness in microbial community coalescence. Proceedings of the National Academy of Sciences **119**:e2111261119.

Dixon, A. P., D. Faber-Langendoen, C. Josse, J. Morrison, and C. J. Loucks. 2014. Distribution mapping of world grassland types. Journal of Biogeography **41**:2003-2019.

Donnison, I. S., and M. D. Fraser. 2016. Diversification and use of bioenergy to maintain future grasslands. Food and Energy Security **5**:67-75.

Dunning, L. T., A.-L. Liabot, J. K. Olofsson, E. K. Smith, M. S. Vorontsova, G. Besnard, K. J. Simpson, M. R. Lundgren, E. Addicott, R. V. Gallagher, Y. Chu, R. T. Pennington, P.-A. Christin, and C. E. R. Lehmann. 2017. The recent and rapid spread of *Themeda triandra*. Botany Letters **164**:327-337.

Durnin, M., E. Dalziell, S. M. Prober, and P. Marschner. 2024. Variable seed quality hampers the use of *Themeda triandra* (Poaceae) for seed production, agriculture, research and restoration: a review. Australian Journal of Botany **72**:-.

Egidi, E., D. Bristol, K. Hassan, D. Tissue, I. J. Wright, and U. N. Nielsen. 2024. The influence of plant traits on soil microbial communities varies between arid and mesic grasslands. Plant and Soil.

Ehleringer, J. R., T. E. Cerling, and B. R. Helliker. 1997. C 4 photosynthesis, atmospheric CO 2, and climate. Oecologia **112**:285-299.

Eskelinen, A., W. S. Harpole, M.-T. Jessen, R. Virtanen, and Y. Hautier. 2022. Light competition drives herbivore and nutrient effects on plant diversity. Nature **611**:301-305.

Facelli, J. M., and E. Facelli. 2022. Effects of slashing on native and invasive species, and community structure of a remnant natural grassland of South Australia. Applied Vegetation Science **25**:e12704.

Fanin, N., D. Lin, G. T. Freschet, A. D. Keiser, L. Augusto, D. A. Wardle, and G. F. Veen. 2021. Home-field advantage of litter decomposition: from the phyllosphere to the soil. New Phytologist **231**:1353-1358.

Farley, G. J., S. M. Bellairs, and S. W. Adkins. 2013. Germination of selected Australian native grass species, with potential for minesite rehabilitation. Australian Journal of Botany **61**:283-290.

Fekete, R., O. Valkó, L. K. Fischer, B. Deák, and V. H. Klaus. 2024. Ecological restoration and biodiversity-friendly management of urban grasslands – A global review on the current state of knowledge. Journal of Environmental Management **368**:122220.

Fletcher, M.-S., R. Hamilton, W. Dressler, and L. Palmer. 2021. Indigenous knowledge and the shackles of wilderness. Proceedings of the National Academy of Sciences **118**:e2022218118.

Gann, G. D., T. McDonald, B. Walder, J. Aronson, C. R. Nelson, J. Jonson, J. G. Hallett, C. Eisenberg, M. R. Guariguata, J. Liu, F. Hua, C. Echeverría, E. Gonzales, N. Shaw, K. Decleer, and K. W. Dixon. 2019. International principles and standards for the practice of ecological restoration. Second edition. Restoration Ecology **27**:S1-S46.

Gao, C., P. M. van Bodegom, T. M. Bezemer, M. P. Veldhuis, R. Mancinelli, and N. A. Soudzilovskaia. 2022. Soil Biota Adversely Affect the Resistance and Recovery of Plant Communities Subjected to Drought. Ecosystems.

GBIF.org. 2023. Occurrence Download. The Global Biodiversity Information Facility.

Gebashe, F., A. O. Aremu, J. F. Finnie, and J. Van Staden. 2019. Grasses in South African traditional medicine: A review of their biological activities and phytochemical content. South African Journal of Botany **122**:301-329.

Godfree, R. C., D. J. Marshall, A. G. Young, C. H. Miller, and S. Mathews. 2017. Empirical evidence of fixed and homeostatic patterns of polyploid advantage in a keystone grass exposed to drought and heat stress. Royal Society Open Science **4**:170934.

Goldstein, A., W. R. Turner, S. A. Spawn, K. J. Anderson-Teixeira, S. Cook-Patton, J. Fargione, H. K. Gibbs, B. Griscom, J. H. Hewson, J. F. Howard, J. C. Ledezma, S. Page, L. P. Koh, J. Rockström, J. Sanderman, and D. G. Hole. 2020. Protecting irrecoverable carbon in Earth's ecosystems. Nature Climate Change **10**:287-295.

Gonzalez, J. B., R. H. Petipas, O. Franken, E. T. Kiers, K. E. Veblen, and A. K. Brody. 2018. Herbivore removal reduces influence of arbuscular mycorrhizal fungi on plant growth and tolerance in an East African savanna. Oecologia **187**:123-133.

González, J. B., R. H. Petipas, O. Franken, E. T. Kiers, K. E. Veblen, and A. K. Brody. 2018. Herbivore removal reduces influence of arbuscular mycorrhizal fungi on plant growth and tolerance in an East African savanna. Oecologia **187**:123-133.

Gopal, M., and A. Gupta. 2016. Microbiome selection could spur next-generation plant breeding strategies. Frontiers in Microbiology **7**.

Grady, K. L., J. W. Sorensen, N. Stopnisek, J. Guittar, and A. Shade. 2019. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. Nature Communications **10**:4135.

Graham, E. B., and J. E. Knelman. 2023. Implications of Soil Microbial Community Assembly for Ecosystem Restoration: Patterns, Process, and Potential. Microbial Ecology **85**:809-819.

Griffith, D. M., T. M. Anderson, C. P. Osborne, C. A. E. Strömberg, E. J. Forrestel, and C. J. Still. 2015. Biogeographically distinct controls on C3 and C4 grass distributions: merging community and physiological ecology. Global Ecology and Biogeography **24**:304-313.

Guo, J., H. Ning, Y. Li, Q. Xu, Q. Shen, N. Ling, and S. Guo. 2024. Assemblages of rhizospheric and root endospheric mycobiota and their ecological associations with functional traits of rice. mBio **15**:e02733-02723.

Habel, J. C., J. Dengler, M. Janišová, P. Török, C. Wellstein, and M. Wiezik. 2013. European grassland ecosystems: threatened hotspots of biodiversity. Biodiversity and Conservation **22**:2131-2138.

Hamilton, R., D. Penny, and T. L. Hall. 2020. Forest, fire & monsoon: investigating the long-term threshold dynamics of south-east Asia's seasonally dry tropical forests. Quaternary Science Reviews **238**:106334.

Hardoim, P. R., L. S. v. Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring, and A. Sessitsch. 2015. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. Microbiology and Molecular Biology Reviews **79**:293-320.

Harris, J. 2009. Soil microbial communities and restoration ecology: Facilitators or followers? Science **325**:573-574.

Hassen, A. I., and N. Labuschagne. 2010. Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. World Journal of Microbiology & Biotechnology **26**:1837-1846.

Hautier, Y., F. Isbell, E. T. Borer, E. W. Seabloom, W. S. Harpole, E. M. Lind, A. S. MacDougall, C. J. Stevens, P. B. Adler, J. Alberti, J. D. Bakker, L. A. Brudvig, Y. M. Buckley, M. Cadotte, M. C. Caldeira, E. J. Chaneton, C. Chu, P. Daleo, C. R. Dickman, J. M. Dwyer, A. Eskelinen, P. A. Fay, J. Firn, N. Hagenah, H. Hillebrand, O. Iribarne, K. P. Kirkman, J. M. H. Knops, K. J. La Pierre, R. L. McCulley, J. W. Morgan, M. Pärtel, J. Pascual, J. N. Price, S. M. Prober, A. C. Risch, M. Sankaran, M. Schuetz, R. J. Standish, R. Virtanen, G. M. Wardle, L. Yahdjian, and A. Hector. 2018. Local loss and spatial homogenization of plant diversity reduce ecosystem multifunctionality. Nature Ecology & Evolution **2**:50-56.

Hayden, H. L., P. M. Mele, D. S. Bougoure, C. Y. Allan, S. Norng, Y. M. Piceno, E. L. Brodie, T. Z. DeSantis, G. L. Andersen, A. L. Williams, and M. J. Hovenden. 2012. Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO2 and warming in an Australian native grassland soil. Environmental Microbiology **14**:3081-3096.

Hayman, D. 1960. The distribution and cytology of the chromosome races of *Themeda australis* in southern Australia. Australian Journal of Botany **8**:58-68.

He, X., D. Wang, Y. Jiang, M. Li, M. Delgado-Baquerizo, C. McLaughlin, C. Marcon, L. Guo, M. Baer, Y. A. T. Moya, N. von Wirén, M. Deichmann, G. Schaaf, H.-P. Piepho, Z. Yang, J. Yang, B. Yim, K. Smalla, S. Goormachtig, F. T. de Vries, H. Hüging, M. Baer, R. J. H. Sawers, J. C. Reif,

F. Hochholdinger, X. Chen, and P. Yu. 2024. Heritable microbiome variation is correlated with source environment in locally adapted maize varieties. Nature Plants.

Hederström, V., J. Ekroos, M. Friberg, T. Krausl, Ø. H. Opedal, A. S. Persson, H. Petrén, Y. Quan, H. G. Smith, and Y. Clough. 2024. Pollinator-mediated effects of landscape-scale land use on grassland plant community composition and ecosystem functioning – seven hypotheses. Biological Reviews **99**:675-698.

Hermann, J.-M., M. Lang, J. Gonçalves, and H. Hasenack. 2016. Forest–grassland biodiversity hotspot under siege: land conversion counteracts nature conservation. Ecosystem Health and Sustainability **2**:e01224.

Herzberger, A. J., S. J. Meiners, J. B. Towey, P. A. Butts, and D. L. Armstrong. 2015. Plant-microbe interactions change along a tallgrass prairie restoration chronosequence. Restoration Ecology **23**:220-227.

Higgs, E. 2017. Novel and designed ecosystems. Restoration Ecology **25**:8-13.

Hobbs, R. J., S. Arico, J. Aronson, J. S. Baron, P. Bridgewater, V. A. Cramer, P. R. Epstein, J. J. Ewel, C. A. Klink, A. E. Lugo, D. Norton, D. Ojima, D. M. Richardson, E. W. Sanderson, F. Valladares, M. Vilà, R. Zamora, and M. Zobel. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography **15**:1-7.

Hobbs, R. J., E. Higgs, C. M. Hall, P. Bridgewater, F. S. Chapin, E. C. Ellis, J. J. Ewel, L. M. Hallett, J. Harris, K. B. Hulvey, S. T. Jackson, P. L. Kennedy, C. Kueffer, L. Lach, T. C. Lantz, A. E. Lugo, J. Mascaro, S. D. Murphy, C. R. Nelson, M. P. Perring, D. M. Richardson, T. R. Seastedt, R. J. Standish, B. M. Starzomski, K. N. Suding, P. M. Tognetti, L. Yakob, and L. Yung. 2014. Managing the whole landscape: historical, hybrid, and novel ecosystems. Frontiers in Ecology and the Environment **12**:557-564.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution **24**:599-605.

Hodapp, D., E. T. Borer, W. S. Harpole, E. M. Lind, E. W. Seabloom, P. B. Adler, J. Alberti, C. A. Arnillas, J. D. Bakker, L. Biederman, M. Cadotte, E. E. Cleland, S. Collins, P. A. Fay, J. Firn, N.

Hagenah, Y. Hautier, O. Iribarne, J. M. H. Knops, R. L. McCulley, A. MacDougall, J. L. Moore, J. W. Morgan, B. Mortensen, K. J. La Pierre, A. C. Risch, M. Schütz, P. Peri, C. J. Stevens, J. Wright, and H. Hillebrand. 2018. Spatial heterogeneity in species composition constrains plant community responses to herbivory and fertilisation. Ecology Letters **21**:1364-1371.

Hönigová, I., D. Vačkář, E. K. Lorencová, J. K. Melichar, M. Götzl, G. Sonderegger, V. Oušková, M. Hošek, K. Chobot, and J. Boháč. 2012. Survey on grassland ecosystem services Report of the European Topic Centre on Biological Diversity.

Hoover, D. L., A. K. Knapp, and M. D. Smith. 2014. Resistance and resilience of a grassland ecosystem to climate extremes. Ecology **95**:2646-2656.

Humphries, T., C. Turville, S. Sinclair, and S. Florentine. 2022. An integrated approach for the restoration of Australian temperate grasslands invaded by Nassella trichotoma. Scientific Reports **12**:21364.

Idris, A., N. Labuschagne, and L. Korsten. 2009. Efficacy of rhizobacteria for growth promotion in sorghum under greenhouse conditions and selected modes of action studies. The Journal of Agricultural Science **147**:17-30.

Isbell, F., D. Craven, J. Connolly, M. Loreau, B. Schmid, C. Beierkuhnlein, T. M. Bezemer, C. Bonin, H. Bruelheide, E. de Luca, A. Ebeling, J. N. Griffin, Q. F. Guo, Y. Hautier, A. Hector, A. Jentsch, J. Kreyling, V. Lanta, P. Manning, S. T. Meyer, A. S. Mori, S. Naeem, P. A. Niklaus, H. W. Polley, P. B. Reich, C. Roscher, E. W. Seabloom, M. D. Smith, M. P. Thakur, D. Tilman, B. F. Tracy, W. H. van der Putten, J. van Ruijven, A. Weigelt, W. W. Weisser, B. Wilsey, and N. Eisenhauer. 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature **526**:574-U263.

Jia, X., F. Dini-Andreote, and J. Falcão Salles. 2018. Community Assembly Processes of the Microbial Rare Biosphere. Trends in Microbiology **26**:738-747.

Jiang, F., J. A. Bennett, K. M. Crawford, J. Heinze, X. C. Pu, A. Luo, and Z. H. Wang. 2024. Global patterns and drivers of plant-soil microbe interactions. Ecology Letters **27**:12.

Jousset, A., C. Bienhold, A. Chatzinotas, L. Gallien, A. Gobet, V. Kurm, K. Küsel, M. C. Rillig, D. W. Rivett, J. F. Salles, M. G. A. van der Heijden, N. H. Youssef, X. Zhang, Z. Wei, and W. H. G. Hol. 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. The ISME Journal **11**:853-862.

Joyce, C. B., M. Simpson, and M. Casanova. 2016. Future wet grasslands: ecological implications of climate change. Ecosystem Health and Sustainability **2**:e01240.

Kardol, P., T. M. Bezemer, and W. H. van der Putten. 2006. Temporal variation in plant-soil feedback controls succession. Ecology Letters **9**:1080-1088.

Kardol, P., G. B. De Deyn, E. Laliberte, P. Mariotte, and C. V. Hawkes. 2013. Biotic plant-soil feedbacks across temporal scales. Journal of Ecology **101**:309-315.

Kelly, C. L., C. M. Pickering, and R. C. Buckley. 2003. Impacts of tourism on threatened plant taxa and communities in Australia. Ecological Management & Restoration **4**:37-44.

Ketzer, D., C. Rösch, and M. Haase. 2017. Assessment of sustainable Grassland biomass potentials for energy supply in Northwest Europe. Biomass and Bioenergy **100**:39-51.

Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, C. R. Fellbaum, G. A. Kowalchuk, M. M. Hart, A. Bago, T. M. Palmer, S. A. West, P. Vandenkoornhuyse, J. Jansa, and H. Bücking. 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science **333**:880-882.

Kiers, E. T., S. A. West, and R. F. Denison. 2002. Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. Journal of Applied Ecology **39**:745-754.

Koziol, L., P. A. Schultz, G. L. House, J. T. Bauer, E. L. Middleton, and J. D. Bever. 2018. The plant microbiome and native plant restoration: The example of native Mycorrhizal fungi. Bioscience **68**:996-1006.

Kretz, L., K. Koll, C. Seele-Dilbat, F. van der Plas, A. Weigelt, and C. Wirth. 2021. Plant structural diversity alters sediment retention on and underneath herbaceous vegetation in a flume experiment. Plos One **16**:e0248320.

Kuťáková, E., L. Mészárošová, P. Baldrian, Z. Münzbergová, and T. Herben. 2023. Plant–soil feedbacks in a diverse grassland: Soil remembers, but not too much. Journal of Ecology **00**:1-15.

Lemoine, R. T., and J.-C. Svenning. 2022. Nativeness is not binary—a graduated terminology for native and non-native species in the Anthropocene. Restoration Ecology **30**:e13636.

Lewis, T., N. Reid, P. J. Clarke, and R. D. B. Whalley. 2010. Resilience of a high-conservation-value, semi-arid grassland on fertile clay soils to burning, mowing and ploughing. Austral Ecology **35**:464-481.

Li, G., C. Fang, Y. Li, Z. Wang, S. Sun, S. He, W. Qi, C. Bao, H. Ma, Y. Fan, Y. Feng, and X. Liu. 2022. Global impacts of future urban expansion on terrestrial vertebrate diversity. Nature Communications **13**:1628.

Liddle, M. J. 1991. Recreation ecology: Effects of trampling on plants and corals. Trends in Ecology & Evolution **6**:13-17.

Lin, Q., Y. Wang, M. Li, Z. Xu, and L. Li. 2022. Ecological niche selection shapes the assembly and diversity of microbial communities in Casuarina equisetifolia L. Frontiers in Plant Science **13**.

Linder, H. P., C. E. R. Lehmann, S. Archibald, C. P. Osborne, and D. M. Richardson. 2018. Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. Biological Reviews **93**:1125-1144.

Ling, N., T. Wang, and Y. Kuzyakov. 2022. Rhizosphere bacteriome structure and functions. Nature Communications **13**:836.

Liu, X., and J. F. Salles. 2024. Drivers and consequences of microbial community coalescence. The ISME Journal **18**.

Louca, S., M. F. Polz, F. Mazel, M. B. N. Albright, J. A. Huber, M. I. O'Connor, M. Ackermann, A. S. Hahn, D. S. Srivastava, S. A. Crowe, M. Doebeli, and L. W. Parfrey. 2018. Function and functional redundancy in microbial systems. Nature Ecology & Evolution **2**:936-943.

Lunt, I. D., and J. W. Morgan. 1999. Vegetation Changes after 10 Years of Grazing Exclusion and Intermittent Burning in a *Themeda triandra* (Poaceae) Grassland Reserve in South-eastern Australia. Australian Journal of Botany **47**:537-552.

Lyons, K. G., P. Török, J.-M. Hermann, K. Kiehl, A. Kirmer, J. Kollmann, G. E. Overbeck, S. Tischew, E. B. Allen, J. D. Bakker, C. Brigham, E. Buisson, K. Crawford, P. Dunwiddie, J. Firn, D. Grobert, K. Hickman, S. L. E. Stradic, and V. M. Temperton. 2023. Challenges and opportunities for grassland restoration: A global perspective of best practices in the era of climate change. Global Ecology and Conservation **46**:e02612.

Lyu, D., J. Zajonc, A. Pagé, C. A. S. Tanney, A. Shah, N. Monjezi, L. A. Msimbira, M. Antar, M. Nazari, R. Backer, and D. L. Smith. 2021. Plant Holobiont Theory: The Phytomicrobiome Plays a Central Role in Evolution and Success. Microorganisms **9**:675.

Ma, H., L. Mo, T. W. Crowther, D. S. Maynard, J. van den Hoogen, B. D. Stocker, C. Terrer, and C. M. Zohner. 2021. The global distribution and environmental drivers of aboveground versus belowground plant biomass. Nature Ecology & Evolution **5**:1110-1122.

Male, D., J. Hunt, C. Celestina, J. Morgan, and D. Gupta. 2022. *Themeda triandra* as a perennial seed crop in south-eastern Australia: What are the agronomic possibilities and constraints, and future research needs? Cogent Food & Agriculture **8**:2153964.

Malinga, R., L. J. Gordon, G. Jewitt, and R. Lindborg. 2015. Mapping ecosystem services across scales and continents – A review. Ecosystem Services **13**:57-63.

McNaughton, S. J. 1985. Ecology of a Grazing Ecosystem: The Serengeti. Ecological Monographs **55**:259-294.

McQuillan, M., R. J. Smernik, and R. Ryba. 2024. Sunlight can have a stronger influence than air temperature on soil solarisation: observational evidence from Australia. Soil Research **62**:-.

Möhl, P., R. S. von Büren, and E. Hiltbrunner. 2022. Growth of alpine grassland will start and stop earlier under climate warming. Nature Communications **13**:7398.

Mohr, J. J., P. A. Harrison, J. Stanhope, and M. F. Breed. 2021. Is the genomics cart before the restoration ecology horse? Insights from qualitative interviews and trends from the literature. bioRxiv:2021.2008.2005.455206.

Montoya, E., U. Lombardo, C. Levis, G. A. Aymard, and F. E. Mayle. 2020. Human Contribution to Amazonian Plant Diversity: Legacy of Pre-Columbian Land Use in Modern Plant Communities. Pages 495-520 *in* V. Rull and A. C. Carnaval, editors. Neotropical Diversification: Patterns and Processes. Springer International Publishing, Cham.

Morgan. 1999. Defining grassland fire events and the response of perennial plants to annual fire in temperate grasslands of south-eastern Australia. Plant Ecology **144**(1): 127-144.

Morgan, J. W., and I. D. Lunt. 1999. Effects of time-since-fire on the tussock dynamics of a dominant grass (*Themeda triandra*) in a temperate Australian grassland. Biological Conservation **88**:379-386.

Moroenyane, I., J. Tremblay, and É. Yergeau. 2020. Temporal and spatial interactions modulate the soybean microbiome. Fems Microbiology Ecology **97**.

Murphy, B. P., A. N. Andersen, and C. L. Parr. 2016. The underestimated biodiversity of tropical grassy biomes. Philosophical Transactions of the Royal Society B: Biological Sciences **371**:20150319.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature **368**:734-737.

Nerlekar, A. N., and J. W. Veldman. 2020. High plant diversity and slow assembly of old-growth grasslands. Proceedings of the National Academy of Sciences **117**:18550-18556.

Neuenkamp, L., S. M. Prober, J. N. Price, M. Zobel, and R. J. Standish. 2019. Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. Fungal Ecology **40**:140-149.

Ngumbi, E., and J. Kloepper. 2016. Bacterial-mediated drought tolerance: Current and future prospects. Applied Soil Ecology **105**:109-125.

O'Mara, F. P. 2012. The role of grasslands in food security and climate change. Annals of Botany **110**:1263-1270.

Öckinger, E., A. K. Eriksson, and H. G. Smith. 2006. Effects of grassland abandonment, restoration and management on butterflies and vascular plants. Biological Conservation **133**:291-300.

Ojima, D. S., B. O. M. Dirks, E. P. Glenn, C. E. Owensby, and J. O. Scurlock. 1993. Assessment of C budget for grasslands and drylands of the world. Water, Air, and Soil Pollution **70**:95-109.

Ott, J. P., B. B. Hanberry, M. Khalil, M. W. Paschke, M. Post van der Burg, and A. J. Prenni. 2021. Energy Development and Production in the Great Plains: Implications and Mitigation Opportunities. Rangeland Ecology & Management **78**:257-272.

Pascoe, B. 2018. Dark Emu: Aboriginal Australia and the Birth of Agriculture. Magabala Books.

Peddle, S. D., R. J. Hodgson, R. J. Borrett, S. Brachmann, T. C. Davies, T. E. Erickson, C. Liddicoat, M. Muñoz-Rojas, J. M. Robinson, C. D. Watson, S. L. Krauss, and M. F. Breed. 2024. Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions. Biological Reviews **100**: 1-18

Petermann, J. S., and O. Y. Buzhdygan. 2021. Grassland biodiversity. Current Biology **31**:R1195-R1201.

Petipas, R. H., M. A. Geber, and J. A. Lau. 2021. Microbe-mediated adaptation in plants. Ecology Letters **00**:1–16.

Petipas, R. H., J. B. González, T. M. Palmer, and A. K. Brody. 2017. Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. Acta Oecologica **78**:71-78.

Pineda, A., I. Kaplan, and T. M. Bezemer. 2017. Steering Soil Microbiomes to Suppress Aboveground Insect Pests. Trends in Plant Science **22**:770-778.

Potapov, P., S. Turubanova, M. C. Hansen, A. Tyukavina, V. Zalles, A. Khan, X.-P. Song, A. Pickens, Q. Shen, and J. Cortez. 2022. Global maps of cropland extent and change show accelerated cropland expansion in the twenty-first century. Nature Food **3**:19-28.

Price, J. N., M. K. Good, N. L. Schultz, L. K. Guja, and J. W. Morgan. 2019. Multivariate drivers of diversity in temperate Australian native grasslands. Australian Journal of Botany **67**:367-380.

Ramankutty, N., A. T. Evan, C. Monfreda, and J. A. Foley. 2008. Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. Global Biogeochemical Cycles **22**.

Reinhart, K. O. 2012. The organization of plant communities: Negative plant-soil feedbacks and semiarid grasslands. Ecology **93**:2377-2385.

Rillig, M. C., J. Antonovics, T. Caruso, A. Lehmann, J. R. Powell, S. D. Veresoglou, and E. Verbruggen. 2015. Interchange of entire communities: microbial community coalescence. Trends in Ecology & Evolution **30**:470-476.

Robinson, J. M., A. Annells, C. Cando-Dumancela, and M. F. Breed. 2024. Sonic restoration: acoustic stimulation enhances plant growth-promoting fungi activity. Biology Letters **20**:20240295.

Robinson, J. M., R. Hodgson, S. L. Krauss, C. Liddicoat, A. A. Malik, B. C. Martin, J. J. Mohr, D. Moreno-Mateos, M. Muñoz-Rojas, S. D. Peddle, and M. F. Breed. 2023. Opportunities and challenges for microbiomics in ecosystem restoration. Trends in Ecology & Evolution.

Rolfe, S. A., J. Griffiths, and J. Ton. 2019. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Current Opinion in Microbiology **49**:73-82.

Ruuskanen, S., B. Fuchs, R. Nissinen, P. Puigbò, M. Rainio, K. Saikkonen, and M. Helander. 2023. Ecosystem consequences of herbicides: the role of microbiome. Trends in Ecology & Evolution **38**:35-43.

Saleem, A., F. U. Hassan, A. Manaf, and M. S. Ahmedani. 2009. Germination of *Themeda triandra* (Kangaroo grass) as affected by different environmental conditions and storage periods. African Journal of Biotechnology **8**:4094-4099.

Sand-jensen, K. 1998. Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. Freshwater Biology **39**:663-679.

Santoyo, G. 2022. How plants recruit their microbiome? New insights into beneficial interactions. Journal of Advanced Research **40**:45-58.

Schlesinger, C., S. White, and S. Muldoon. 2013. Spatial pattern and severity of fire in areas with and without buffel grass (enchrus ciliaris) and effects on native vegetation in central Australia. Austral Ecology **38**:831-840.

Shade, A., and N. Stopnisek. 2019. Abundance-occupancy distributions to prioritize plant core microbiome membership. Current Opinion in Microbiology **49**:50-58.

Shi, B., M. Delgado-Baquerizo, A. K. Knapp, M. D. Smith, S. Reed, B. Osborne, Y. Carrillo, F. T. Maestre, Y. Zhu, A. Chen, K. Wilkins, M. C. Holdrege, A. Kulmatiski, C. Picon-Cochard, C. Roscher, S. Power, K. M. Byrne, A. C. Churchill, A. Jentsch, H. A. L. Henry, K. H. Beard, M. A. Schuchardt, N. Eisenhauer, R. Otfinowski, Y. Hautier, H. Shen, Y. Wang, Z. Wang, C. Wang, D. F. Cusack, A. Petraglia, M. Carbognani, T. a. G. W. Forte, S. Flory, P. Hou, T. Zhang, W. Gao, and W. Sun. 2024. Aridity drives the response of soil total and particulate organic carbon to drought in temperate grasslands and shrublands. Science Advances **10**:eadq2654.

Shipley, J. R., E. R. Frei, A. Bergamini, S. Boch, T. Schulz, C. Ginzler, M. Barandun, P. Bebi, J. Bolliger, K. Bollmann, N. Delpouve, M. M. Gossner, C. Graham, F. Krumm, M. Marty, N. Pichon, A. Rigling, and C. Rixen. 2024. Agricultural practices and biodiversity: Conservation policies for semi-natural grasslands in Europe. Current Biology **34**:R753-R761.

Simpson, K. J., B. S. Ripley, P.-A. Christin, C. M. Belcher, C. E. R. Lehmann, G. H. Thomas, and C. P. Osborne. 2016. Determinants of flammability in savanna grass species. Journal of Ecology **104**:138-148.

Sloan, S., C. N. Jenkins, L. N. Joppa, D. L. A. Gaveau, and W. F. Laurance. 2014. Remaining natural vegetation in the global biodiversity hotspots. Biological Conservation **177**:12-24.

Sloan, W. T., S. Woodcock, M. Lunn, I. M. Head, and T. P. Curtis. 2007. Modeling Taxa-Abundance Distributions in Microbial Communities using Environmental Sequence Data. Microbial Ecology **53**:443-455.

Smith, A. L., R. L. Barrett, and R. N. C. Milner. 2018. Annual mowing maintains plant diversity in threatened temperate grasslands. Applied Vegetation Science **21**:207-218.

Smith, M. E., T. R. Cavagnaro, M. J. Christmas, L. M. Pound, and J. M. Facelli. 2021. Site preparation impacts on soil biotic and abiotic properties, weed control, and native grass establishment. Restoration Ecology **29**:e13297.

Smith, S. W., J. D. M. Speed, J. Bukombe, S. N. Hassan, R. D. Lyamuya, P. J. Mtweve, A. Sundsdal, and B. J. Graae. 2019. Litter type and termites regulate root decomposition across contrasting savanna land-uses. Oikos **128**:596-607.

Snyman, H. A., L. J. Ingram, and K. P. Kirkman. 2013. *Themeda triandra*: a keystone grass species. African Journal of Range & Forage Science **30**:99-125.

Snyman, H. A., W. L. J. van Rensburg, and W. D. Venter. 1997. Transpiration and water-use efficiency in response to water stress in *Themeda triandra* and Eragrostis lehmanniana. South African Journal of Botany **63**:55-59.

Soliveres, S., P. Manning, D. Prati, M. M. Gossner, F. Alt, H. Arndt, V. Baumgartner, J. Binkenstein, K. Birkhofer, S. Blaser, N. Blüthgen, S. Boch, S. Böhm, C. Börschig, F. Buscot, T. Diekötter, J. Heinze, N. Hölzel, K. Jung, V. H. Klaus, A.-M. Klein, T. Kleinebecker, S. Klemmer, J. Krauss, M. Lange, E. K. Morris, J. Müller, Y. Oelmann, J. Overmann, E. Pašalić, S. C. Renner, M. C. Rillig, H. M. Schaefer, M. Schloter, B. Schmitt, I. Schöning, M. Schrumpf, J. Sikorski, S. A. Socher, E. F. Solly, I. Sonnemann, E. Sorkau, J. Steckel, I. Steffan-Dewenter, B. Stempfhuber, M. Tschapka, M. Türke, P. Venter, C. N. Weiner, W. W. Weisser, M. Werner, C. Westphal, W. Wilcke, V. Wolters, T. Wubet, S. Wurst, M. Fischer, and E. Allan. 2016. Locally rare species influence grassland ecosystem multifunctionality. Philosophical Transactions of the Royal Society B: Biological Sciences **371**:20150269.

Stegen, J. C., X. Lin, J. K. Fredrickson, X. Chen, D. W. Kennedy, C. J. Murray, M. L. Rockhold, and A. Konopka. 2013. Quantifying community assembly processes and identifying features that impose them. The ISME Journal **7**:2069-2079.

Stegen, J. C., X. Lin, A. E. Konopka, and J. K. Fredrickson. 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. The ISME Journal **6**:1653-1664.

Stopnisek, N., and A. Shade. 2021. Persistent microbiome members in the common bean rhizosphere: an integrated analysis of space, time, and plant genotype. The ISME Journal **15**:2708-2722.

Subedi, Y. R., P. Kristiansen, and O. Cacho. 2022. Drivers and consequences of agricultural land abandonment and its reutilisation pathways: A systematic review. Environmental Development **42**:100681.

Suding, K. N., K. L. Gross, and G. R. Houseman. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology & Evolution **19**:46-53.

Tang, M., J. Liu, W. Hou, R. M. Stubbendieck, H. Xiong, J. Jin, J. Gong, C. Cheng, X. Tang, Y. Liu, Z. Li, J. Wang, and Y. Yi. 2021. Structural variability in the bulk soil, rhizosphere, and root endophyte fungal communities of Themeda japonica plants under different grades of karst rocky desertification. Plant and Soil.

te Beest, M., J. J. Le Roux, D. M. Richardson, A. K. Brysting, J. Suda, M. Kubešová, and P. Pyšek. 2011. The more the better? The role of polyploidy in facilitating plant invasions. Annals of Botany **109**:19-45.

Theunissen, J. D. 1992. An ecosystematic investigation of *Themeda triandra* (Poaceae: Andropogoneae) in the semi-arid grasslands of southern Africa. Journal of Arid Environments **23**:35-44.

UN General Assembly. 2007. United Nations Declaration on the Rights of Indigenous Peoples :resolution. UN, [New York] :.

UNEP. 1992. World Atlas of Desertification.

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

Valkó, O., B. Deák, P. Török, A. Kelemen, T. Miglécz, K. Tóth, and B. Tóthmérész. 2016. Abandonment of croplands: problem or chance for grassland restoration? case studies from hungary. Ecosystem Health and Sustainability **2**:e01208.

van der Putten, W. H., M. A. Bradford, E. Pernilla Brinkman, T. F. J. van de Voorde, and G. F. Veen. 2016. Where, when and how plant–soil feedback matters in a changing world. Functional Ecology **30**:1109-1121.

Vandenkoornhuyse, P., A. Quaiser, M. Duhamel, A. Le Van, and A. Dufresne. 2015. The importance of the microbiome of the plant holobiont. New Phytologist **206**:1196-1206.

Verbruggen, E., M. G. A. van der Heijden, M. C. Rillig, and E. T. Kiers. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytologist **197**:1104-1109.

Vetterlein, D., A. Carminati, I. Kögel-Knabner, G. P. Bienert, K. Smalla, E. Oburger, A. Schnepf, T. Banitz, M. T. Tarkka, and S. Schlüter. 2020. Rhizosphere Spatiotemporal Organization–A Key to Rhizosphere Functions. Frontiers in Agronomy **2**.

Volaire, F., A. Morvan-Bertrand, M.-P. Prud'homme, M.-L. Benot, A. Augusti, M. Zwicke, J. Roy, D. Landais, and C. Picon-Cochard. 2019. The resilience of perennial grasses under two climate scenarios is correlated with carbohydrate metabolism in meristems. Journal of Experimental Botany **71**:370-385.

Vujanović, D., G. Losapio, M. Mészáros, S. Popov, Z. Markov Ristić, S. Mudri Stojnić, J. Jović, and A. Vujić. 2023. Forest and grassland habitats support pollinator diversity more than wildflowers and sunflower monoculture. Ecological Entomology **48**:421-432.

Wagg, C., S. F. Bender, F. Widmer, and M. G. A. van der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences **111**:5266-5270.

Wakeling, J. L., M. D. Cramer, and W. J. Bond. 2012. The savanna-grassland 'treeline': why don't savanna trees occur in upland grasslands? Journal of Ecology **100**:381-391.

Wang, G. Z., P. Schultz, A. Tipton, J. L. Zhang, F. S. Zhang, and J. D. Bever. 2019. Soil microbiome mediates positive plant diversity-productivity relationships in late successional grassland species. Ecology Letters **22**:1221-1232.

Wang, X., L. J. Wang, W. Wu, Y. Chen, and R. Tian. 2024. Tourists' environmentally responsible behavior: A new path of sustainable development of grassland tourism. Business Strategy and the Environment **33**:2143-2158.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**:1629.

Weidlich, E. W. A., F. G. Flórido, T. B. Sorrini, and P. H. S. Brancalion. 2020. Controlling invasive plant species in ecological restoration: A global review. Journal of Applied Ecology **57**:1806-1817.

West, J. R., and T. Whitman. 2022. Disturbance by soil mixing decreases microbial richness and supports homogenizing community assembly processes. Fems Microbiology Ecology **98**.

Williams, N. S. G., A. Marshall, and J. W. Morgan. 2015. Land of Sweeping Plains : Managing and Restoring the Native Grasslands of South-Eastern Australia. CSIRO Publishing, Collingwood, AUSTRALIA.

Williams, N. S. G., M. J. McDonnell, and E. J. Seager. 2005. Factors influencing the loss of an endangered ecosystem in an urbanising landscape: a case study of native grasslands from Melbourne, Australia. Landscape and Urban Planning **71**:35-49.

Willmer, P. G., and G. N. Stone. 2004. Behavioral, Ecological, and Physiological Determinants of the Activity Patterns of Bees. Pages 347-466 Advances in the Study of Behavior. Academic Press.

Wilson, J. B., R. K. Peet, J. Dengler, and M. Pärtel. 2012. Plant species richness: the world records. Journal of Vegetation Science **23**:796-802.

Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences **106**:13875-13879.

Wright, C. K., and M. C. Wimberly. 2013. Recent land use change in the Western Corn Belt threatens grasslands and wetlands. Proceedings of the National Academy of Sciences **110**:4134-4139.

Wubs, E. R. J., W. H. van der Putten, S. R. Mortimer, G. W. Korthals, H. Duyts, R. Wagenaar, and T. M. Bezemer. 2019. Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. Ecology Letters **22**:1145-1151.

Xia, H., S. Yuan, and A. V. Prishchepov. 2023. Spatial-temporal heterogeneity of ecosystem service interactions and their social-ecological drivers: Implications for spatial planning and management. Resources, Conservation and Recycling **189**:106767.

Zhang, M., J. Sun, Y. Wang, Y. Li, and J. Duo. 2024. State-of-the-art and challenges in global grassland degradation studies. Geography and Sustainability.

Zhao, Y., Z. Liu, and J. Wu. 2020. Grassland ecosystem services: a systematic review of research advances and future directions. Landscape Ecology **35**:793-814.

Zhao, Y., J. Wang, G. Zhang, L. Liu, J. Yang, X. Wu, C. Biradar, J. Dong, and X. Xiao. 2023. Divergent trends in grassland degradation and desertification under land use and climate change in Central Asia from 2000 to 2020. Ecological Indicators **154**:110737.

Zhu, K., Y. Song, J. C. Lesage, J. C. Luong, J. W. Bartolome, N. R. Chiariello, J. Dudney, C. B. Field, L. M. Hallett, M. Hammond, S. P. Harrison, G. F. Hayes, R. J. Hobbs, K. D. Holl, P. Hopkinson, L. Larios, M. E. Loik, and L. R. Prugh. 2024. Rapid shifts in grassland communities driven by climate change. Nature Ecology & Evolution.

Zomer, R. J., J. Xu, and A. Trabucco. 2022. Version 3 of the Global Aridity Index and Potential Evapotranspiration Database. Scientific Data **9**:409.

Chapter two: Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C4 grass, *Themeda triandra*

Riley J. Hodgson*, Craig Liddicoat, Christian Cando-Dumancela, Nicole W. Fickling, Shawn D. Peddle, Sunita Ramesh & Martin F. Breed 2024. *Applied soil ecology* **201**, 105514

https://doi.org/10.1016/j.apsoil.2024.105514 **(Published manuscript)**

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The candidate was the primary author of the manuscript. The contributions of all co-authors are as follows; Project design: **RJH,** CL, MFB; Field work: **RJH**, MFB, CCD, SP, NWF; Lab work: **RJH**, CCD; Statistical analysis: **RJH**, CL; Writing manuscript: **RJH**; Revisions and editing: **RJH**, MFB, CL, NWF, SR, SP, CCD.

Percentage contribution: **RJH: 89%,** CL: 1%, CCD: 1%, NWF: 1%, SDP: 1%, SR: 1%, MFB: 6%

Throughout this chapter I have used the pronoun 'we' to acknowledge the contributions of my coauthors, following the customary etiquette in published manuscripts.

Chapter two: Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C4 grass, Themeda triandra

Abstract

Understanding belowground plant-microbial interactions is fundamental to predicting how plant species respond to climate change, particularly in global drylands. However, these interactions are poorly understood, especially for keystone grass species like the pan-palaeotropical *Themeda triandra*. Here, we used 16S rRNA amplicon sequencing to characterise microbiota in rhizospheres and bulk soils associated with *T. triandra*. We applied this method to eight native sites across a 3-fold aridity gradient (aridity index range = 0.318 to 0.903 = 87% global aridity distribution) in southern Australia. By examining the relative contributions of climatic, edaphic, ecological, and host specific phenotypic traits, we identified the ecological drivers of core *T. triandra*-associated microbiota. We show that aridity had the strongest effect on shaping these core microbiotas, and report that a greater proportion of bacterial taxa that were from the core rhizosphere microbiomes were also differentially abundant in more arid *T. triandra* regions. These results suggest that *T. triandra* naturally growing in soils under more arid conditions have greater reliance on rhizosphere core taxa than plants growing under wetter conditions. Our study underscores the likely importance of targeted recruitment of bacteria into the rhizosphere by grassland keystone species, such as *T. triandra*, when growing in arid conditions. This bacterial soil recruitment is expected to become even more important under climate change.

2.1 Introduction

Soil is the most biodiverse habitat on Earth – home to ~59% of all species – and supports animals, plants, and, crucially, their associated microbiota (Anthony et al. 2023). Soil microbiota support ecosystem productivity and stability and are often impacted by ecosystem degradation (Naeem et al. 1994, Schnitzer et al. 2011). These microbiota often form strong relationships with plant hosts and contribute to improved ecosystem resilience via plant-soil feedbacks, which help their host plants mitigate the effects of environmental stressors, such as drought (de Vries et al. 2020, Thiergart et al. 2020, Yang et al. 2021). Plant-soil feedbacks can also aid plant nutrient acquisition, altering plant metabolic activity, and antagonising competitive plant species (Bever et al. 2010, Pineda et al. 2017, Hubbard et al. 2019). These positive feedbacks can therefore promote assemblages of microbes that confer species-specific benefits to plants (Smith et al. 2018), and create pressures that shape plant community composition (Wardle et al. 2004). These vital plant-soil feedbacks, however, are expected to be adversely impacted by climate change (Dudenhöffer et al. 2022) and require greater consideration to predict future host plant fitness and ecosystem functioning.

Plant-soil interactions are increasingly recognised as key components of host plant ecology, with many plants sustaining core microbiomes – specific microbial communities associated with a host species or a specific environment. Numerous studies have characterised persistent core microbiomes of host plants to better understand the mechanisms of microbial community assembly and ecological drivers shaping community composition and function (Bulgarelli et al. 2012, Hamonts et al. 2018, Urbina et al. 2018). Characterising the core microbiome of plant species is important as it underscores how soil microbiota can drive plant population dynamics, but only a few studies have investigated core microbiomes across broad geographic scales (e.g., at ranges of ~10s km) (Shade and Stopnisek 2019, Risely 2020, Neu et al. 2021). Studying plant-soil interactions at these larger scales is required to understand their potential role in facilitating host plant adaptation to climate change, and other landscape-scale ecosystem pressures. Core microbiota could, for example, provide positive feedbacks to host plants that buffer shifts in fitness landscapes of ecologically important plant species due to climate change (Brinkman et al. 2017, Wolfsdorf et al. 2021).

The pan-palaeotropical C4 grass species *Themeda triandra* is a keystone species with high ecological value in many grasslands across the world (Linder et al. 2018). It is an ecologically and culturally significant grass species in Australia, for example, where it has been used and consumed by Indigenous Australians for 10,000s of years (Pascoe 2018). It is also an important feedstock for grazing animals (McNaughton 1985, Snyman et al. 2013). This grass is often a dominant species in many southern Australian grasslands, which are increasingly arid due to climate change-driven shifts in rainfall and evapotranspiration patterns (Guerin et al. 2018, Nishant et al. 2021, DEW 2022). Thus, the soil microbiota associated with *T. triandra*, including the growth-promoting bacteria (Hassen and Labuschagne 2010) and fungi (Petipas et al. 2017) that it cultivates around its roots, are at risk of being impacted by changing CO_2 and temperature (Hayden et al. 2012), with potential flow-on impacts to the plant host. Indeed, the congeneric *T. japonica,* considered by some to be synonymous to *T. triandra* (POWO 2023), has already seen shifts in its associated soil microbiota in response to increasing desertification across Asia (Tang et al. 2021). Together, this suggests that *T. triandra* may be exposed to climate change impacts directly, but also via climate change causing a shift in its associated microbiota.

Better understanding the interaction between *T. triandra* root systems and its belowground microbial communities, as well as determining whether the composition of these microbiota change along an aridity gradient, is crucial for anticipating climate impacts on *T. triandra*. Accordingly, we characterised the core soil and rhizosphere bacterial microbiomes of *T. triandra* across a strong aridity gradient in southern Australia, which is representative of the global distribution of *T. triandra*. We addressed the following research questions: (a) how do *T. triandra*-associated soil and rhizosphere bacterial diversity and community composition change across a strong aridity gradient? and (b) what is the relative contribution of climatic, soil abiotic, ecological, and host related phenotypic traits on structuring the core *T. triandra*-associated microbiota?

2.2 Methods

2.2.1 Study species

Themeda triandra (Forssk.) is a pan-palaeotropical perennial C4 grass species (Dunning et al. 2017). This grass has an important fire ecology and its seed germination rates have seen improvements when exposed to smoke and heat (Baxter et al. 1994, Ghebrehiwot et al. 2012). *T. triandra* is a difficult species to grow at scale, as the seed has been known to germinate best after long dormancy periods, with substantial variation across regions (Saleem et al. 2009, Farley et al. 2013, Hancock and Hughes 2014). As a keystone species in palaeotropical grasslands, *T. triandra* is important for the deposition of organic matter into the soil through its roots, facilitating invertebrate communities, and is relied on by grazing herbivores. Moreover, the abundance of *T. triandra* within grasslands can be an indicator of ecosystem health, and can highlight overgrazing risk or biodiversity decline (Snyman et al. 2013).

2.2.2 Sampling design

In December 2021, we collected soil and plant tissue samples from six replicate *T. triandra* individuals across eight regions along an aridity gradient in southern Australia (Figure 2.1a; Figure S2.1).

Site selection

We obtained aridity index data used for modelling associations across the Australian study sites from the Atlas of living Australia (ALA) Spatial portal (Belbin 2011) using the Mean annual aridity index layer (ALA 2014). This annual mean aridity index data was based on monthly ratios of precipitation to potential evaporation; adapted from the UNEP World Atlas of Desertification (UNEP 1992, Middleton and Thomas 1997). Sites spanned aridity index values of 0.318–0.903 (higher to lower aridity, respectively; Table S2.1). We note that low aridity index values correspond to low water availability of an area, however, in common parlance this would usually be expressed as 'higher aridity'. Regions with higher aridity index values, correspond to higher water availability of an area., We ran a Mantel test to compare site pairwise geographical distances with site pairwise aridity index distances to investigate potential bias due to spatial autocorrelation (i.e., a positive correlation between site geographic and enviornmental distances, in this case). We implemented this analysis to check this potential bias in our sampling design (Mantel test: r = -0.021; *p-value* = 0.489; Figure 2.1b). We conducted a second test for spatial autocorrelation in the aridity data using Moran's I, implemented via the ape R package (Paradis and Schliep, 2019). Spatial weights were based on the inverse of a Haversine distance matrix between sites, with diagonal values set to zero to avoid infinite weights. Statistical significance was assessed using a permutation test with 999 iterations. The results indicated no significant spatial autocorrelation in aridity (Moran's I = 0.159, expected = –0.143, SD = 0.227, p = 0.183).

In selecting our sites, we compared the global aridity distributions of *T. triandra* to our sample sites using the R package terra (Hijmans 2023) based on aridity data from version 3 of the Global Aridity Index and Potential Evapotranspiration Database (Global-AI_PET; Figure 2.1c) (Zomer et al. 2022). We compared the global occurrence for *T. triandra* based on records from the Global Biodiversity Information Facility between the years 2000-2023 (GBIF.org 2023). Using the upper and lower limits from the Atlas of Living Australia aridity index data, we found that the sampled aridity gradient covered 87% of all global occurrences (Belbin 2011), whereas when reevaluating our sampling sites using values from the Global-AI_PET database, the aridity gradient covered 41% of the recorded global occurence (Figure 2.1d) (Zomer et al. 2022). We note this discrepancy may be due to data smoothing in the coarser global climatic data compared to more detailed fine-resolution patterns in the Atlas of Living Austral data. However, we argue this allows for a well replicated and representative aridity gradient within which we could study *T. triandra* microbial dynamics.

Field sampling

At each site, we placed a 25 x 25 m quadrat, from which we randomly selected *T. triandra* plants using coordinates generated from a random number table transposed to our map. We collected whole plant specimens from six randomly chosen individuals and measured aboveground biomass to give an indication of plant growth. We also performed a range of vegetation assessments at each population (see *below*). We profiled the diversity and community composition of bacteria in the rhizospheres and bulk soils at 30 cm and 2 m from the base of the sampled individual using 16S rRNA amplicon sequencing to understand how bacterial communities change with proximity to their host plant (described below; Figure S2.2a). This sampling design was intended to explore the spatial limits of *T. triandra's* direct influence on its surrounding soil environment, comparing microbial communities at near (30 cm) and far (2 m) distances. We collected *T. triandra* samples and analysed them for nutrient concentrations, as were the bulk soil samples that were collected at 30 cm and 2 m from the base of these plants, along with other physicochemical conditions (see *below;* Figure S2.2a).

Sampling methodology

At each study population, *T. triandra* density estimates and vegetation assessments were taken alongside plant and soil samples within 25 m x 25 m quadrats. *T. triandra* density was measured from five 4 m x 4 m quadrats within the target area (Figure S2.2b). To characterise vegetation at each population, we ran six point-intercept transects within our 25 x 25 m quadrats (Bonham 2013). Each transect was spaced 5 m apart in a North-South direction and involved observations of the occurrence of plant species found every meter (Figure S2.2c). Functional categories for the vegetation were as follows: graminoids, herbs (forbs), shrubs, trees/canopy cover, litter, and bare earth (exposed dirt or rock). Where more than one functional unit occurred at a given point, all were recorded. The occurrences of each of these functional categories for each transect replicate at each population were contrasted using principal components analysis (PCA). These data were used to describe differences in functional vegetation across the sampling sites (Figure S2.3).

2.2.4 Plant and soil physicochemical analysis

We analysed a range of soil physicochemical conditions from around the six randomly chosen plant individuals at 30 cm and 2 m from host plants (i.e., 6 plants x 8 sites = 48 soils at 30 cm, and 48 soils at 2 m, n = 96; Figure S2.2a). These included: phosphorus and potassium (Colwell 1965), sulphur (KCl 40 method) (Blair et al. 1991), organic carbon (Walkley and Armstrong 1934), nitrate, ammonium, electrical conductivity and pH ($CaCl_2$) at CSBP Laboratories (Bibra Lake, Australia). We also measured the mean gravimetric water content (McPherson et al. 2018) which correlated with mean aridity index values across sampling sites (Figure S2.4). Soil collected in each sample sent to CSBP was about 200 g per sample. Nutrient analysis of *T. triandra* root and leaf samples (n = 48) were also conducted at CSBP Laboratories (Bibra Lake, Australia) using inductively coupled plasma (ICP) spectroscopy to measure trace elements and macronutrients within the plant, including: nitrogen, phosphorus, potassium, sulphur, copper, zinc, manganese, calcium, magnesium, sodium, iron, and boron. Here, we prioritised the use of the youngest fully mature leaves; however, due to field limitations, we often used all available live leaves to obtain sufficient tissue for these tests (approximately 4 g tissue per plant).

2.2.5 DNA extraction and sequencing

Microbiota from the *T. triandra* rhizospheres were characterised following the protocol from McPherson et al. (2018). Briefly, roots collected from each sampled plant were washed in 0.02% Silwet L-77 amended PBS buffer and vigorously shaken in the field, before being transferred to lab facilities on ice. Within 72 hours of collection, the buffer solutions were filtered using 100 µm filters and centrifuged prior to DNA extraction. Frozen rhizosphere and soil samples were left to thaw at room temperature before DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany). Amplicon libraries of the 16S rRNA V3-4 gene region were developed by the Australian Genome Research Facility (AGRF, Melbourne, Australia). Samples were PCR amplified with the forward primer, 341F (CCTAYGGGRBGCASCAG), and reverse primer, 806R (GGACTACNNGGGTATCTAAT). Sequences were determined using the 300 base pairs paired end run of Illumina MiSeq sequence production.

2.2.6 Bioinformatic processing

We used the standard DADA2 bioinformatic pipeline to infer identity profiles from amplicon sequence data from SILVA (Version 138.1) (Wang et al. 2007, Quast et al. 2013), a small subunit rRNA 16S/18S database used for taxonomic Assignment, using a naïve Bayesian classifier (Wang et al. 2007, Callahan et al. 2016). All taxa that were not assigned as Bacteria, unassigned at the Phylum level, or associated to mitochondria or chloroplasts were removed. We also removed taxa that did not occur in at least two samples across our whole soil and rhizosphere datasets to avoid unrepresentative and potentially contaminating sequences/taxa. The resulting dataset was used for all downstream analyses, described below.

2.2.7 Statistical analysis

All statistics were performed using R version 4.0.2 (R Core Team 2022). To produce a taxonomic list of candidate core microbiome taxa in *T. triandra* rhizospheres, we used an abundance-occupancy selection strategy using methods and code from Shade and Stopnisek (2019). Using rarefied data, as per methods detailed in Shade and Stopnisek (2019), we first ranked taxa by their occupancy, accounting for detection as a proportion across all sites and consistency of replication. We then assessed the percent contribution of these potential core taxa to the overall beta diversity in order from most to least abundant. From this list, we ranked the contribution of each top ranked taxa sequentially against the Bray-Curtis similarity for the whole dataset in a cumulative stepwise manner. The taxa were included for core microbiome membership until the taxa saw a final 2% increase in explanatory value. Finally, we compared the selected candidate core taxa to a neutral theory model of microbial community assembly to visualise selection of these taxa by the host plant. Candidate core taxa were also determined for the bulk soil samples collected at 30 cm and 2 m from the host plants.

Alpha diversity analysis

We used rarefaction without replacement to normalise for variation in library sizes across samples after sequencing. Samples were rarefied to 11,336 reads (Figure S2.5). The effective number of ASVs was estimated by taking the exponential transformation of Shannon's diversity (Jost 2006), as a measure of bacterial alpha diversity.

We used linear mixed-effects modelling with the *lmer* function in the lme4 package in R (Bates et al. 2015) to test the effect of sampling population, and belowground zones on alpha diversity via the effective number of ASVs. These attempt to account for site differences and resampling of the individual plants across bulk soils and rhizospheres. In our first model we modelled effective number of ASVs as our response variable, belowground zone was included as a fixed effect, and we included plant ID nested within sampling population as random intercepts. Our second model also explored how the effective number of ASVs changed with belowground zone and sampling site, which were included as fixed effects, where plant ID was also included as a random slope. Unlike in our first model, we did not use a nested design (e.g. Plant ID nested within Site) because this would have reduced the effective sample size to n = 1 for each group – one observation per plant compartment, per site, per individual – eliminating replication and preventing reliable estimation of variance components. Model assumptions were assessed using diagnostic plots, and the Shapiro-Wilk test to explore normality. Model significance was tested using the afex package in R (Singmann 2023), via the *mixed* function which ran likelihood ratio tests against all fixed effects against reduced models.

Pairwise tests were made using multiple comparisons with Tukey contrasts through the *glht* function of the multcomp package (Hothorn et al. 2008).

Beta diversity analysis

Bacterial community composition was visualised using non-metric multidimensional scaling ordination (NMDS) with Bray-Curtis distances on our bacterial 16S rRNA dataset, following standard bioinformatic and rarefaction processing. Bray-Curtis distance metrics were chosen because they are sensitive to differences in relative abundances between samples, making them well-suited for analysing ecological community composition based on count data, including rarefied datasets such as those used in this analysis. Treatment effects on the bacterial communities were estimated via permutational multivariate analysis of variance (PERMANOVA) using the *adonis2* function in vegan (Oksanen et al. 2019), followed by testing for homogeneity of group dispersion with the *betadisper* function. We also compared site differences within and across compartments using subsets of the dataset to address assumptions of independence in our analyses. Visualisations of the relative estimated abundance of major phyla across treatments were performed using the *plot_bar* function in Phyloseq (McMurdie P. J. and Holmes 2013). Rare phyla that had less than 0.7% total relative estimated abundance were grouped as ‘other minor phyla’. We tested for significant changes in the relative abundance of the major phyla across the aridity gradient for all samples using Pearson’s correlation via the *cor.test* function in R, while Spearman’s rank order correlation was used for data that failed normality assumptions.

Host plant analysis

To compare differences between *T. triandra* biomass across sites, we used analysis of variance (ANOVA) after log transforming data to meet model assumptions. Pairwise comparisons were made with Tukey’s honest significant differences test (Tukey’s HSD) (Tukey 1949). Linear model assumptions were failed when comparing *T. triandra* density differences among sampling areas, and so we used the Kruskal-Wallis rank sum test, with the Dunn test for pairwise comparisons.

Differential abundance

Differential abundance of bacterial ASVs were explored in rarefied rhizosphere samples across the sampled aridity gradient once for our whole dataset following all the bioinformatic processes, described previously. We classified samples across sites into three classes: low, medium, and high, based on their population aridity index values. Sites with a mean aridity index ≤0.4 were considered in the high aridity class, whereas those >0.6 were categorised as low, and sites between these values (>0.4, but ≤0.6) were categorised in the medium class.

Using the function *ancombc* from the R package ANCOMBC (Lin and Peddada 2020) we ran assessments of differentially abundant ASVs by comparing log-fold changes in our aridity classes. Only statistically significant ASVs from our prior differential abundance analysis (at the 0.05 significance level) were included here. For each sample, we then calculated the sum of reads for the rhizosphere samples across two treatments: differentially abundant ASVs; and ASVs that were both differentially abundant but also identified as core microbiome candidates. We report the combined relative abundance of all these taxa. We then modelled the relationship between aridity index and the sum of reads for core versus non-core differentially abundant taxa using linear models, bootstrapping 95% confidence intervals 2,000 times to assess whether the estimates overlapped zero for each group. This enabled us to rigorously test how the relative abundance (percentage of all summed sequence reads) changed with aridity index across these two treatments: the taxonomic reads which were just differentially abundant; versus those that were both differentially abundant and core-candidates.

Canonical correspondence analysis

To estimate and visualise the drivers of bacterial community structure, we ran canonical correspondence analysis (CCA) for bacterial rhizosphere and bulk soil samples on all ASVs, and just those selected for candidate core microbiome inclusion. Null and full models incorporating explanatory variables were calculated. For our analysis of bulk soil communities, these explanatory variables included: all soil physicochemical variables sampled, prevalence of key functional vegetation categories, in addition to host plant variables such as population density, aboveground biomass, and aridity index (Table S2.2). Correlated explanatory variables were removed before we performed forward and backward selection of these explanatory variables using the *ordistep* function in the R package vegan (Oksanen et al. 2019). Where we observed saturation of the distance metrics in our CCAs via observed 'horseshoes' (Morton et al. 2017), we presented and interpreted our figures and according to the constrained components, CCA2 and CCA3, ensuring all data generated was available in the supplementary informatoin.

2.3. Results

2.3.1 Bacterial diversity

We observed 11 bacterial phyla across all samples, which represented 98.8% of reads and had abundance estimates of >2% (Figure S2.6). In the rhizospheres, we saw significant correlations between increasing aridity and decreases in the relative abundance of acidobacteriota, armatimonadota, gemmatimonadota, proteobacteria, and verrucomicrobiota, and increases in actinobacteriota and chloroflexi (Figure S2.7; Table S2.3).

Linear mixed-effects models revealed strong differences between bacterial alpha diversity levels across the rhizospheres and the bulk soils (LMEM: df = 2, X^2= 28.74, p <0.001). Rhizospheres averaged (mean ± SE) an effective number of ASVs of 491 ± 35, which was lower than 657 ± 26 in bulk soil at 30 cm (p <0.001) and 647 ± 24 at 2 m (p <0.001). The two bulk soil groups were not significantly different in their alpha diversity values (Figure 2.2a). Effective number of ASVs showed strong differences across sampling sites (Figure S2.8; LMEM: $X^2_{(7)}= 42.77$, $p <0.001$). Interestingly, we observed 2,296 ASVs that were uniquely found in the rhizospheres, whereas there were fewer ASVs found in the 2 m and 30 cm soils alone (2 m = 1139 ASVs versus 30 cm = 1364, respectively), but combined found 7,547 ASVs unique to just bulk soils (bulk soil ASVs in 2 m plus ASVs in 30 cm). Overall, the greatest partition was that of all shared ASVs across the rhizospheres and bulk soils (7,796; Figure 2.2b).

We observed no effect of aridity on effective number of ASVs across the two bulk soil groups at 30 cm or 2 m from host plants (Figure 2.2c). In rhizospheres, we observed a weak effect of aridity on decreasing effective number of ASVs, which reduced by 308.8 ± 188.5 ASVs (± SE) per aridity index unit (LM: $t_{(1,42)} = 2.685$, $p = 0.109$) (Muff et al. 2022).

There were strong bacterial communities structures between bulk soils at 30 cm and 2 m and plant rhizospheres (Figure 2.2d; Figure S2.9a; PERMANOVA: $F_{(2, 136)}= 2.308$, $R^2= 0.0628$, = 0.002, $n= 48$ samples per group). Bacterial communities were also well organised based aridity levels (Figure 2.2d; Figure 2.3a-c; PERMANOVA: $F_{(1,137)} = 9.360$, $R = 0.064$, $p = 0.001$), and by sampling population (Figure S2.9b; PERMANOVA: $df = 1$, $R^2=0.355$, $F= 10.297$, $p= 0.001$, $n= 6$ per population). We also found that sites had comparable beta dispersions across these models (PERMANOVA: $df= 7$, F= 1.495, $p= 0.182$, n = 6 per population).

We identified 193 and 177 candidate core ASVs in bulk soils at 2 m and 30 cm, respectively, from across 6 bacterial phyla (Figure 2.4a-b; Figure S2.10a-b), and 71 core ASVs from 5 bacterial phyla in rhizosphere samples (Figure 2.4c; Figure 2.5a). Our rhizosphere core bacteria occupancy levels ranged from 0.27-0.96 where the mean occupancy was 0.5 ± 0.0162 (occupancies of 1 would mean present across all samples; Figure S2.11). Of these taxa, 20 ASVs in the rhizosphere were present in at least one replicate across all sites, whereas there were 13 ASVs found across all soil 30 cm from host plants, and 19 ASVs in soil 30 cm from host plants (Figure S2.12).

2.3.2 Environmental associations

We found significant differences in the aboveground biomass of the sampled *T. triandra* plants (ANOVA: $F_{(7,42)}$= 10.297, *p*< 0.001; Figure S2.13), and density of *T. triandra* among sampling sites (Kruskal-Wallis: X^2 = 21.826, *df*= 7, *p*= 0.003; Figure S2.14).

2.3.3 Differential abundance

We found 1,234 ASVs were differentially abundant across the three aridity classes (low, medium and high; Figure 2.5b). Aridity had no effect on the mean relative abundance of these ASVs as the bootstrapped 95% confidence intervals predicted a relative abundance change of between -6.267% to 8.513% per aridity index unit (i.e. confidence intervals did overlap zero; Figure 2.5c). However, we observed a negative relationship between aridity index and the relative abundance of ASVs that were both differentially abundant and core selected. Here, we saw a decrease in mean relative abundance by between -41.16% to -10.58% per unit of the aridity index (i.e. did not overlap zero; Figure 2.5c). The abundance of these ASVs was higher in rhizosphere communities from more arid sites, compared to less arid sites.

2.3.4 Canonical correspondence analysis

Canonical correspondence analysis (CCA) revealed significant associations between microbial communities with a suite of environmental variables at 2 m from the host plant (Figure 2.3d; Figure S2.15; Permutation test: permutations=999, X^2= 3.358, $F_{(8,37)}$= 2.090, $P \leq 0.001$). The highest rate of change for the bacterial communities was observed with population aridity, relative abundance of litter, and population longitude. Other variables of note included: canopy cover, relative abundance of herbs and graminoids, latitude, and electrical conductivity. Together, these factors were summarised as having explained 16.55% and 13.90% of variation in CCA2 and CCA3, respectively. Highly correlated covariates with correlations of ≥0.75 were removed from the analysis, and included bare soil, ammonium nitrogen, elevation, pH, and organic carbon (Figure S2.21).

For microbial communities in bulk soil at 30 cm from host plant, CCA revealed significant effects of population aridity index, longitude, phosphorus and density of host plants (Figure 2.3e; Figure S2.16; Permutation test: permutations=999, X^2= 3.633, $F_{(9,37)}$= 1.823, $P \leq 0.001$). Additionally, important variables identified for potentially shaping the bacterial community structures included latitude, electrical conductivity, pH ($CaCl_2$) and the relative abundance of graminoids, herbs and litter. This CCA reported 15.51% and 12.24 % of variation in CCA2 and CCA3, respectively. Removed covariates included: elevation, bare soil, organic carbon and nitrogen (Figure S2.22).

In the *T. triandra* rhizospheres, CCA revealed significant associations between select environmental variables and microbial communities (Figure 2.3f; Figure S2.17; Permutation test: permutations=999, X^2=3.767, $F_{(7,37)}$= 1.345, $P \leq 0.001$). The variables that explained the highest rate of change in microbial communities included aridity index and host magnesium. Furthermore, population canopy cover, host calcium, longitude and the relative abundance of herbs and litter were all identified in the analysis as having some influence on shaping the rhizosphere bacterial communities. This analysis accounted 15.41% and 14.86% of variation in CCA2 and CCA3 respectively. Excluded covariates included: elevation, host nitrogen, and bare soil, which correlated with population aridity index (Figure S2.23).

We ran a CCA on just the candidate core bacteria in the bulk soils at 2 m from the host plants, which included 193 ASVs, which showed changes in bacterial community structures with population aridity and longitude, among other variables (Figure 2.4d; Figure S2.18). Components explained 17.54%

(CCA2) and 5.89% (CCA3) of variation in the data. Again, covariates were removed prior to analysis (Figure S2.21).

CCAs on the candidate core selected bacteria found in the bulk soils at 30 cm, considered 177 ASVs. The analysis identified associations between bacterial communities and environmental factors such as aridity index, canopy cover, and population-wise longitudinal differences, among others (Figure 2.4e; Figure S2.19). Variations explained by the components were 14.76% and 7.4% for these bulk soils, which excluded all covariates removed before analysis (Figure S2.22).

CCAs for the candidate core bacteria from the host rhizospheres (containing 71 ASVs) revealed that differences among host calcium and aridity, and canopy cover within the sampled population were important factors associated with bacteria in the rhizospheres (Figure 2.4f; Figure S2.20). Covariates were removed prior to the analysis (Figure S2.23), and the CCA1 and CCA2 components explained 51.08% and 17.18% of variation.

2.4. Discussion

We show that aridity strongly associated with the rhizospheres and bulk soil microbiomes of *T. triandra*, a pan-palaeotropical C4 grass species that dominates many global grassland ecosystems. We identified many candidate core microbiome taxa that are likely functionally important to *T. triandra* within their rhizospheres. Interestingly, we show that aridity had a strong effect on increasing the relative abundance of bacterial taxa that were both differentially abundant (arid-indicating taxa) but were also candidate core microbiome members in *T. triandra* rhizospheres. This pattern suggests arid conditions are likely to be driving stronger symbiotic relationships between *T. triandra* and soil microbiota in this plant-soil interface. Climate change is already affecting regional temperatures, evapotranspiration, and rainfall patterns (DEW 2022), which underscores the importance of understanding and preserving the role of *T. triandra* and its associated bacterial microbiomes within its natural environment. These symbioses likely play a crucial role in not only aiding the functioning and competitive dynamics of the host plant, but could also be crucial to maintaining soil health and productivity in recognised keystone plant species, like *T. triandra,* under current and anticipated future climates.

2.4.1 Aridity strengthens core microbiome associations

Our results point to stronger combined selection from both the environment (via differential abundance analysis) and the plant host (via candidate core prioritisation) on shaping rhizosphere bacterial communities in more arid regions. More arid conditions appeared to lead to more plant-microbe symbiotic relationships probably due to the resource-limited nature of increasingly arid environments. Low nitrogen and nutrient levels often correlate with high aridity (Abdelfattah et al. 2018, Chen et al. 2021, Liu et al. 2022) which can drive increased diversity of nitrogen-fixing bacteria to such an extent that aridity can be a better predictor of nitrogen-fixing capacity than even soil nitrogen levels (Doby et al. 2022). The resource limitations of arid ecosystems could mean that *T. triandra* may require more specialist microbes to maintain higher fitness rates within these more arid communities, in line with other studies where soil microbes have been seen to have an effect under arid conditions (Chen et al. 2021, Dadzie et al. 2022, Zhong et al. 2022). Interestingly, the prevalence of mycorrhizal fungi is found to decrease across aridity scenarios, and these conditions favour plants with lower reliance on fungal communities (Pérez-Ramos et al. 2021). Yet, warming and reduced rainfall can lead to higher carbon and nutrient exchange rates between mycorrhizal fungi and plant roots (Pérez-Ramos et al. 2021), suggesting a more active role of mycorrhizal fungi impacting on plant fitness. We expect that these patterns could also be reflective of soil bacteria, though this would require further investigation of the functional activity and biomass of bacterial microbiota (Muff et al. 2022). Overall, our findings contribute to a growing literature that highlights the relationships between plants, their microbial communities, and how they are shaped by environmental influences.

2.4.2 Core microbiome predicts functional importance of ASVs

Our candidate core microbiome taxa revealed 71 ASVs as likely having important roles on *T. triandra* fitness and plant-soil interactions. Of these ASVs, 20 were present in at least one rhizosphere replicate at every sampling population, giving a population-occupancy of 100%. We propose each of the candidate core taxa may have particularly important functions for the host plant, either by providing a competitive advantage or mitigating environmental limitations, thereby raising their importance to host plant fitness compared to other non-candidate core taxa (Hamonts et al. 2018, Estendorfer et al. 2020, Risely 2020). Common microbial functions within rhizosphere often reflect pathogen protection, phytohormone production and nutrient cycling (Bulgarelli et al. 2013), and whether our candidate core microbial taxa support these functions should be examined. It is important to note, however, that candidate core taxa occurred at an average occupancy of 50% of all samples across the aridity gradient, so there is still considerable bacterial community turnover across the environmental niche space we studied.

Many other grass species also display changes in bacterial communities across environmental gradients with provision of important functional properties that may be provided by rare or site-specific bacterial taxa. Escobar Rodríguez et al. (2018), for instance, showed that bacterial taxa in the rhizospheres of the perennial C4-grass, *Setaria viridis,* had high relative abundances, despite low site occupancy. They suggest, as we also do, that these bacteria could be important for fitness of grass species, and hence less frequently observed taxa can be important members of core microbiomes. Furthermore, we expected to see the composition of candidate core rhizosphere taxa being influenced by abiotic soil resources (carbon and nutrients), vegetation and population variation, and our finding emphasises the particular importance of micro-site predictors for bacterial communities in this study (Turlure et al. 2014, Estendorfer et al. 2020, Zhong et al. 2022). Importanatly, variation in bacterial communities across ecotypes is also found in similar grasses like the dominant perennial prairie grass, *Andropogon gerardii*, where bacterial variation is greater than that of fungi (Sarkar et al. 2022). Future work to develop understanding of the comparative patterns of candidate core bacterial versus fungal community and functional change, will therefore also be an important research avenue for understanding *T. triandra* soil interactions.

2.4.3 Environmental drivers of bacterial community structures

In exploring the impact of *T. triandra* on its immediate soil environment, we noted distinguishable differences between bacterial community composition and diversity in the rhizospheres compared to the bulk soil, but these differences were not observed within bulk soils between 30 cm and 2 m from *T. triandra* at depths of 0-10 cm. While we expected that rhizospheres would foster different bacterial communities to bulk soils due to preferential selection of specific bacteria to meet host plant needs (Bulgarelli et al. 2012, Bulgarelli et al. 2013, Urbina et al. 2018), it was interesting to learn that *T. triandra* had no detectable effect on the bacterial communities of proximal surrounding soils. The alternative interpretation is that *T. triandra* had a profound legacy in soils that extended at least 2 m from the plants, however we consider this explanation unlikely due to the significant shift in composition only seen in rhizospheres. Bacterial legacies in soil fade quickly when compared to those of fungi, as evidence suggests fungal community legacies can persist for months after the loss of a host plant (Hannula et al. 2021), and bacterial community structures are often driven by seasonal influences rather than long-term precipitation changes (Yuste et al. 2014). This could explain the absence of detectable difference in microbial communities within the 0-10 cm soil columns at 30 cm and 2 m from host plants. Research by Kuťáková et al. (2023) argued that the strongest influence of plant-soil feedbacks from dominant deep-rooted grass species may occur deeper in the soil profile, while shallow-rooted and less abundant plants can have a greater impact in the surface 0-10 cm layer. As such, these dynamics may be shaping the soil environment around *T. triandra*, which has a deep taproot, in a way that allows shallower-rooted species to exert more influence within 30 cm of *T. triandra* individuals – similar to the microbial composition observed in soils up to 2 m away. It is therefore likely that any legacy effects of *T. triandra* at 30 cm and 2 m distances were diminished by the influence of other plant species (including *T. triandra* individuals themselves) present in the soil at the time of sampling, which were not accounted for in this study (Wubs and Bezemer 2018; Kuťáková et al. 2023). Here, we suggest that bacterial legacies in bulk soil may fade quickly, or are quickly masked by co-occurring plants, and greater controls for this confounder would improve subsequent research on this topic.

In total, we identified 1,234 differentially abundant taxa across the aridity gradient, with substantially different microbiota profiles in high versus low aridity regions, suggesting a high degree of bacterial community turnover for this plant species across the natural range we studied. While our data revealed various environmental influences on soil and rhizosphere communities, aridity consistently emerged as a significant factor shaping bacterial communities. Canopy cover and leaf litter were important vegetation components for driving the microbial communities. The relationship between litter, vegetation and soil microbiota is a well-studied and important driver for ecosystem functioning (Liu et al. 2023). Litter dynamics, particularly in grasslands, influence nutrient and carbon inputs and mediate plant competition by affecting light availability and biotic factors (Prescott and Grayston 2013, Hassan et al. 2021). Microbial communities present on foliage, otherwise known as the phyllosphere microbiota, can be an important driver of litter decomposition (Fanin et al. 2021). Additionally, the taxonomic makeup of the phyllosphere communities during foliage deposition or the senescence of annual plants can modify the taxonomic make-up of subsequent colonising communities in deposed litter (Voříšková and Baldrian 2013, Purahong et al. 2016). As such, litter present in similar ecosystems via niche modification, or successional dynamics, could explain the relative importance of litter for driving microbial community assemblage in the *T. triandra* rhizospheres or soils (Fang et al. 2019, Fanin et al. 2021), and future research might further explore the modulation role of leaf litter on *T. triandra* microbiomes.

2.5 Conclusion

Our study demonstrates the increasing strength of relationships between *T. triandra* plants and their associated rhizosphere bacteria in more arid conditions. These findings are contributions to better understanding plant-soil feedbacks, especially in the context of climate change and aridity more broadly (O'Mara 2012, Hodgins and Moore 2016, Breed et al. 2019). Our study not only shows that plant-microbiota associations are strongly influenced by aridity levels, but we also identify key microbiota that have putative functional importance to *T. triandra* that promote strong symbiotic relationships under these arid conditions. Increasing aridity due to climate change will mean that a greater proportion of conservation and restoration efforts may need to consider these plant-soil feedbacks across species and ecosystems (Kardol and Wardle 2010, van der Putten et al. 2016, Valliere et al. 2020). Similarly to how we consider collecting high fitness seed sources for revegetation plantings under climate change (Jordan et al. 2016, Breed et al. 2019), it may be important to consider the importance of soil microbiotas that help support greater climate resilience during conservation or restoration interventions.

Acknowledgements

We acknowledge the traditional custodians of the lands on which we conducted our study, these include the Kaurna Pangkarra, Peramangk, Ngarrindjeri, Narangga, and Nukunu peoples. This work was supported by funding from the Ecological Society of Australia (Holsworth Wildlife Research Endowment), the Biological Society of South Australia and Nature Conservation Society of South Australia (Conservation Biology Grant), the Australian Research Council (grant numbers LP190100051, LP190100484) and the New Zealand Ministry of Business Innovation and Employment (grant UOWX2101). We thank Joel Brame, Carl Watson, Tarryn Davies, Billy Miller, and Victoria Drysdale for assistance in the field.

Figures

Figure 2.1. Overview of sampling and aridity gradient. (a) Map of *Themeda triandra* sampling sites (points) across an aridity gradient in southern Australia. (b) The association between the geographic distances between sites (Haversine distance matrix) and mean annual aridity index distances (Euclidian distance matrix). (c) Pan-palaeotropical distribution of *T. triandra* based on observations (points) from the Global Biodiversity Information Facility between years 2000-2023 (GBIF.org 2023), and aridity index data from version 3 of the Global Aridity Index and Potential Evapotranspiration Database (Global-AI_PET) (Zomer et al. 2022). (d) Density plot showing global *T. triandra* distribution with aridity index. Dashed blue lines represent the upper and lower limits of the aridity gradient sampled within this study, acording to the aridity data from the Atlas of living australia (Belbin 2011), where as the red lines represent limits according to Global-AI_PET database (Zomer et al. 2022), and 8% and 41% of the recorded global occurrence lay within these bounds, respectively.

Figure 2.2. Bacterial diversity and community differences. (a) Effective number of ASVs across different belowground zones: bulk soil at 2 m from host plant (purple), bulk soil at 30 cm from host plant (turquoise), and rhizospheres (orange); dashed white line denotes mean average. (b) Venn diagram showing the number and proportion of unique taxa across all sampling sites according to each belowground zone associated with *T. triandra* plants (bulk soils at 2 m and 30 cm from host plants, and rhizospheres) pooled across all sampling sites. (c) Effective number of ASVs across different belowground zones against mean annual aridity levels at each sampling population. Colour denotes belowground zone: bulk soil at 2 and, 30 cm from host plant, and rhizospheres. (d) Non-metric multi-dimensional scaling (NMDS) plot of differences between bacterial community composition using Bray-Curtis distances (stress = 0.1091). The colour of each point represents the mean annual aridity index, whereas coloured hulls and point shapes reveal belowground zones.

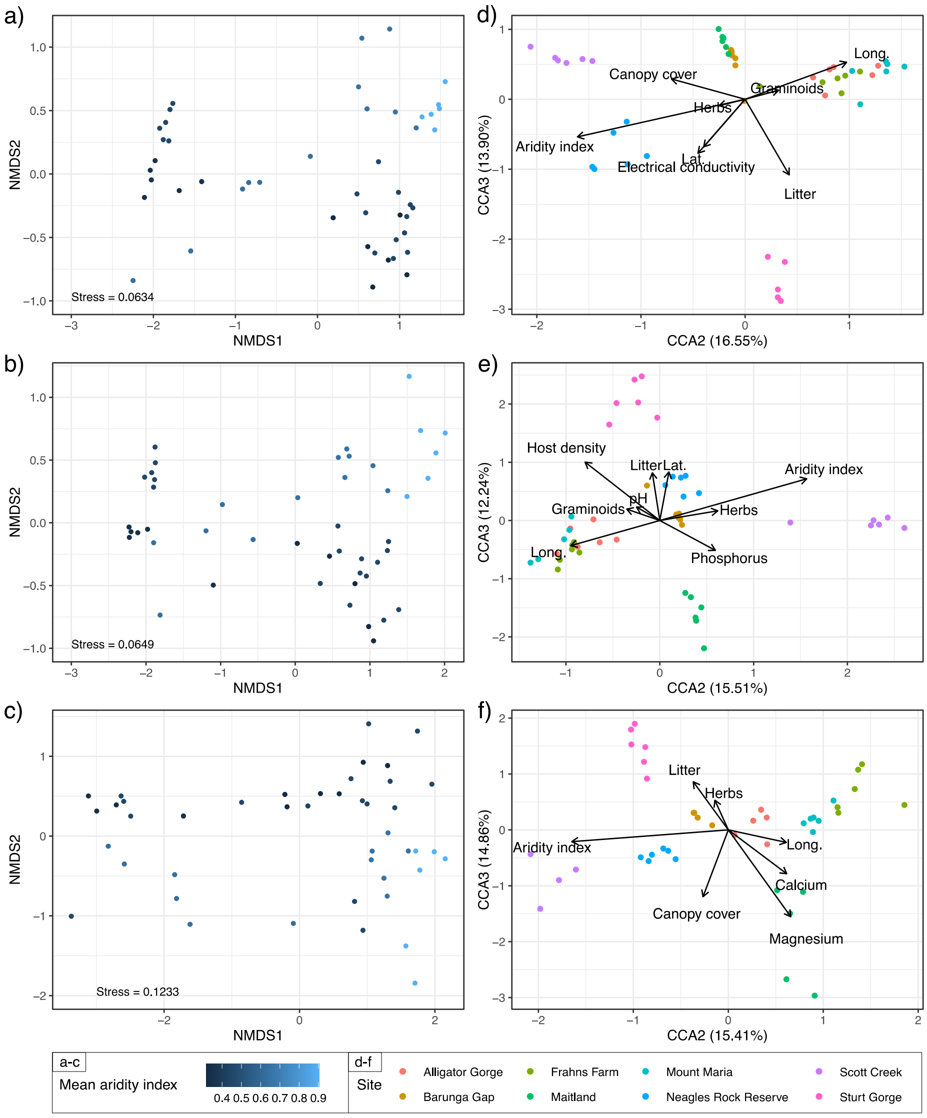

Figure 2.3. Treatment differences and environmental associations with whole bacterial communities. Non-metric multi-dimensional scaling (NMDS) plot of Bray-Curtis distances of bacterial community composition for: (a) bulk soil communities at 2 m from host plant (stress = 0.064), (b) bulk soil communities at 30 cm from host plant (stress = 0.0649), and (c) rhizosphere bacterial communities (stress = 0.1233). Canonical correspondence analysis (CCA) of the effect of the environmental variables on bacterial 16S community structure for: (d) soil at 2 m from host plant, (e) soil at 30 cm from host plant, and (f) *T. triandra* rhizospheres. Importantly, bulk soil analyses (d-e) considered soil physicochemical conditions potential environmental drivers for community structure, whereas the rhizospheres (f) analysis considered nutrients concentrations in the host plant (Table S2.2).

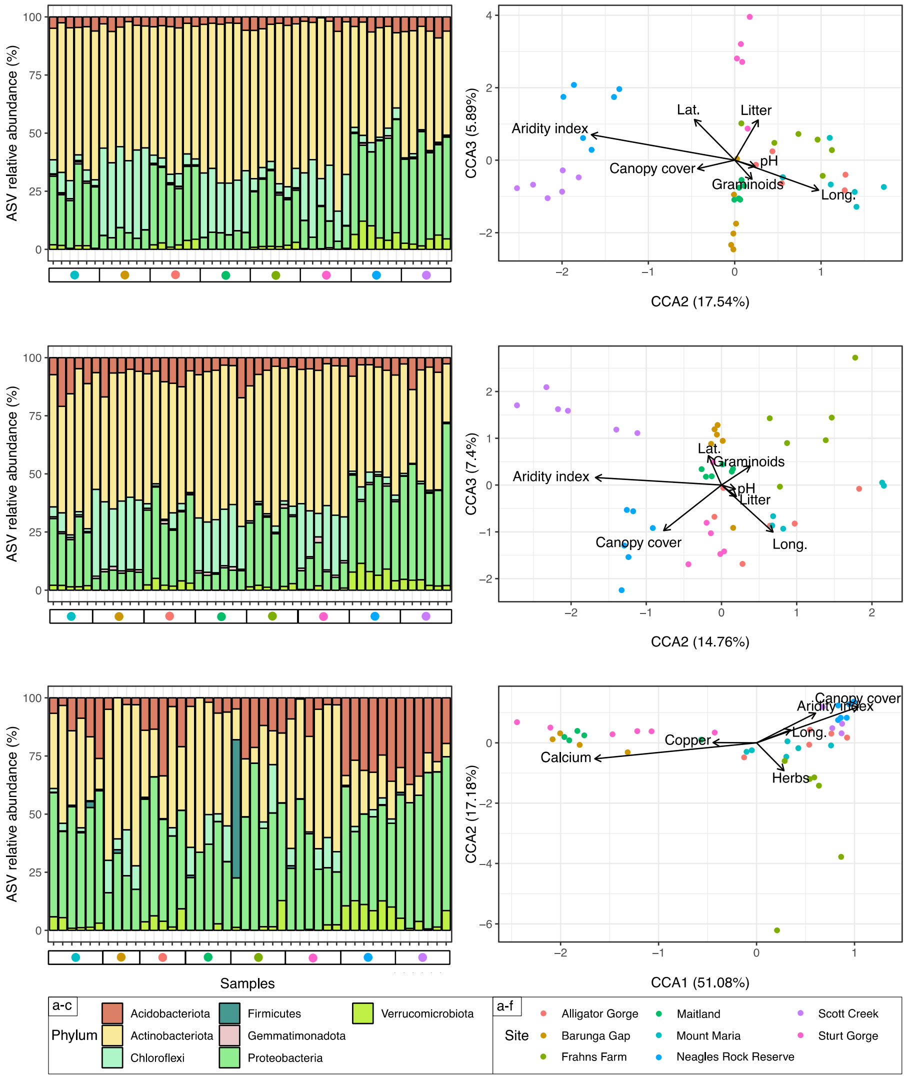

Figure 2.4. Core microbiome communities and CCAs. ASVs relative estimated abundance of bacterial phyla in candidate core microbiomes across: (a) soil samples at 2 m from host plant, (b) 30 cm from host plant, and (c) rhizosphere samples. Canonical correspondence analysis (CCA) of the effect of the climatic, edaphic geographic, and host related variables on bacterial 16S communities including only candidate core included ASVs. These include (d) soils at 2 m from host plant, (e) soils at 30 cm from host plant, and (f) T. triandra rhizospheres. Analysis of the bulk soil groups considered physicochemical properties of the soils as potential environmental drivers, whereas the rhizosphere communities considered nutrients in the host plant rather than soil factors (Table S2.2).

Figure 2.5. Aridity strengthens core rhizosphere microbiome associations. (a) Abundance occupancy curves to identify candidate bacteria for core microbiome membership across *T. triandra* rhizospheres. Blue points represent amplicon sequence variants (ASVs) selected as candidates for core microbiome membership in the rhizosphere (71 ASVs), white points represent taxa that were not selected as candidates for core microbiome membership. Core taxa were selected by first ranking taxa by occupancy, and then comparing their contribution to the Bray-Curtis similarity of the dataset until they provide a final 2% increase (see Methods for full details). The solid grey line represents a neutral model, with dashed lines revealing 95% confidence intervals. (b) Log fold change for differentially abundant ASVs (1,234 ASVs) across three aridity classes at the 0.05 significance level. (c) Relative abundance of sequences across each rhizosphere sample (points) made up of taxa identified as differentially abundant

(red), and taxa that are differentially abundant but are also candidates for core microbiome membership (orange), across an aridity gradient. Relationships between relative abundance and aridity index were tested by bootstrapping 95% confidence intervals 2000. Bootstrapped estimates are represented for each group by each transparent regression line. Only the relative abundance of differentially abundant, candidates core microbial communities (orange), had sufficient evidence for a negative relationship between aridity index and the relative, with a decrease in mean relative abundance by between -41.16% to -10.58% per unit of the aridity index (i.e. confidence intervals did not overlap 0).

References

Abdelfattah, A., A. Malacrinò, M. Wisniewski, S. O. Cacciola, and L. Schena. 2018. Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. Biological Control **120**:1-10.

ALA. 2014. Mean annual aridity index. Atlas of Living Australia.

Anthony, M. A., S. F. Bender, and M. G. A. van der Heijden. 2023. Enumerating soil biodiversity. Proceedings of the National Academy of Sciences **120**:e2304663120.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1-48.

Baxter, B. J. M., J. Van Staden, J. E. Granger, and N. A. C. Brown. 1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. Environmental and Experimental Botany **34**:217-223.

Belbin, L. 2011. The Atlas of Livings Australia's Spatial Portal.*in* In, Proceedings of the Environmental Information Management Conference 2011 (EIM 2011).

Bever, J., I. Dickie, E. Facelli, J. Facelli, J. Klironomos, M. Moora, M. Rillig, W. Stock, M. Tibbett, and M. Zobel. 2010. Rooting Theories of Plant Community Ecology in Microbial Interactions. Trends in Ecology & Evolution **25**:468-478.

Blair, G., N. Chinoim, R. Lefroy, G. Anderson, and G. Crocker. 1991. A soil sulfur test for pastures and crops. Soil Research **29**:619-626.

Breed, M. F., P. A. Harrison, C. Blyth, M. Byrne, V. Gaget, N. J. C. Gellie, S. V. C. Groom, R. Hodgson, J. G. Mills, T. A. A. Prowse, D. A. Steane, and J. J. Mohr. 2019. The potential of genomics for restoring ecosystems and biodiversity. Nature Reviews Genetics **20**:615-628.

Brinkman, E. P., C. E. Raaijmakers, W. de Boer, and W. H. van der Putten. 2017. Changing soil legacies to direct restoration of plant communities. AoB PLANTS **9**.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature **488**:91-95.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. v. Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology **64**:807-838.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods **13**:581-583.

Chen, Y., J. W. Neilson, P. Kushwaha, R. M. Maier, and A. Barberán. 2021. Life-history strategies of soil microbial communities in an arid ecosystem. The ISME Journal **15**:649-657.

Colwell, J. D. 1965. An automatic procedure for the determination of Phosphorus in sodium hydrogen carbonate extracts of soils. Chemistry Industry **22**:893-895.

Dadzie, F. A., A. T. Moles, T. E. Erickson, E. Slavich, and M. Muñoz-Rojas. 2022. Native bacteria and cyanobacteria can influence seedling emergence and growth of native plants used in dryland restoration. Journal of Applied Ecology **59**:2983-2992.

de Vries, F. T., R. I. Griffiths, C. G. Knight, O. Nicolitch, and A. Williams. 2020. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science **368**:270.

DEW. 2022. Guide to Climate Projections for Risk Assessment and Planning in South Australia 2022.*in* D. f. E. a. Water, editor., Government of South Australia, through the Department for Environment and Water, Adelaide.

Doby, J. R., D. J. Li, R. A. Folk, C. M. Siniscalchi, and R. P. Guralnick. 2022. Aridity drives phylogenetic diversity and species richness patterns of nitrogen-fixing plants in North America. Global Ecology and Biogeography **31**:1630-1642.

Dudenhöffer, J.-H., N. C. Luecke, and K. M. Crawford. 2022. Changes in precipitation patterns can destabilize plant species coexistence via changes in plant–soil feedback. Nature Ecology & Evolution **6**:546-554.

Dunning, L. T., A.-L. Liabot, J. K. Olofsson, E. K. Smith, M. S. Vorontsova, G. Besnard, K. J. Simpson, M. R. Lundgren, E. Addicott, R. V. Gallagher, Y. Chu, R. T. Pennington, P.-A. Christin, and C. E. R. Lehmann. 2017. The recent and rapid spread of *Themeda triandra*. Botany Letters **164**:327-337.

Escobar Rodríguez, C., B. Mitter, L. Antonielli, F. Trognitz, S. Compant, and A. Sessitsch. 2018. Roots and panicles of the C4 model grasses Setaria viridis (L). and S. pumila jost distinct bacterial assemblages with core taxa conserved across host genotypes and sampling sites. Frontiers in Microbiology **9**.

Estendorfer, J., B. Stempfhuber, G. Vestergaard, S. Schulz, M. C. Rillig, J. Joshi, P. Schroder, and M. Schloter. 2020. Definition of Core Bacterial Taxa in Different Root Compartments of Dactylis glomerata, Grown in Soil under Different Levels of Land Use Intensity. Diversity **12**:18.

Fang, K., L. Chen, J. Zhou, Z.-P. Yang, X.-F. Dong, and H.-B. Zhang. 2019. Plant-soil-foliage feedbacks on seed germination and seedling growth of the invasive plant *Ageratina adenophor*a. Proceedings of the Royal Society B: Biological Sciences **286**:20191520.

Fanin, N., D. Lin, G. T. Freschet, A. D. Keiser, L. Augusto, D. A. Wardle, and G. F. Veen. 2021. Home-field advantage of litter decomposition: from the phyllosphere to the soil. New Phytologist **231**:1353-1358.

Farley, G. J., S. M. Bellairs, and S. W. Adkins. 2013. Germination of selected Australian native grass species, with potential for minesite rehabilitation. Australian Journal of Botany **61**:283-290.

GBIF.org. 2023. Occurrence Download. The Global Biodiversity Information Facility.

Ghebrehiwot, H. M., M. G. Kulkarni, K. P. Kirkman, and J. Van Staden. 2012. Smoke and heat: influence on seedling emergence from the germinable soil seed bank of mesic grassland in South Africa. Plant Growth Regulation **66**:119-127.

Guerin, G. R., P. J. O'Connor, B. Sparrow, and A. J. Lowe. 2018. An ecological climate change classification for South Australia. Transactions of the Royal Society of South Australia **142**:70-85.

Hamonts, K., P. Trivedi, A. Garg, C. Janitz, J. Grinyer, P. Holford, F. C. Botha, I. C. Anderson, and B. K. Singh. 2018. Field study reveals core plant microbiota and relative importance of their drivers. Environmental Microbiology **20**:124-140.

Hancock, N., and L. Hughes. 2014. Turning up the heat on the provenance debate: Testing the 'local is best' paradigm under heatwave conditions. Austral Ecology **39**:600-611.

Hannula, S. E., R. Heinen, M. Huberty, K. Steinauer, J. R. De Long, R. Jongen, and T. M. Bezemer. 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. Nature Communications **12**:5686.

Hassan, N., K. Sher, A. Rab, I. Abdullah, U. Zeb, I. Naeem, M. Shuaib, H. Khan, W. Khan, and A. Khan. 2021. Effects and mechanism of plant litter on grassland ecosystem: A review. Acta Ecologica Sinica **41**:341-345.

Hassen, A. I., and N. Labuschagne. 2010. Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. World Journal of Microbiology & Biotechnology **26**:1837-1846.

Hayden, H. L., P. M. Mele, D. S. Bougoure, C. Y. Allan, S. Norng, Y. M. Piceno, E. L. Brodie, T. Z. DeSantis, G. L. Andersen, A. L. Williams, and M. J. Hovenden. 2012. Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO2 and warming in an Australian native grassland soil. Environmental Microbiology **14**:3081-3096.

Hijmans, R. 2023. terra: Spatial Data Analysis}.

Hodgins, K. A., and J. L. Moore. 2016. Adapting to a warming world: Ecological restoration, climate change, and genomics. American Journal of Botany **103**:590-592.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal **50**:346-363.

Hubbard, C. J., B. Li, R. McMinn, M. T. Brock, L. Maignien, B. E. Ewers, D. Kliebenstein, and C. Weinig. 2019. The effect of rhizosphere microbes outweighs host plant genetics in reducing insect herbivory. Molecular Ecology **28**:1801-1811.

Jordan, R., S. K. Dillon, S. M. Prober, and A. A. Hoffmann. 2016. Landscape genomics reveals altered genome wide diversity within revegetated stands of Eucalyptus microcarpa (Grey Box). New Phytologist **212**:992-1006.

Jost, L. 2006. Entropy and diversity. Oikos **113**:363-375.

Kardol, P., and D. A. Wardle. 2010. How understanding aboveground-belowground linkages can assist restoration ecology. Trends in Ecology & Evolution **25**:670-679.

Kuťáková, E., L. Mészárošová, P. Baldrian, Z. Münzbergová, and T. Herben. 2023. Plant–soil feedbacks in a diverse grassland: Soil remembers, but not too much. Journal of Ecology **00**:1-15.

Lin, H., and S. D. Peddada. 2020. Analysis of compositions of microbiomes with bias correction. Nature Communications **11**:3514.

Linder, H. P., C. E. R. Lehmann, S. Archibald, C. P. Osborne, and D. M. Richardson. 2018. Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. Biological Reviews **93**:1125-1144.

Liu, C., X. Li, F. R. P. Mansoldo, J. An, Y. Kou, X. Zhang, J. Wang, J. Zeng, A. B. Vermelho, and M. Yao. 2022. Microbial habitat specificity largely affects microbial co-occurrence patterns and functional profiles in wetland soils. Geoderma **418**:115866.

Liu, S., C. Plaza, R. Ochoa-Hueso, C. Trivedi, J. Wang, P. Trivedi, G. Zhou, J. Piñeiro, C. S. C. Martins, B. K. Singh, and M. Delgado-Baquerizo. 2023. Litter and soil biodiversity jointly drive ecosystem functions. Global Change Biology **n/a**.

McMurdie P. J., and S. Holmes. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data.*in* P. ONE, editor. PLoS ONE.

McNaughton, S. J. 1985. Ecology of a Grazing Ecosystem: The Serengeti. Ecological Monographs **55**:259-294.

McPherson, M. R., P. Wang, E. L. Marsh, R. B. Mitchell, and D. P. Schachtman. 2018. Isolation and Analysis of Microbial Communities in Soil, Rhizosphere, and Roots in Perennial Grass Experiments. JoVE:e57932.

Middleton, N., and D. Thomas. 1997. World atlas of desertification. Arnold: UNEP.

Morton, J. T., L. Toran, A. Edlund, J. L. Metcalf, C. Lauber, and R. Knight. 2017. Uncovering the Horseshoe Effect in Microbial Analyses. mSystems **2**:e00166-00116.

Muff, S., E. B. Nilsen, R. B. O'Hara, and C. R. Nater. 2022. Rewriting results sections in the language of evidence. Trends in Ecology & Evolution **37**:203-210.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature **368**:734-737.

Neu, A. T., E. E. Allen, and K. Roy. 2021. Defining and quantifying the core microbiome: Challenges and prospects. Proceedings of the National Academy of Sciences of the United States of America **118**:10.

Nishant, N., J. P. Evans, G. Di Virgilio, S. M. Downes, F. Ji, K. K. W. Cheung, E. Tam, J. Miller, K. Beyer, and M. L. Riley. 2021. Introducing NARCliM1.5: Evaluating the Performance of Regional Climate Projections for Southeast Australia for 1950–2100. Earth's Future **9**:e2020EF001833.

O'Mara, F. P. 2012. The role of grasslands in food security and climate change. Annals of Botany **110**:1263-1270.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, L. P., M. D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. S., Szoecs E., and W. H. 2019. vegan: Community Ecology Package.

Pascoe, B. 2018. Dark Emu: Aboriginal Australia and the Birth of Agriculture. Magabala Books.

Pérez-Ramos, I. M., A. Álvarez-Méndez, K. Wald, L. Matías, M. D. Hidalgo-Galvez, and C. M. Navarro-Fernández. 2021. Direct and indirect effects of global change on mycorrhizal associations of savanna plant communities. Oikos **130**:1370-1384.

Petipas, R. H., J. B. González, T. M. Palmer, and A. K. Brody. 2017. Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. Acta Oecologica **78**:71-78.

Pineda, A., I. Kaplan, and T. M. Bezemer. 2017. Steering Soil Microbiomes to Suppress Aboveground Insect Pests. Trends in Plant Science **22**:770-778.

POWO. 2023. Plants of the World Online (2023). Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 10 July 2023.

Prescott, C. E., and S. J. Grayston. 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. Forest Ecology and Management **309**:19-27.

Purahong, W., T. Wubet, G. Lentendu, M. Schloter, M. J. Pecyna, D. Kapturska, M. Hofrichter, D. Krüger, and F. Buscot. 2016. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Molecular Ecology **25**:4059-4074.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research **41**:D590-D596.

Risely, A. 2020. Applying the core microbiome to understand host-microbe systems. Journal of Animal Ecology **89**:1549-1558.

Saleem, A., F. U. Hassan, A. Manaf, and M. S. Ahmedani. 2009. Germination of *Themeda triandra* (Kangaroo grass) as affected by different environmental conditions and storage periods. African Journal of Biotechnology **8**:4094-4099.

Sarkar, S., A. Kamke, K. Ward, A. K. Rudick, S. G. Baer, Q. Ran, B. Feehan, S. Thapa, L. Anderson, M. Galliart, A. Jumpponen, L. Johnson, and S. T. M. Lee. 2022. Bacterial but Not Fungal Rhizosphere Community Composition Differ among Perennial Grass Ecotypes under Abiotic Environmental Stress. Microbiology Spectrum **10**:e02391-02321.

Schnitzer, S. A., J. N. Klironomos, J. HilleRisLambers, L. L. Kinkel, P. B. Reich, K. Xiao, M. C. Rillig, B. A. Sikes, R. M. Callaway, S. A. Mangan, E. H. van Nes, and M. Scheffer. 2011. Soil microbes drive the classic plant diversity-productivity pattern. Ecology **92**:296-303.

Shade, A., and N. Stopnisek. 2019. Abundance-occupancy distributions to prioritize plant core microbiome membership. Current Opinion in Microbiology **49**:50-58.

Singmann, H., Bolker, B, Westfall, J, Aust, F Ben-Shachar, MS. 2023. afex: Analysis of Factorial Experiments.

Smith, M. E., S. Delean, T. R. Cavagnaro, and J. M. Facelli. 2018. Evidence for species-specific plant responses to soil microbial communities from remnant and degraded land provides promise for restoration. Austral Ecology **43**:301-308.

Snyman, H. A., L. J. Ingram, and K. P. Kirkman. 2013. *Themeda triandra*: a keystone grass species. African Journal of Range & Forage Science **30**:99-125.

Tang, M., J. Liu, W. Hou, R. M. Stubbendieck, H. Xiong, J. Jin, J. Gong, C. Cheng, X. Tang, Y. Liu, Z. Li, J. Wang, and Y. Yi. 2021. Structural variability in the bulk soil, rhizosphere, and root endophyte fungal communities of Themeda japonica plants under different grades of karst rocky desertification. Plant and Soil.

Thiergart, T., P. Durán, T. Ellis, N. Vannier, R. Garrido-Oter, E. Kemen, F. Roux, C. Alonso-Blanco, J. Ågren, P. Schulze-Lefert, and S. Hacquard. 2020. Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. Nature Ecology & Evolution **4**:122-131.

Tukey, J. W. 1949. Comparing Individual Means in the Analysis of Variance. Biometrics **5**:99-114.

Turlure, C., S. Vandewoestijne, and M. Baguette. 2014. Conservation genetics of a threatened butterfly: comparison of allozymes, RAPDs and microsatellites. Bmc Genetics **15**:11.

UNEP. 1992. World Atlas of Desertification.

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

Valliere, J. M., W. S. Wong, P. G. Nevill, H. Zhong, and K. W. Dixon. 2020. Preparing for the worst: Utilizing stress-tolerant soil microbial communities to aid ecological restoration in the Anthropocene. Ecological Solutions and Evidence **1**:e12027.

van der Putten, W. H., M. A. Bradford, E. Pernilla Brinkman, T. F. J. van de Voorde, and G. F. Veen. 2016. Where, when and how plant–soil feedback matters in a changing world. Functional Ecology **30**:1109-1121.

Voříšková, J., and P. Baldrian. 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. The ISME Journal **7**:477-486.

Walkley, A., and B. I. Armstrong. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science **37**:29-38.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology **73**:5261-5267.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**:1629.

Wolfsdorf, G., A. Abrahão, A. M. D'Angioli, M. de Sá Dechoum, S. T. Meirelles, L. F. L. Pecoral, L. Rowland, L. da Silveira Verona, I. B. Schmidt, A. B. Sampaio, and R. S. Oliveira. 2021. Inoculum origin and soil legacy can shape plant–soil feedback outcomes for tropical grassland restoration. Restoration Ecology **n/a**:e13455.

Wubs, E. R. J., and T. M. Bezemer. 2018. Temporal carry-over effects in sequential plant–soil feedbacks. Oikos **127**:220-229.

Yang, G. W., J. Roy, S. D. Veresoglou, and M. C. Rillig. 2021. Soil biodiversity enhances the persistence of legumes under climate change. New Phytologist **229**:2945-2956.

Yuste, J. C., A. J. Fernandez-Gonzalez, M. Fernandez-Lopez, R. Ogaya, J. Penuelas, J. Sardans, and F. Lloret. 2014. Strong functional stability of soil microbial communities under semiarid

Mediterranean conditions and subjected to long-term shifts in baseline precipitation. Soil Biology & Biochemistry **69**:223-233.

Zhong, Y., P. O. Sorensen, G. Zhu, X. Jia, J. Liu, Z. Shangguan, R. Wang, and W. Yan. 2022. Differential microbial assembly processes and co-occurrence networks in the soil-root continuum along an environmental gradient. iMeta **n/a**:e18.

Zomer, R. J., J. Xu, and A. Trabucco. 2022. Version 3 of the Global Aridity Index and Potential Evapotranspiration Database. Scientific Data **9**:409.

Chapter three: Strong host modulation of rhizosphere-to-endosphere microbial colonisation in natural populations of the pan-palaeotropical keystone grass species, *Themeda triandra*

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https://doi.org/10.1002/ece3.71595

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The candidate was the primary author of the manuscript. Project design: **RJH**, SR, MFB; Field work: **RJH**, CCD, MFB; Lab work: **RJH**, CCD; Statistical analysis: **RJH,** CL; Writing manuscript: **RJH**; Revisions and editing: **RJH**, CL, CCD, SR, MFB. All authors gave approvals before final submission.

Percentage contribution: **RJH: 90%,** CCD: 1%, CL: 1%, SR: 1%, RAE 1%, MFB: 6%

Throughout this chapter I have used the pronoun ‘we’ to acknowledge the contributions of my coauthors, following the customary etiquette in published manuscripts.

Chapter three: Strong host modulation of rhizosphere-to-endosphere microbial colonisation in natural populations of the pan-palaeotropical keystone grass species, Themeda triandra

Abstract

Soil microbiota can colonise plant roots via a two-step selection process, which involves the recruitment of microbiota first from bulk soil into plant rhizospheres, then into root endospheres. This process is poorly understood in all but a few model species (i.e., *Arabidopsis)*, which is surprising given its fundamental role in plant and soil ecology. Here we examined the microbial assembly processes across the rhizospheres and root endospheres in eight natural populations of the pan-palaeotropical C4 grass, *Themeda triandra*, in southern Australia. We assessed whether root endosphere colonisation patterns aligned with the two step-selection process using space-for-time substitutions to compare their bacterial communities across these root compartments, and test whether their assembly was dominated by deterministic versus stochastic processes. We show that the two-step selection was the dominant recruitment dynamic across these natural *T. triandra* populations, and present clear evidence that host plants influenced microbial assembly via deterministic pressures that produced strong convergence of endospheres.. Furthermore, the *T. triandra* endospheres were strongly shaped by the host plant and displayed patterns consistent with the two-step selection process. These findings raise intriguing questions about the functions of this 'core' microbial endosphere, but our limited understanding of their ecology hinders our ability to harness these important relationships to, for example, improve plant propagation and revegetation practices.

3.1 Introduction

Soil microbiota have important roles in ecosystem functioning as they help to drive ecological processes (e.g., nutrient cycling) and make important contributors to plant growth and fitness (David et al. 2019, Wang et al. 2019, Choi et al. 2021). These soil microbiota commonly interact with plants via plant-soil feedbacks, where plants release organic exudates into the soil via their roots which then influence microbial community structure and diversity patterns (Bever et al. 2010). In turn, microbiota can provide their host plants with essential nutrients, protection against pathogens, and growth or fitness advantages via the release of metabolites and/or hormones (de Vries et al. 2020, Thiergart et al. 2020, Yang et al. 2021). Though generally poorly understood in non-model systems, a better understanding of these plant-soil feedbacks has promise to help ecosystem managers make more informed decisions about how to reintroduce or promote plant species (Breed et al. 2019, de Vries et al. 2020, Thiergart et al. 2020), especially during plant propagation, translocation and revegetation efforts (Peixoto et al. 2022, Robinson et al. 2023).

Soil microbiota can colonise plant roots via a two-step selection process, where certain soil microbiota are selectively recruited from bulk soil into plant rhizospheres (the soil and associated microbiota surrounding roots), and then into the root endosphere (the microbiota inside roots) via plant regulation processes (Lundberg et al. 2012, Bulgarelli et al. 2013, Urbina et al. 2018). This two-step selection process is promoted by the deposition of cells and organic exudates that attract microbiota into rhizospheres from bulk soils. From the rhizosphere, microbiota can enter into plant roots to form root endospheres via regulation of the plant's immune system (Bulgarelli et al. 2013). The differentiation in beneficial microbiota observed across plant rhizospheres and endospheres can be linked to how microbiota are selected by their host plants (Urbina et al. 2018, Stopnisek and Shade 2021). However, the assembly dynamics responsible for the microbial composition of rhizospheres and root endospheres are poorly explored, especially in non-model organisms (e.g., unlike for *Arabidopsis thaliana*) (Naylor and Coleman-Derr 2018, Sasse et al. 2018, Thiergart et al.

2020). Indeed, in natural systems, we might expect assembly processes to depend strongly on local environmental conditions, such as site-level aridity, but these dynamics remain poorly understood (Petipas et al. 2017, Hodgson et al. 2024).

Rhizospheres and endospheres can impact on host plant fitness (Zhang et al. 2020, Durán et al. 2022, Ling et al. 2022). Indeed, it is not only the most abundant microbial taxa that are important for plants; rare microbial taxa can also promote plant health and affect microbial community dynamics (Jousset et al. 2017, Neu et al. 2021, Custer et al. 2023). Identifying rare and abundant taxa, and taxa whose abundances are variable across ecological contexts (i.e., conditionally rare and/or abundant), can provide insight into rhizosphere and endosphere recruitment (Logares et al. 2014, Xue et al. 2018, Zhang et al. 2018). By separately considering these components of microbial communities, we can determine potential differences in their recruitment (i.e., how important are rare vs. abundant taxa?). Highly diverse recruitment strategies can highlight the importance of microbiota fulfilling multiple functions for their hosts, by for example, offering long term protection against stress or disturbance through generating functional redundancy (Naeem et al. 1994, Louca et al. 2018). Therefore, characterising the structure of microbial communities – plus microbial taxa that are selected for by plant hosts – can identify functionally important microbial taxa, plus the recruitment strategies used by the host plants (Hamonts et al. 2018, Risely 2020, Ling et al. 2022).

Neutral ecological theory has previously been used to describe the assembly of microbial communities in terms of deterministic versus neutral processes (Ofițeru et al. 2010, Stopnisek and Shade 2021). While neutral models are built on assumptions of functional equivalence among taxa (Zhou and Ning 2017, Rocha 2018), they enable direct comparisons of microbial communities based on whether taxa follow patterns expected under neutral processes that operate stochastically through random birth, death, and dispersal (Burns et al. 2016, Stopnisek and Shade 2021). Indeed, taxa deviating from these models may be affected more by environment/host selection. Different

types of deterministic selection processes can also be inferred by predicting rates of phylogenetic or taxonomic community turnover among communities, which would be expected under random population fluctuations (i.e., ecological drift) (Stegen et al. 2013). These include heterogenous selection (environments lead to greater phylogenetic turnover), or homogeneous selection (environments reduce phylogenetic turnover) (Stegen et al. 2012; Stegen et al. 2013; Ning et al. 2020). Furthermore, taxonomic turnover can also be used to predict dispersal rates via processes like homogenising dispersal (communities become more similar than expected due to high movement) or dispersal limitation (drift causes greater differentiation in communities). Overall, these methods offer a useful option to understand the complex processes shaping different microbiota.
Themeda triandra is a pan-palaeotropical C4 grass species that is globally dominant in many grassland ecosystems (Snyman et al. 2013). While this plant is widely distributed, grasslands are in global decline (Murphy et al. 2016, Bardgett et al. 2021), and there is a need to build new knowledge that assists the recovery of grassland ecosystems that are resilient to climate change (Gopal and Gupta 2016, Brinkman et al. 2017, Larson et al. 2022). Soil microbiota are known to strongly associate with *T. triandra* (Hodgson et al. 2024), and can aid its growth and fitness of this plant (Hassen and Labuschagne 2010, Petipas et al. 2017). Microbial communities linked to *T. triandra* fitness may also be susceptible to climate change impacts, including warming temperatures, increased CO2 and desertification (Hayden et al. 2012, Tang et al. 2021). Therefore, further understanding the composition of the microbial communities that directly interact with *T. triandra* root structures – such as those surrounding (i.e., rhizospheres) and within (i.e., endospheres) roots – across a diversity of climatic and soil conditions is a key step for identifying the microbial taxa and environmental circumstances that should promote the growth and fitness of this plant (Hayden et al. 2012, Snyman et al. 2013, Gonzalez et al. 2018).

Here, we examined the two-step selection process of *T. triandra* through a microbial community assembly lens, focusing on regional variation in its rhizospheres and endospheres. This study builds on our previous research Hodgson et al. (2024), which investigated the first step of the selection process – the movement of microbiota from bulk soil into rhizospheres using a space-for-time substitution – and found that rhizospheres exhibited lower diversity and distinct bacterial communities compared to surrounding soils. While we do not examine bulk soils in this study, we aim to address whether the bacterial communities within the endospheres of *T. triandra* originate from the diversity found in rhizospheres and whether regional differences affect patterns of endosphere colonisation by rhizosphere microbiota. Using 16S rRNA amplicon sequencing, we characterised the bacterial communities within rhizospheres and root endospheres of eight naturally occurring *T. triandra* populations along a southern Australian aridity gradient. Our focus is on the changing patterns between the rhizosphere and endosphere bacterial communities, the second step of the two-step selection process. To further investigate these dynamics, we used neutral theory models and diversity-based analyses to explore the different processes driving selection and bacterial colonisation across these different ecological populations. We posed the following research questions: (1) Do *T. triandra* rhizosphere and root endosphere bacterial communities align with the processes described in the two-step selection process, with reduced bacterial diversity in the endosphere compared to the rhizosphere? (2) Is there evidence of different deterministic or stochastic assembly processes within each site influencing the assembly of rhizosphere and endosphere bacterial communities? And (3), are the bacterial communities in the *T. triandra* endosphere within each site entirely constrained by the diversity of bacteria available in the rhizosphere, or are there other sources of bacterial recruitment?

3.2 Methods

3.2.1 Study species

Themeda triandra (Forssk.) is a pan-palaeotropical, perennial, C4 grass species that forms tussocks and generally reaches heights of 1 m, often dominating other species (Snyman et al. 2013, Dunning et al. 2017). As a keystone species, *T. triandra* is important for supporting invertebrate communities across stable environments (Snyman et al. 2013), and it has important associations with fire – for instance, it deposits flammable leaf litter that accumulate during growth, and its seeds respond well to smoke and high temperatures (Baxter et al. 1994, Ghebrehiwot et al. 2012). The seeds of *T. triandra* have been known to germinate best after long dormancy periods with substantial variation across regions, making it a difficult species to cultivate (Saleem et al. 2009, Farley et al. 2013, Hancock and Hughes 2014).

3.2.2 Observational field study

In December 2021, soil and plant tissue samples were collected from six *T. triandra* individuals across eight sites along an aridity gradient in southern Australia (aridity index values 0.318–0.903), as described in Hodgson et al. (2024) (Table 3.1; Figure 3.1a). There was no correlation between pairwise geographic distances and aridity differences between sites (Mantel: p = 0.489; r = -0.021). Mean annual aridity index data (annual precipitation/annual potential evaporation) was obtained from the Atlas of living Australia (Belbin 2011, ALA 2014) spatial portal, using the Aridity index layer (UNEP 1992, Middleton and Thomas 1997).

The six *T. triandra* plants were sampled from within a 25 x 25 m area at each study site using coordinates generated from a random number table, where for each coordinate the nearest plant was sampled. We profiled the diversity and community composition of bacteria in the rhizospheres and root endospheres of these plants using 16S rRNA amplicon sequencing (described below).

Isolation and extraction of rhizosphere and endosphere DNA

Microbial DNA from the rhizospheres was obtained following the protocol outlined in McPherson et al. (2018) and detailed in Hodgson et al. (2024). Briefly, sampled roots were washed in 0.02% Silwet L-77 amended PBS buffer and vortexed, before being filtered at 100 µm and centrifuged, prior to DNA extraction. *T. triandra* endospheres were extracted by removing as many bacteria and DNA as possible from root surfaces and subsequently extracting the DNA directly from these 'cleaned' root tissues. To determine the best methods of isolating *T. triandra* endosphere DNA, we ran a pilot study to compare methods of root cleaning via washing, bleaching and sonicating root surfaces (see *Supplementary Information: Chapter three* for further detail, Figures S3.1-S3.3). Based on our pilot study, roots were sonicated on ice in 0.02% Silwet L-77 amended PBS buffer at 30% amplitude for five 30 sec alternating burst and rest periods over 5 minutes. Following this, roots underwent a series of five washes in this sterilised amended PBS buffer solution. Root endosphere samples were pulverised with metal beads for 1 min in bead beating solution (PowerSoil Kit, Qiagen, Hilden, Germany). DNA extractions were then performed on rhizosphere and endosphere samples using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols.

3.2.3 Amplification, sequencing and bioinformatics

Amplicon libraries of the 16S rRNA V3-4 gene region were developed by the Australian Genome Research Facility (AGRF, Melbourne, Australia). Samples were PCR amplified with the forward primer, 341F (CCTAYGGGRBGCASCAG), and reverse primer, 806R (GGACTACNNGGGTATCTAAT). DNA libraries were sequenced on an Illumina MiSeq sequence platform with 300 base pair paired-end sequences. We used the DADA2 bioinformatic pipeline through QIIME2 (Bolyen et al. 2019) to infer identity profiles from amplicon sequence data by matching against the SILVA database (Version 138.1) (Wang et al. 2007, Quast et al. 2012) using a naïve Bayesian classifier (Wang et al. 2007, Callahan et al. 2016). We produced a table of unique amplicon sequence variants (ASVs) with abundance data and taxonomic annotations. All ASVs that were not assigned as Bacteria, and associated to mitochondria or chloroplasts were removed. We also removed taxa that did not occur in at least two samples across our datasets to avoid unrepresentative and potentially contaminating sequences/taxa .

3.2.4 Statistics

All analyses were done in R version 4.0.2 (R Core Team 2022). We rarefied samples to 11,491 reads without replacement to normalise for variation in library size across samples and to maintain a constant sampling effort for downstream analyses, both within and across sites (Figure S3.4). We estimated alpha diversity as effective number of ASVs (eff. no. ASVs.), which were calculated as the exponential transformation of Shannon's diversity index (Jost 2006), and Faith's phylogenetic diversity (Kembel et al. 2010). We compared these values across rhizospheres and endospheres using linear mixed-effects models (LMEM) with the lmer function in the lme4 package in R (Bates et al. 2015). Plant compartment (i.e., endosphere, rhizosphere) was treated as a fixed effect, and plant ID was included as a random effect to account for resampling the same individuals across rhizospheres and endospheres. Model significance was tested using a Walk chi-squared test, and pairwise tests were made using multiple comparisons with Tukey contrasts through the glht function of the multcomp package (Hothorn et al. 2008). We also assessed the number of unique taxa within each belowground compartment, and across sites using the Microeco package in R (Liu et al. 2020).

We visualised bacterial communities using non-metric multidimensional scaling ordination (NMDS) with Bray-Curtis distances. We also performed NMDS ordinations with β mean nearest taxon distance (βMNTD), and weighted unifrac (wunifrac) distances, to account for phylogenetic influences (Kembel et al. 2010). Compositional differences between bacterial community compartments in the endospheres and rhizospheres samples were tested via permutational multivariate analysis of variance (PERMANOVA) using the adonis2 function in vegan (Oksanen et al. 2019). Here, plant ID was included as a strata variable to account for repeated sampling of individual plants across both the rhizospheres and endospheres. When testing the effect of aridity on community composition, we also used compartment (rhizosphere or endosphere) as a strata variable to account for variation between these two community types. We also assessed for homogeneity of group dispersion with vegan's betadisper function (Oksanen et al. 2019).Visualisations of the relative abundance of the top 11 phyla across treatments were performed using the plot_bar function in Phyloseq (McMurdie P. J. and Holmes 2013). Rare phyla that made up less than 0.5% total relative abundance were grouped as 'Other minor phyla.'

Neutral theory models

We compared bacterial ASVs found in rhizospheres and endospheres to a neutral model of microbial community assembly to assess host plant selection processes on these taxa (Sloan et al. 2007, Burns et al. 2016, Stopnisek and Shade 2021). This was done via comparison to the Sloan neutral model, which assumes that community structures are principally driven by stochastic processes (i.e., reproduction, mortality, speciation, extinction, colonisation) (Sloan et al. 2007). Although these models can underrepresent taxa or species that are deterministically selected (Stopnisek and Shade 2021), we are still able to use them to hypothesise functionally useful ASVs that may play key roles in *T. triandra* microbiomes. ASVs outside the upper confidence intervals of the neutral model were inferred as those to have undergone positive selection, whereas ASVs outside the lower confidence interval were assumed to have undergone negative selection pressures in that environment.

We also tested the contribution of different community assembly processes on shaping microbiota across the rhizospheres and endospheres within and across each sampling site. Using the R package iCAMP (Ning et al. 2020), we first assessed pairwise sample differences using a null model of the β nearest taxon index (βNTI). A βNTI < -2 indicates homogeneous selection, while a βNTI > 2 suggests heterogeneous selection. Sample comparisons yielding βNTI values between -2 and 2 were considered not to be under any significant influence of selection (Stegen et al. 2013, Ning et al. 2020). These comparisons were further evaluated using Raup-Crick values based on Bray-Curtis dissimilarity (RCbray). RCbray values > 0.95 indicated the presence of dispersal limitation alongside heightened influence of drift, while values < -0.95 suggest homogenising dispersal. RCbray values between -0.95 and 0.95 indicate that drift was operating alone. To compare how different community assembly processes influenced rhizosphere and endosphere communities, we calculated the percentage of connections associated with each process in each sampling site: homogeneous selection, heterogeneous selection, homogenising dispersal, dispersal limitation acting with drift, and drift acting alone.

Conditional abundances of bacterial ASVs

To examine the rarity of bacteria across our dataset, we assigned ASVs to three groups – abundant, moderate and rare – according to whether they met relative abundance thresholds according to Xue et al. (2018). ASVs that had ≥1% relative abundance within their sampling sites were considered abundant taxa (AT), while ASVs with <0.01% relative abundance were considered rare taxa (RT). ASVs between these values (i.e., ≥0.01 but <1%) were considered moderate taxa (MT). Bacterial ASVs that were of relative abundances of ≥0.01% in all sites, but ≥1% in at least one site, were considered conditionally abundant taxa (CAT), whereas those that were found to be of <1% relative abundance in all sites, but <0.01% in at least one site, were conditionally rare taxa (CRT). The ASVs that had instances where relative abundance was at least <0.01% in one site, but ≥1% in another, were considered conditionally rare and abundant taxa (CRAT) The community composition of ASVs in these categories was visualised at the phylum level with chord diagrams using the R package circlize (Gu et al. 2014).

The different abundance categories were then compared against neutral models (described above) to investigate whether these ASVs underwent selection by the host plants. ASVs which were MT, RT, and CRT were examined separately, whereas ASVs that were CRAT and CAT were combined due to the low numbers of taxa in these groupings.

Differentially abundant taxa

We determined differentially abundant taxa across compartments and sites using the R package ANCOMBC (Lin and Peddada 2020) with function ancombc2 to reveal phyla and ASVs that were disproportionally present in endospheres versus rhizospheres communities. In this model, plant compartment (i.e., endosphere vs. rhizosphere) – with samples from all sites – was treated as a fixed effect, and plant ID was included as a random effect. We visualised differentially abundant taxa using log-fold changes, maintaining only statistically significant taxa at a 0.05 significance threshold.

We then conducted this analysis again, but for each individual sampling site separately, identifying differentially abundant taxa using the ancombc function across rhizospheres and endospheres, separately across each of the eight sites. Following software instructions, this differential abundance testing was based on non-rarefied data, and we used the false discovery rate for p-value adjustment for multiple comparisons (Benjamini and Hochberg method) at both phylum-level and ASV-level of our data to identify differing taxa between endospheres and rhizospheres. Comparisons across sites were then made to identify differences in the rhizosphere to endosphere recruitment dynamics within sampling sites.

We then used a three-step approach to explore the neutral and deterministic selection dynamics of the rhizosphere and endosphere differentially abundant ASVs. First, we isolated endosphere and rhizosphere ASVs from our dataset. Secondly, we created lists of ASVs that were disproportionately more abundant in the rhizosphere (= those with significant positive log fold change), those more abundant in the endosphere (= significant negative log fold change), and those that were not differentially abundant (non-significant effect). Finally, we examined neutral assembly models based on these three lists of ASVs, using rarefied data for making model comparisons (as used in our diversity analyses), to separately compare selection in the endospheres and rhizospheres. We used this approach to show how selection on ASVs changed from the rhizosphere to endosphere. Model fits were assessed using the coefficient of determination (R2).

Co-occurrence network analysis

We used co-occurrence network analysis of bacterial ASVs to determine interactions between taxa and derive an indication of community structure within endospheres and rhizospheres. This analysis explores connections between different specific ASVs (i.e., nodes) via their correlative connections to one another (i.e., edges) by estimating a significant positive or negative relationship between these taxa. ASVs were filtered to the number of associations within the communities to give a measure of community complexity and to compare patterns of occurrence of taxa within endospheres and rhizospheres. We used SparCC to define absolute abundance associations between taxa at the ASV level, using the Spiec-Easi R package (Friedman and Alm 2012, Kurtz et al. 2015).

For visualisations and computational processing of the network analyses, we only report ASVs with >100 sequences. Randomly permuted (n = 1000) data were used to estimate the statistical significance of associations. Taxon associations were included using SparCC correlations at ≥0.65, with $p < 0.05$. We used the R package Matrix (Bates et al. 2023) to create a matrix from the given set of values and igraph (Csardi et al. 2006) to visualise and evaluate the plots. We identified 'hub' taxa as the top 30 bacterial ASVs with the highest number of positive or negative connected edges (node degrees). Of the significant relationships, these have the strongest effect sizes. These taxa likely have important roles within a community, based on their links to other taxa.

3.3 Results

3.3.1 Bacterial diversity in belowground compartments

Across all compartments (rhizospheres and endospheres), we observed 11 bacterial phyla that represented 99.5% of reads and had abundance estimates of >2% (Figure 3.2a).

The alpha diversity of endospheres at the ASV level was 48.3% lower than rhizosphere diversity (effective number of ASVs was 153 in endospheres, versus 296 in rhizospheres) (LMEM: $X^2_{(1)}$= 56.220, p <0.001; Figure 3.1b), and there were many differences in alpha diversity across the sampling sites (LMEM: $X^2_{(7)}$= 24.522, p <0.001). Interestingly, there was no difference between the rhizospheres and endospheres using Faith's phylogenetic diversity (Figure S3.5: LMEM: $X^2_{(1)}$= 0.7781, p <0.378). Additionally, 96% of ASVs were shared by both compartments (Figure 3.3a), and 3.6% of taxa were unique to the endospheres (= 1031 ASVs), whereas only 0.3% of taxa were unique to rhizospheres (= 158 ASVs).

The composition of bacterial communities in *T. triandra* endospheres and rhizospheres based on Bray-Curtis dissimilarity (Figure 3.1c; PERMANOVA: $F_{(1, 76)}$ = 10.888, R^2= 0.078, p = 0.001, n= 48 samples per group), and communities were tightly clustered by sampling site (Figure S3.6; PERMANOVA: R^2= 0.287, $F_{(7,76)}$ = 5.741, p= 0.001, n= 6 per site). We also found that bacterial community composition changed with the aridity levels of our sampling sites across both the rhizospheres and endospheres (Figure S3.7; PERMANOVA: R^2= 0.049, $F_{(1,90)}$ = 4.626, p < 0.001).

We also found differences between rhizospheres and endosphere bacterial communities based on phylogenetically informed metrics: βMNTD (Figure S3.8a; PERMANOVA: R^2= 0.078, $F_{(1,90)}$ = 7.660, p < 0.001), and wunifrac (Figure S3.9a; PERMANOVA: R^2= 0.034, $F_{(1,90)}$ = 3.129, p < 0.001). Bacterial community composition also changed with differing site aridity using wunifrac distances (Figure S3.9b; PERMANOVA: R^2= 0.065, $F_{(1,42)}$ = 2.914, p < 0.001), and βMNTD (Figure S3.8ba; PERMANOVA: R^2= 0.040, $F_{(1,90)}$ = 3.719, p < 0.001).

Endospheres were less varied than rhizospheres based on Bray-Curtis distances (Figure 3.2d; PemuTest : $F_{(1,90)}$=36.24, p <0.001), βMNTD (Figure S3.8c; PemuTest: $F_{(1,90)}$= 17.475, p <0.001), and wunifrac distances (Figure S3.9c; PemuTest: $F_{(1,90)} = 22.486$, *p* <0.001), suggesting reduced heterogeneity, and converging bacterial communities in *T. triandra* endospheres.

3.3.2 Taxonomic rarity and abundance

Bacterial ASVs were delineated into different rarity and abundance categories (see Methods for details; Table 3.2). Only one ASV was abundant in the endospheres across all samples (unidentified ASV from the genus *Bradyrhizobium*, phylum: Proteobacteria), and no ASVs were abundant in the rhizosphere (>1% abundance). As a proportion of the whole community, the greatest difference between endospheres and rhizospheres was in the conditionally rare taxa (<1% in all sites, but <0.01% in some), which comprised 62% of bacterial sequences in the endospheres (5,070 ASVs) and 79% of sequences in the rhizospheres (5,705 ASVs; Table 3.2). The rare taxa (<0.01% in all sites) also showed a large difference between compartments, comprising 37% of sequences in the endospheres (2,970 ASVs) and 21% of sequences in the rhizospheres (1,477 ASVs; Table 3.2).

Across the rhizospheres and endospheres, all ASVs from both communities had similar taxonomic compositions when considering their phyla across MT, CAT, and CRAT categories (Figure 3.2b-c). However, we did see a change in the relative number of bacterial sequences in the RT to CRT categories between rhizospheres and endospheres (Figure 3.2b-c).

3.3.3 Differentially abundant taxa among endospheres and rhizospheres

We found that 13 bacterial phyla were differentially abundant across the endospheres and rhizospheres using the ANCOMBC approach (analysis conducted at the phylum level: Figure S3.10a; Table S3.1). These were phyla with log fold change level differences in their abundances across these two groups. For instance, phyla that were more abundant in the rhizosphere and reduced in the endosphere, and included bacteria attributed to: Verrucomicrobiota, WSP2, Chloroflexi, Armatimonadota, RCP2-54, Acidobacteriota, Gemmatimonadota, and Planctomycetota (Figure 3.2d).

The endosphere abundant phyla included: Patescibacteria, Actinobacteriota, Proteobacteria and Myxococcota (Figure 3.2d). In a separate differential abundance analysis at the ASV level, we found 218 ASVs were differently abundant (Figure 3.4a; Table S3.2). Additional findings describing differentially abundant phyla at each site can be found in the *Supplementary Information* (see Figure S3.7, Figure S3.11-S3.12)

When we conducted this analysis at the ASV level for each site independently, we found 388 differentially abundant ASVs that were significantly different between endospheres and rhizospheres (Figure S3.13). We observed 182 ASVs were more abundant in endospheres (negative log fold changes) and that 217 ASVs were more abundant in rhizospheres (positive log fold changes; Figure 3.4b). Interestingly, the differentially abundant ASVs between rhizosphere and endospheres at the site level were often unique to each site. Only 1 common ASV was differentially abundant between the rhizospheres and endospheres in every site, whereas a mean of 197 ASVs were uniquely differentially abundant across all sites (Figure 3.4c). The remaining ASVs were shared across two or more sites in various combinations, with diminishing counts as site-site comparisons became more inclusive (Figure S3.13-3.14).

3.3.4 Selection of microbiota under neutral theory of community assembly

We fitted neutral assembly models to our rhizosphere and endosphere samples including samples from all sites, to explore the neutral versus deterministic influences that shape their assembly. Endospheres fitted the neutral model to a lower degree than rhizospheres (Figure 3.5; endosphere, $R^2 = 0.317$, rhizosphere, $R^2 = 0.464$). This reveals greater deterministic influence for shaping the selection of microbiota into the rhizosphere.

We then applied the neutral model to the different rarity and abundance categories of bacterial ASVs in rhizospheres and endospheres of all sites to explore differences in the assembly of the unique structural elements of the microbiota (i.e., rare and abundant taxa) (Figure S3.15). The abundance patterns of MT and CRT had better fit to the neutral models in the rhizospheres ($R^2 = 0.469$, Figure S3.15a; $R^2 = 0.49$, Figure S3.15c, respectively) compared to the endospheres ($R^2 = 0.12$, Figure S3.15e; $R^2 = 0.424$, Figure S3.15g, respectively). The CRAT+CAT and RT neutral models had poorer fits in rhizospheres (albeit with RT producing a poor fitting model; $R^2 = 0.295$, Figure S3.15d; $R^2 = -0.129$, indicating failure to fit a model, Figure S3.15b, respectively), compared to the endospheres ($R^2 = 0.175$, Figure S3.15f; $R^2 = 0.466$, Figure S3.15h, respectively). Additional information on how the neutral models fit other subsets of our differential abundance analyses can be found in the *Supplementary information* (Figures S3.12, S3.16).

In the rhizospheres and endospheres of each sampling site we tested the influence of phylogenetic and bacterial community turnover to investigate the different deterministic and stochastic influences acting on these microbial communities. The endosphere microbiota were influenced by stronger selection pressures compared to the rhizospheres, with an average selection effect of 72.5% (±7.6% SE) for βNTI values > 2 or < -2, compared to 44.6% (±6.5% SE) for the rhizospheres (Figure 3.5c; Figure S3.17). Specifically, the endospheres were driven by homogeneous selection which identify lower rates of phylogenetic turnover than expected under our null hypothesis (Figure 3.5d). Only rhizosphere communities were under any influence of heterogeneous selection (7.1% ±3.4% SE), though they were mainly driven by homogeneous selection 37.5 % (±7.1% SE). The dominance of stochastic processes also differed between rhizospheres and endospheres in the different sites. The effect of dispersal limitation plus drift (RCbray > 0.95), on average, was stronger for stronger in the rhizospheres 50.8% (±6.6% SE) than the endospheres 8.3% (±4.3% SE), but interestingly the drift acting alone (RCbray ≥ -0.95, but ≤ 0.95) had, on average, a stronger influence on the endosphere communities 19.2% (±8.2% SE) than the rhizospheres 4.6% (±1.9% SE) (Figure 3.5d). We did not detect an influence of homogenising dispersal in the rhizospheres or endospheres (RCbray < -0.95).

3.3.5 Network analysis and hub taxa

Our network analysis included only associations between nodes (ASVs) with SparCC correlations ≥ 0.65 and p-values < 0.05. We then removed any isolated nodes, resulting in endosphere networks with 81 nodes (ASVs) and rhizosphere networks with 60 nodes (Figure 3.6, Tables S3.3-S3.6). The rhizosphere networks had a lower average node degree (4.43 ± 0.63 SE vs. 8.44 ± 1.09 SE; Figure 3.6), indicating fewer significant associations between microbiota in rhizospheres compared to endospheres. Rhizospheres also showed lower average edge weight values (0.27 ± 0.06 SE vs. 0.34 ± 0.03 SE; Figure 3.6), suggesting more negative associations between taxa, while endospheres exhibited more positive associations among ASVs.

3.4 Discussion

We investigated the patterns of microbial assembly in rhizospheres and root endospheres in natural populations of the non-model pan-palaeotropical C4 grass species, *Themeda triandra*. We found that rhizosphere and endosphere diversity patterns were consistent with the second step of the two-step selection process (Bulgarelli et al. 2013) – endospheres were less diverse than rhizospheres. We also observed convergence in endospheres across populations, where these bacterial communities were more homogeneous than rhizospheres. Despite this convergence, endosphere recruitment was also influenced by site-specific factors, including aridity (with differences among bacterial community compositions across the gradient). We found more unique bacterial ASVs in endospheres than rhizospheres, which suggests a potential role of vertical transmission (i.e., parent to offspring transfer) and/or life stage dependency on endosphere colonisation. Finally, we found that assembly processes in endospheres had stronger deterministic influence in the rhizosphere, and that there was a core microbiome (taxa that persistently occur within a given environment) in these endospheres that probably supports the functioning of *T. triandra*. A deeper understanding of these microbial interactions would help inform plant and soil resource management during conservation and restoration efforts (e.g., propagation, translocation, revegetation).

3.4.1 Two-step selection process

We observed that overall bacterial diversity in *T. triandra* endospheres was approximately 48% lower than in rhizospheres. Both endospheres and rhizospheres had distinct community compositions from each other, which is consistent with expectations under the two-step selection process. Our diversity and composition findings are in line with previous work on *Arabidopsis thaliana*, where several studies have now shown the selection of microbiota across soil and rhizosphere environments into root endospheres (Bulgarelli et al. 2013, Urbina et al. 2018, Barajas et al. 2020). These previous studies suggest that the controlled release of exudates by the plant attracts and supports the recruited microbiota (Bai et al. 2022). Though we did not directly measure root exudates, we do find compelling results from a bacterial community perspective which is supported by our previous *T. triandra* soil-rhizosphere study (Hodgson et al. 2024). Here, we present strong evidence that two-step selection process outlined by Bulgarelli et al. (2013) is active in the rhizospheres and endospheres of this non-model, keystone grass species. Importantly, we report these results from naturally occurring populations of this grass species, which is a noteworthy difference to previous studies, which generally focussed on plants growing *ex situ* and under controlled lab or greenhouse conditions.

Our detailed investigation into assembly patterns revealed stronger deterministic processes in the endospheres compared to the rhizospheres, which is consistent with the general assumptions of the two-step selection process – the host plant is expected to exert greater regulatory and selective control over microbiota entering the roots than those in the rhizosphere (Bulgarelli et al. 2013). Additionally, the endospheres also contained more ASVs that deviated from the neutral theory model than the rhizospheres, and homogeneous selection processes were dominant for explaining the assembly of endosphere communities. As such, endospheres recruit phylogenetically similar bacterial communities, likely for distinct roles that target similar bacterial traits according to the eco-physiological needs of the host plant or required to pass host immune system filtering (Stegen et al. 2012, Zhang et al. 2021, Wang et al. 2023).

Alongside the homogeneous selection acting on the endosphere communities, we found a stronger than expected influence of ecological drift (random population changes) on the endosphere microbial communities. Microbe-microbe interactions, specifically priority effects, may explain this, as early colonisers likely create conditions in the endospheres that facilitate the establishment of other species (Rillig et al. 2015). These interactions could alter the endosphere environment through resource competition and/or metabolic processes that limit the strength of host-imposed selection processes. As such, stochastic events – such as random fluctuations in microbial populations – can play a larger role in community assembly than expected in such a regulated environment (Rillig et al. 2015, Debray et al. 2022). In contrast to the endospheres, the rhizosphere microbiota were more strongly influenced by dispersal limitation (low dispersal rates) compared to ecological drift alone (Stegen et al. 2013). The low community turnover in the rhizospheres suggests that these bacterial communities were strongly shaped by the diversity and dispersal potential of local soil microbiota (Zhang et al. 2021). This highlights the constraining influence of local conditions on the two-step selection process. Future work should focus on identifying the root exudates involved and assessing the fitness consequences of these assembly processes by characterising the functional processes of microbiota involved.

3.4.2 Endosphere convergence

We report that local site conditions influenced endosphere recruitment dynamics, which resulted in a unique assortment of differentially abundant bacterial ASVs in the endospheres across sites – an effect also observed in previous studies on the trees *Populus deltoides* and *Taxodium distichum* (Gottel et al. 2011, Lumibao et al. 2020). Observing different endospheres across sites suggests that either stochastic effects, local conditions and/or resource availability affected how *T. triandra* regulates inbound microbiota (Vandenkoornhuyse et al. 2015). These influences were consistent with our earlier work which showed that *T. triandra* bulk soil microbial communities and rhizospheres were strongly shaped by soil nutrient levels, aridity and moisture availability (Hodgson et al. 2024). Local conditions are well known to shape bulk soil, rhizospheres and endospheres, however, in our study, these site-specific effects did not appear to impede the development of a convergent root endosphere across populations. Factors that shape internal microbial profiles could also shape preferential niches created by the host plant or some combination of other influences, such as microbe-mediated priority effects (Rillig et al. 2015). This raises intriguing questions about the functional potential of the 'core' microbial endosphere, and follow-up studies should investigate this further.

We reported higher overall complexity (based on node degree in our network analysis) and positive associations of ASVs in endospheres compared to rhizospheres, indicating remarkable symbiosis inherent in the convergence of bacterial communities in these root compartments. The top connected ASVs (= hub taxa) are often hypothesised to be keystone species that support or facilitate the recruitment of other microbiota (Rillig et al. 2015, Trivedi et al. 2020, Debray et al. 2022). Additionally, a decrease in the ratio of conditionally rare taxa to rare taxa within rhizospheres compared with endospheres (1.71 versus 3.84, respectively) shows that rhizospheres often support highly varied microbial community structures that are also more diverse (i.e., greater alpha diversity). As expected, we report new evidence of bacterial symbioses in *T. triandra* endospheres (i.e., less influenced by local soil and/or climatic conditions) relative to rhizospheres, which supported ASVs with fewer key microbe-microbe associations (Trivedi et al. 2020).

3.4.3 Vertical transmission of microbiota

The high count of bacterial ASVs that were unique to root endospheres were likely populated via vertical transmission (i.e., from parent plant flowers to their offspring during seed development)

(Bulgarelli et al. 2013, Escobar Rodríguez et al. 2018, Abdelfattah et al. 2023); or transferred across host compartments (e.g., leaves or stems into roots) (Chi et al. 2005). There is strong evidence of vertically transmitted bacterial endophytes being involved in mobilising plant nutrients and affect phytohormone signalling inside roots (Bulgarelli et al. 2013, Santoyo 2022). Future research should explore whether unique ASVs within each site are inherited through vertical transmission due to local adaptation of *T. triandra* populations (Thiergart et al. 2020, Durán et al. 2022). As such, this form of parent to offspring transfer could be important to *T. triandra* fitness, where microbiota cannot survive independently in soil environments and host plants may have evolved traits that facilitate the persistence of a portion of the microbial community (Johnston-Monje and Raizada 2011, Lumibao et al. 2020, Lyu et al. 2021).

It is worth considering that the ASVs suspected of vertical transmission in this study could still be a product of the two-step selection process, especially if we simply did not observe them in the rhizosphere during sequencing due to insufficient sequence depth or the changing nature of rhizospheres across plant developmental stages. Further research could investigate how horizontally transferred bacterial taxa (i.e., soil to root endosphere colonisation) are supported in soil environments and whether they require their plant hosts for completion of their lifecycles (i.e., are they obligate symbionts?) (Vandenkoornhuyse et al. 2015). These ASVs may have a dormant, protected life stage (e.g., spore-forming) (van Vliet 2015), or could perhaps be microbiota that are influenced by host plant demographics and local adaptation (Ledeganck et al. 2003, Hannula et al. 2021). Further investigations should consider the vertical transmission of root endospheres.

3.5 Conclusions

We show that the microbiomes of natural populations of *T. triandra* growing across diverse environments retain assembly processes consistent with root endosphere colonisation from rhizospheres. We show that deterministic assembly processes acted strongly on these endospheres, as they were strongly affected by both environmental factors (e.g., aridity) plus host selection for similar microbial communities and traits within sampling sites (homogeneous selection). Additionally, while numerous endosphere taxa were likely from the plant rhizospheres, we present evidence for probable vertical transmission of microbiota from parent to offspring. Our limited understanding of the complex roles of plant-associated microbiota hinders our ability to harness the ecology of these important relationships in applied ecology context (e.g., propagation, translocation, revegetation). Future investigations should consider the functional roles and inheritance patterns of root endosphere microbiota in non-model plant species, and assess how these plant-microbe interactions effect host fitness.

Acknowledgements

We thank Antonino Malacrinò and an anonymous reviewer for their comments on an earlier version of this manuscript. We thank Victoria Drysdale, Chris Franco, Shawn Peddle, Nicole Fickling, Joel Brame, Carl Watson, Billy Miller, and Tarryn Davies for their assistance in the field. We acknowledge the traditional custodians of the lands on which we conducted our study: the Kaurna Pangkarra, Peramangk, Ngarrindjeri, Narangga, and Nukunu Peoples.

This work was supported by funding from Holsworth Wildlife Research Endowment with the Ecological Society of Australia, the Conservation Biology Grant 2022 with the Biological Society of South Australia and Nature Conservation Society of South Australia, the Lirabenda Wildlife Research Fund from the Field Naturalists Society of South Australia, the Australian Research Council (grant numbers LP190100051, LP190100484) and the New Zealand Ministry of Business Innovation and Employment (grant UOWX2101).

Tables

Table 3.1. *T. triandra* sampling sites across southern Australia.

Site name	Latitude, longitude	Aridity index	Sampling date
Alligator Gorge	-32.71487, 138.10172	0.445	15 Dec 2021
Barlunga Gap	-33.82000, 138.17392	0.347	14 Dec 2021
Frahn's Farm	-35.07231, 139.09781	0.454	19 Dec 2021
Maitland	-34.37366, 137.71203	0.453	21 Dec 2021
Mount Maria	-32.65862, 138.08985	0.318	16 Dec 2021
Neagles Rock Reserve	-33.85031, 138.60674	0.651	14 Dec 2021
Scott Creek	-35.08720, 138.67266	0.903	19 Dec 2021
Sturt Gorge	-35.03311, 138.57324	0.634	13 Dec 2021

Table 3.2. Bacterial ASVs allocated to six relative abundance categories

Compartment	Category	Number of ASVs	Number of sequences
Rhizosphere ~	Abundant taxa (AT)	0	0
	Moderate taxa (MT)	11 (0.15%)	7821 (1.55%)
	Rare taxa (RT)	1477 (20.45%)	5518 (1.09%)
	Conditionally abundant taxa (CAT)	4 (0.06%)	19238 (3.81%)
	Conditionally rare taxa (CRT)	5705 (78.99%)	429588 (84.97%)
	Conditionally rare and abundant taxa (CRAT)	25 (0.35%)	43439 (8.59%)
Endosphere ~	Abundant taxa (AT)	1 (0.01%)	13094 (2.37%)
	Moderate taxa (MT)	6 (0.07%)	6755 (1.23%)
	Rare taxa (RT)	2970 (36.69%)	11938 (2.16%)
	Conditionally abundant taxa (CAT)	4 (0.05%)	31755 (5.76%)
	Conditionally rare taxa (CRT)	5070 (62.63%)	322711 (58.51%)
	Conditionally rare and abundant taxa (CRAT)	44 (0.54%)	165315 (29.97%)

Figures

Figure 3.1. *T. triandra* sampling sites, and bacterial diversity. (a) Map showing Australia and the sampling locations of *Themeda triandra* populations (blue points) across a strong aridity gradient in southern Australia. (b) Bacterial alpha diversity as effective number of ASVs in *T. triandra* rhizospheres. (c) NMDS ordination showing the differences in bacterial community composition between rhizospheres (blue) and endospheres (red). (d) Distance to centroid of samples comparing rhizosphere (blue) and endosphere (red) samples, calculated from Bray-Curtis dissimilarity.

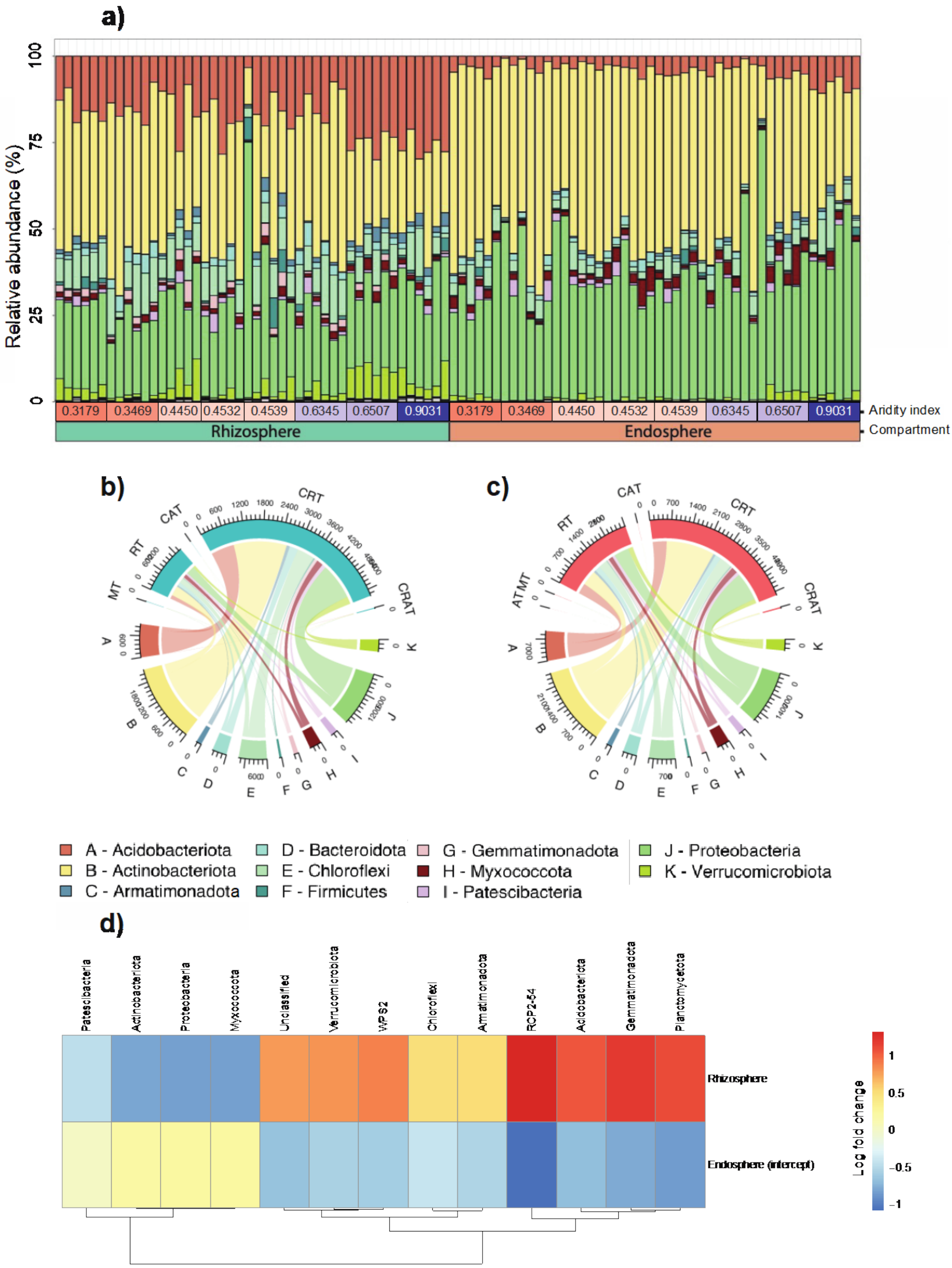

Figure 3.2. Bacterial ASV relative abundances visualised at the phylum level in endospheres and rhizospheres. (a) Stacked bars represent samples, grouped by aridity index of their sampling site. The bar colours represent the bacterial phylum. Chord diagrams for (b) rhizospheres and (c)

endospheres, showing the relative proportion of each bacterial ASV within each phylum (groupings: A-K) found within bacterial abundance categories (AT – abundant taxa, MT – moderate taxa, RT – rare taxa, CAT – categorically abundant taxa, CRT – categorically rare taxa, and CRAT – categorically rare and abundant taxa). (d) Differential abundance analysis of major and minor bacterial phyla across the rhizospheres and endospheres.

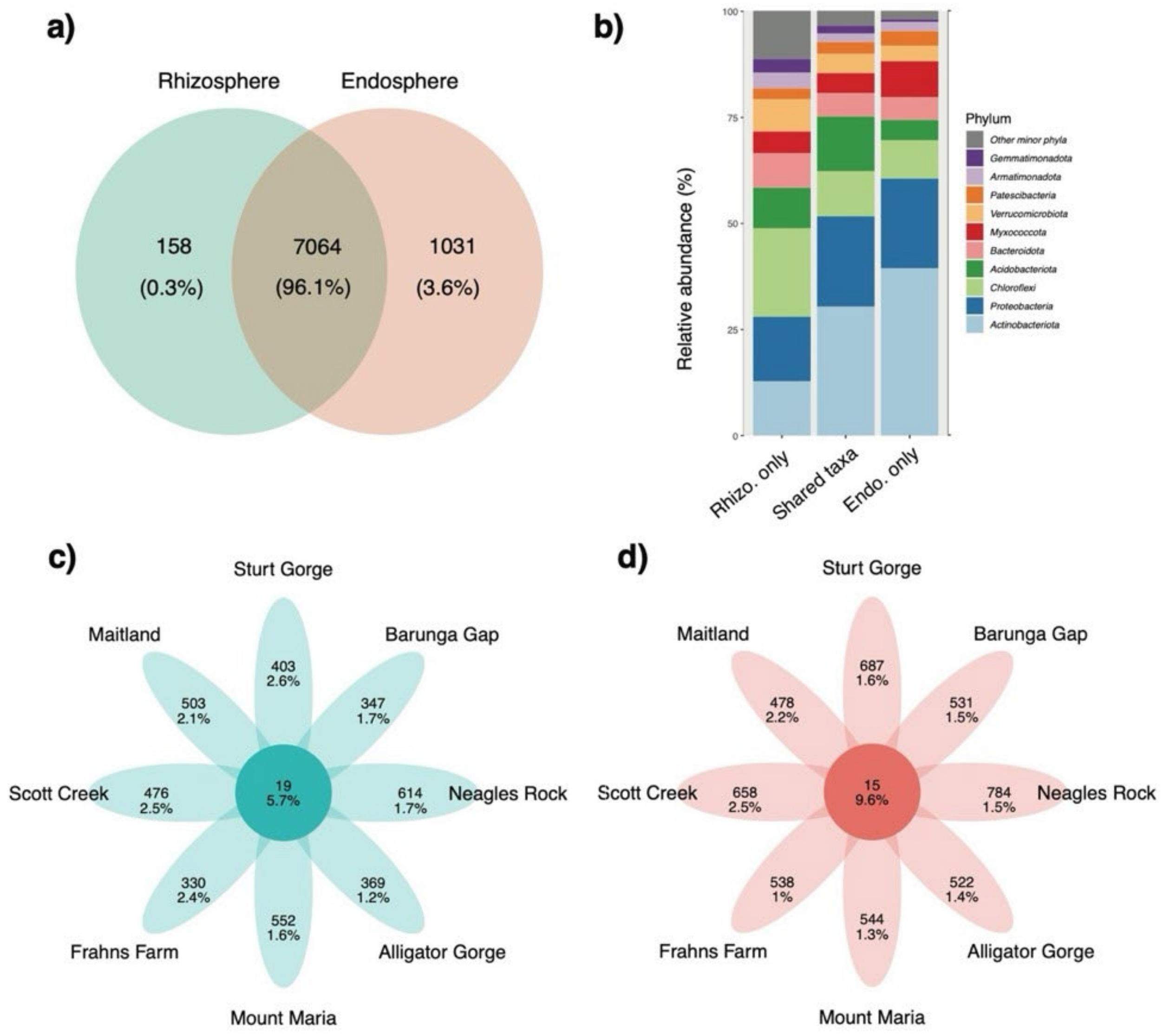

Figure 3.3. Shared bacterial ASVs across rhizospheres and endospheres. (a) Venn diagram of unique ASVs across *T. triandra* endospheres and rhizospheres showing number of unique ASVs and percentage of reads within each grouping, and (b) plot summarising relative abundance of phyla for the unique and shared ASVs in the endospheres and rhizospheres. (c) Partial Venn diagram showing unique *T. triandra* rhizospheres ASVs across each sampling site, and shared across all sites, and percentage of reads within each grouping; and (d) partial Venn diagram of unique ASV across *T. triandra* endospheres in each site, and shared across all sites, showing number of unique ASVs and percentage of reads within each grouping.

Figure 3.4. Differentially abundant ASVs across rhizospheres and endospheres (a) Heatmap showing 218 differentially abundant ASVs across *T. triandra* rhizospheres and endospheres, with clustering of ASVs with high and low log fold changes represented by the dendrogram, and (b) the number of differentially abundant ASVs calculated within each sampling site. The negative grouping includes those ASVs favoured in endospheres (negative log fold change), whereas the positive grouping includes ASVs favoured in rhizospheres (positive log fold change). Sites are ordered from most to least arid (top to bottom, respectively). (c) Upset plot showing the number of shared and unique bacterial ASVs across each site. This plot shows the first 30 most populated ASV intersections between sites (see Figure S14 for all site intersections).

Figure 3.5. Abundance-occupancy curves fitted with the Sloan neutral model in *T. triandra* rhizospheres and endospheres. Each point represents a bacterial ASV that occurs above (blue), below (red), or within (white) neutral model predictions in (a) rhizospheres and (b) endospheres. ASVs that occur at greater occupancies than predicted by the neutral model (blue) are hypothesised to be positively selected by the environment, and those occurring with lower occupancies than predicted by the neutral model (red) are hypothesised to be negatively selected by the environment. (c) βNTI values across the rhizospheres and endospheres of each sampling site in order of aridity index, and (d) Stacked bars plot illustrating the relative contribution of ecological assembly processes across rhizospheres and endospheres in each sampling site in order of aridity index. Heterogeneous and homogeneous selection is attributed βNTI values of > +2 or < -2, respectively. Communities without significant βNTI deviations (|βNTI | <2) were investigated for homogenising dispersal or limiting dispersal with RCbray values of < -0.95 or > 0.95, respectively.

Figure 3.6. Network analysis of bacterial ASVs in rhizospheres and endospheres, sampled across *T. triandra* aridity gradient. Vertex colour indicates taxonomic groups at the ASV level for both (a) rhizospheres (blue), and (b) endospheres (red). Positive associations are represented by blue edges, and negative associations are represented by red edges. The average degree and average edge weight is shown below of each network with their respective standard error.

References

Abdelfattah, A., A. J. M. Tack, C. Lobato, B. Wassermann, and G. Berg. 2023. From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. Trends in Microbiology **31**:346-355.

ALA. 2014. Mean annual aridity index. Atlas of Living Australia.

Bai, B., W. Liu, X. Qiu, J. Zhang, J. Zhang, and Y. Bai. 2022. The root microbiome: Community assembly and its contributions to plant fitness. Journal of Integrative Plant Biology **64**:230-243.

Barajas, H. R., S. Martínez-Sánchez, M. F. Romero, C. H. Álvarez, L. Servín-González, M. Peimbert, R. Cruz-Ortega, F. García-Oliva, and L. D. Alcaraz. 2020. Testing the Two-Step Model of Plant Root Microbiome Acquisition Under Multiple Plant Species and Soil Sources. Frontiers in Microbiology **11**.

Bardgett, R. D., J. M. Bullock, S. Lavorel, P. Manning, U. Schaffner, N. Ostle, M. Chomel, G. Durigan, E. L. Fry, D. Johnson, J. M. Lavallee, G. Le Provost, S. Luo, K. Png, M. Sankaran, X. Hou, H. Zhou, L. Ma, W. Ren, X. Li, Y. Ding, Y. Li, and H. Shi. 2021. Combatting global grassland degradation. Nature Reviews Earth & Environment **2**:720-735.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1-48.

Bates, D., M. Maechler, and M. Jagan. 2023. Matrix: Sparse and Dense Matrix Classes and Methods.

Baxter, B. J. M., J. Van Staden, J. E. Granger, and N. A. C. Brown. 1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. Environmental and Experimental Botany **34**:217-223.

Belbin, L. 2011. The Atlas of Livings Australia's Spatial Portal.*in* In, Proceedings of the Environmental Information Management Conference 2011 (EIM 2011).

Bever, J., I. Dickie, E. Facelli, J. Facelli, J. Klironomos, M. Moora, M. Rillig, W. Stock, M. Tibbett, and M. Zobel. 2010. Rooting Theories of Plant Community Ecology in Microbial Interactions. Trends in Ecology & Evolution **25**:468-478.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology **37**:852-857.

Breed, M. F., P. A. Harrison, C. Blyth, M. Byrne, V. Gaget, N. J. C. Gellie, S. V. C. Groom, R. Hodgson, J. G. Mills, T. A. A. Prowse, D. A. Steane, and J. J. Mohr. 2019. The potential of genomics for restoring ecosystems and biodiversity. Nature Reviews Genetics **20**:615-628.

Brinkman, E. P., C. E. Raaijmakers, W. de Boer, and W. H. van der Putten. 2017. Changing soil legacies to direct restoration of plant communities. AoB PLANTS **9**.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. v. Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology **64**:807-838.

Burns, A. R., W. Z. Stephens, K. Stagaman, S. Wong, J. F. Rawls, K. Guillemin, and B. J. M. Bohannan. 2016. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. The ISME Journal **10**:655-664.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods **13**:581-583.

Chi, F., S.-H. Shen, H.-P. Cheng, Y.-X. Jing, G. Yanni Youssef, and B. Dazzo Frank. 2005. Ascending migration of endophytic Rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Applied and environmental microbiology **71**:7271-7278.

Choi, K., R. Khan, and S. W. Lee. 2021. Dissection of plant microbiota and plant-microbiome interactions. Journal of Microbiology **59**:281-291.

Csardi, G., T. Nepusz, K. Müller, S. Horvát, V. Traag, F. Zanini, and D. Noom. 2006. The igraph software package for complex network research. Complex Systems.

Custer, G. F., M. Gans, L. T. A. van Diepen, F. Dini-Andreote, and C. A. Buerkle. 2023. Comparative Analysis of Core Microbiome Assignments: Implications for Ecological Synthesis. mSystems **8**.

David, A. S., P. F. Quintana-Ascencio, E. S. Menges, K. B. Thapa-Magar, M. E. Afkhami, and C. A. Searcy. 2019. Soil microbiomes underlie population persistence of an endangered plant species. American Naturalist **194**:488-494.

de Vries, F. T., R. I. Griffiths, C. G. Knight, O. Nicolitch, and A. Williams. 2020. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science **368**:270.

Debray, R., R. A. Herbert, A. L. Jaffe, A. Crits-Christoph, M. E. Power, and B. Koskella. 2022. Priority effects in microbiome assembly. Nature Reviews Microbiology **20**:109-121.

Dunning, L. T., A.-L. Liabot, J. K. Olofsson, E. K. Smith, M. S. Vorontsova, G. Besnard, K. J. Simpson, M. R. Lundgren, E. Addicott, R. V. Gallagher, Y. Chu, R. T. Pennington, P.-A. Christin, and C. E. R. Lehmann. 2017. The recent and rapid spread of *Themeda triandra*. Botany Letters **164**:327-337.

Durán, P., T. J. Ellis, T. Thiergart, J. Ågren, and S. Hacquard. 2022. Climate drives rhizosphere microbiome variation and divergent selection between geographically distant Arabidopsis populations. New Phytologist **236**:608-621.

Escobar Rodríguez, C., B. Mitter, L. Antonielli, F. Trognitz, S. Compant, and A. Sessitsch. 2018. Roots and panicles of the C4 model grasses Setaria viridis (L). and S. pumila jost distinct bacterial assemblages with core taxa conserved across host genotypes and sampling sites. Frontiers in Microbiology **9**.

Farley, G. J., S. M. Bellairs, and S. W. Adkins. 2013. Germination of selected Australian native grass species, with potential for minesite rehabilitation. Australian Journal of Botany **61**:283-290.

Friedman, J., and E. J. Alm. 2012. Inferring Correlation Networks from Genomic Survey Data. Plos Computational Biology **8**:e1002687.

Ghebrehiwot, H. M., M. G. Kulkarni, K. P. Kirkman, and J. Van Staden. 2012. Smoke and heat: influence on seedling emergence from the germinable soil seed bank of mesic grassland in South Africa. Plant Growth Regulation **66**:119-127.

Gonzalez, J. B., R. H. Petipas, O. Franken, E. T. Kiers, K. E. Veblen, and A. K. Brody. 2018. Herbivore removal reduces influence of arbuscular mycorrhizal fungi on plant growth and tolerance in an East African savanna. Oecologia **187**:123-133.

Gopal, M., and A. Gupta. 2016. Microbiome selection could spur next-generation plant breeding strategies. Frontiers in Microbiology **7**.

Gottel, N. R., H. F. Castro, M. Kerley, Z. Yang, D. A. Pelletier, M. Podar, T. Karpinets, E. Uberbacher, G. A. Tuskan, R. Vilgalys, M. J. Doktycz, and C. W. Schadt. 2011. Distinct microbial communities within the endosphere and rhizosphere of Populus deltoides roots across contrasting soil types. Applied and environmental microbiology **77**:5934-5944.

Gu, Z., L. Gu, R. Eils, M. Schlesner, and B. Brors. 2014. Circlize implements and enhances circular visualization in R. Bioinformatics **30**:2811-2812.

Hamonts, K., P. Trivedi, A. Garg, C. Janitz, J. Grinyer, P. Holford, F. C. Botha, I. C. Anderson, and B. K. Singh. 2018. Field study reveals core plant microbiota and relative importance of their drivers. Environmental Microbiology **20**:124-140.

Hancock, N., and L. Hughes. 2014. Turning up the heat on the provenance debate: Testing the 'local is best' paradigm under heatwave conditions. Austral Ecology **39**:600-611.

Hannula, S. E., R. Heinen, M. Huberty, K. Steinauer, J. R. De Long, R. Jongen, and T. M. Bezemer. 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. Nature Communications **12**:5686.

Hassen, A. I., and N. Labuschagne. 2010. Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. World Journal of Microbiology & Biotechnology **26**:1837-1846.

Hayden, H. L., P. M. Mele, D. S. Bougoure, C. Y. Allan, S. Norng, Y. M. Piceno, E. L. Brodie, T. Z. DeSantis, G. L. Andersen, A. L. Williams, and M. J. Hovenden. 2012. Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO2 and warming in an Australian native grassland soil. Environmental Microbiology **14**:3081-3096.

Hodgson, R. J., C. Liddicoat, C. Cando-Dumancela, N. W. Fickling, S. D. Peddle, S. Ramesh, and M. F. Breed. 2024. Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C4 grass, *Themeda triandra*. Applied Soil Ecology **201**:105514.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal **50**:346-363.

Johnston-Monje, D., and M. N. Raizada. 2011. Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. Plos One **6**:e20396.

Jost, L. 2006. Entropy and diversity. Oikos **113**:363-375.

Jousset, A., C. Bienhold, A. Chatzinotas, L. Gallien, A. Gobet, V. Kurm, K. Küsel, M. C. Rillig, D. W. Rivett, J. F. Salles, M. G. A. van der Heijden, N. H. Youssef, X. Zhang, Z. Wei, and W. H. G. Hol. 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. The ISME Journal **11**:853-862.

Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics **26**:1463-1464.

Kurtz, Z. D., C. L. Müller, E. R. Miraldi, D. R. Littman, M. J. Blaser, and R. A. Bonneau. 2015. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. Plos Computational Biology **11**:e1004226.

Larson, J. L., R. C. Venette, and D. L. Larson. 2022. Restoration for Resilience: The Role of Plant-Microbial Interactions and Seed Provenance in Ecological Restoration. Natural Areas Journal **42**:152-159.

Ledeganck, P., I. Nijs, and L. Beyens. 2003. Plant functional group diversity promotes soil protist diversity. Protist **154**:239-249.

Lin, H., and S. D. Peddada. 2020. Analysis of compositions of microbiomes with bias correction. Nature Communications **11**:3514.

Ling, N., T. Wang, and Y. Kuzyakov. 2022. Rhizosphere bacteriome structure and functions. Nature Communications **13**:836.

Liu, C., Y. Cui, X. Li, and M. Yao. 2020. microeco: an R package for data mining in microbial community ecology. Fems Microbiology Ecology **97**.

Logares, R., S. Audic, D. Bass, L. Bittner, C. Boutte, R. Christen, J.-M. Claverie, J. Decelle, John R. Dolan, M. Dunthorn, B. Edvardsen, A. Gobet, Wiebe H. C. F. Kooistra, F. Mahé, F. Not, H. Ogata, J. Pawlowski, Massimo C. Pernice, S. Romac, K. Shalchian-Tabrizi, N. Simon, T. Stoeck, S. Santini, R. Siano, P. Wincker, A. Zingone, Thomas A. Richards, C. de Vargas, and R. Massana. 2014. Patterns of rare and abundant marine microbial eukaryotes. Current Biology **24**:813-821.

Louca, S., M. F. Polz, F. Mazel, M. B. N. Albright, J. A. Huber, M. I. O'Connor, M. Ackermann, A. S. Hahn, D. S. Srivastava, S. A. Crowe, M. Doebeli, and L. W. Parfrey. 2018. Function and functional redundancy in microbial systems. Nature Ecology & Evolution **2**:936-943.

Lumibao, C. Y., E. R. Kimbrough, R. H. Day, W. H. Conner, K. W. Krauss, and S. A. Van Bael. 2020. Divergent biotic and abiotic filtering of root endosphere and rhizosphere soil fungal communities along ecological gradients. Fems Microbiology Ecology **96**.

Lundberg, D. S., S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. d. Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe, and J. L. Dangl. 2012. Defining the core Arabidopsis thaliana root microbiome. Nature **488**:86-90.

Lyu, D., J. Zajonc, A. Pagé, C. A. S. Tanney, A. Shah, N. Monjezi, L. A. Msimbira, M. Antar, M. Nazari, R. Backer, and D. L. Smith. 2021. Plant Holobiont Theory: The Phytomicrobiome Plays a Central Role in Evolution and Success. Microorganisms **9**:675.

McMurdie P. J., and S. Holmes. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data.*in* P. ONE, editor. PLoS ONE.

McPherson, M. R., P. Wang, E. L. Marsh, R. B. Mitchell, and D. P. Schachtman. 2018. Isolation and Analysis of Microbial Communities in Soil, Rhizosphere, and Roots in Perennial Grass Experiments. JoVE:e57932.

Middleton, N., and D. Thomas. 1997. World atlas of desertification. Arnold: UNEP.

Murphy, B. P., A. N. Andersen, and C. L. Parr. 2016. The underestimated biodiversity of tropical grassy biomes. Philosophical Transactions of the Royal Society B: Biological Sciences **371**:20150319.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature **368**:734-737.

Naylor, D., and D. Coleman-Derr. 2018. Drought Stress and Root-Associated Bacterial Communities. Frontiers in Plant Science **8**.

Neu, A. T., E. E. Allen, and K. Roy. 2021. Defining and quantifying the core microbiome: Challenges and prospects. Proceedings of the National Academy of Sciences of the United States of America **118**:10.

Ning, D., M. Yuan, L. Wu, Y. Zhang, X. Guo, X. Zhou, Y. Yang, A. P. Arkin, M. K. Firestone, and J. Zhou. 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nature Communications **11**:4717.

Ofiţeru, I. D., M. Lunn, T. P. Curtis, G. F. Wells, C. S. Criddle, C. A. Francis, and W. T. Sloan. 2010. Combined niche and neutral effects in a microbial wastewater treatment community. Proceedings of the National Academy of Sciences **107**:15345-15350.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, L. P., M. D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. S., Szoecs E., and W. H. 2019. vegan: Community Ecology Package.

Peixoto, R. S., C. R. Voolstra, M. Sweet, C. M. Duarte, S. Carvalho, H. Villela, J. E. Lunshof, L. Gram, D. C. Woodhams, J. Walter, A. Roik, U. Hentschel, R. V. Thurber, B. Daisley, B. Ushijima, D. Daffonchio, R. Costa, T. Keller-Costa, J. S. Bowman, A. S. Rosado, G. Reid, C. E. Mason, J. B. Walke, T. Thomas, and G. Berg. 2022. Harnessing the microbiome to prevent global biodiversity loss. Nature Microbiology **7**:1726-1735.

Petipas, R. H., J. B. González, T. M. Palmer, and A. K. Brody. 2017. Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. Acta Oecologica **78**:71-78.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research **41**:D590-D596.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rillig, M. C., J. Antonovics, T. Caruso, A. Lehmann, J. R. Powell, S. D. Veresoglou, and E. Verbruggen. 2015. Interchange of entire communities: microbial community coalescence. Trends in Ecology & Evolution **30**:470-476.

Risely, A. 2020. Applying the core microbiome to understand host-microbe systems. Journal of Animal Ecology **89**:1549-1558.

Robinson, J. M., R. Hodgson, S. L. Krauss, C. Liddicoat, A. A. Malik, B. C. Martin, J. J. Mohr, D. Moreno-Mateos, M. Muñoz-Rojas, S. D. Peddle, and M. F. Breed. 2023. Opportunities and challenges for microbiomics in ecosystem restoration. Trends in Ecology & Evolution.

Rocha, E. P. C. 2018. Neutral Theory, Microbial Practice: Challenges in Bacterial Population Genetics. Molecular Biology and Evolution **35**:1338-1347.

Saleem, A., F. U. Hassan, A. Manaf, and M. S. Ahmedani. 2009. Germination of *Themeda triandra* (Kangaroo grass) as affected by different environmental conditions and storage periods. African Journal of Biotechnology **8**:4094-4099.

Santoyo, G. 2022. How plants recruit their microbiome? New insights into beneficial interactions. Journal of Advanced Research **40**:45-58.

Sasse, J., E. Martinoia, and T. Northen. 2018. Feed your friends: Do plant exudates shape the root microbiome? Trends in Plant Science **23**:25-41.

Sloan, W. T., S. Woodcock, M. Lunn, I. M. Head, and T. P. Curtis. 2007. Modeling Taxa-Abundance Distributions in Microbial Communities using Environmental Sequence Data. Microbial Ecology **53**:443-455.

Snyman, H. A., L. J. Ingram, and K. P. Kirkman. 2013. *Themeda triandra*: a keystone grass species. African Journal of Range & Forage Science **30**:99-125.

Stegen, J. C., X. Lin, J. K. Fredrickson, X. Chen, D. W. Kennedy, C. J. Murray, M. L. Rockhold, and A. Konopka. 2013. Quantifying community assembly processes and identifying features that impose them. The ISME Journal **7**:2069-2079.

Stegen, J. C., X. Lin, A. E. Konopka, and J. K. Fredrickson. 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. The ISME Journal **6**:1653-1664.

Stopnisek, N., and A. Shade. 2021. Persistent microbiome members in the common bean rhizosphere: an integrated analysis of space, time, and plant genotype. The ISME Journal **15**:2708-2722.

Tang, M., J. Liu, W. Hou, R. M. Stubbendieck, H. Xiong, J. Jin, J. Gong, C. Cheng, X. Tang, Y. Liu, Z. Li, J. Wang, and Y. Yi. 2021. Structural variability in the bulk soil, rhizosphere, and root endophyte fungal communities of Themeda japonica plants under different grades of karst rocky desertification. Plant and Soil.

Thiergart, T., P. Durán, T. Ellis, N. Vannier, R. Garrido-Oter, E. Kemen, F. Roux, C. Alonso-Blanco, J. Ågren, P. Schulze-Lefert, and S. Hacquard. 2020. Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. Nature Ecology & Evolution **4**:122-131.

Trivedi, P., J. E. Leach, S. G. Tringe, T. Sa, and B. K. Singh. 2020. Plant–microbiome interactions: from community assembly to plant health. Nature Reviews Microbiology **18**:607-621.

UNEP. 1992. World Atlas of Desertification.

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

van Vliet, S. 2015. Bacterial dormancy: how to decide when to wake up. Current Biology **25**:R753-R755.

Vandenkoornhuyse, P., A. Quaiser, M. Duhamel, A. Le Van, and A. Dufresne. 2015. The importance of the microbiome of the plant holobiont. New Phytologist **206**:1196-1206.

Wang, G. Z., P. Schultz, A. Tipton, J. L. Zhang, F. S. Zhang, and J. D. Bever. 2019. Soil microbiome mediates positive plant diversity-productivity relationships in late successional grassland species. Ecology Letters **22**:1221-1232.

Wang, J., Z. Pan, J. Yu, Z. Zhang, Y.-z. Li, and A. Shade. 2023. Global assembly of microbial communities. mSystems **8**.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology **73**:5261-5267.

Xue, Y., H. Chen, J. R. Yang, M. Liu, B. Huang, and J. Yang. 2018. Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. The ISME Journal **12**:2263-2277.

Yang, G. W., J. Roy, S. D. Veresoglou, and M. C. Rillig. 2021. Soil biodiversity enhances the persistence of legumes under climate change. New Phytologist **229**:2945-2956.

Zhang, G., G. Wei, F. Wei, Z. Chen, M. He, S. Jiao, Y. Wang, Y. Yu, L. Dong, and S. Chen. 2021. Homogeneous selection shapes rare biosphere in rhizosphere of medicinal plant. Ecological Indicators **129**:107981.

Zhang, W., Y. Pan, J. Yang, H. Chen, B. Holohan, J. Vaudrey, S. Lin, and G. B. McManus. 2018. The diversity and biogeography of abundant and rare intertidal marine microeukaryotes explained by environment and dispersal limitation. Environmental Microbiology **20**:462-476.

Zhang, X., S. Liu, J. Wang, Y. Huang, Z. Freedman, S. Fu, K. Liu, H. Wang, X. Li, M. Yao, X. Liu, and J. Schuler. 2020. Local community assembly mechanisms shape soil bacterial β diversity patterns along a latitudinal gradient. Nature Communications **11**:5428.

Zhou, J., and D. Ning. 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiology and Molecular Biology Reviews **81**:10.1128/mmbr.00002-00017.

Chapter four: Contrasting microbial taxonomic and functional colonisation patterns in wild populations of the pan-palaeotropical C4 grass, *Themeda triandra*

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Percentage contribution: **RJH: 86%,** CCD: 1%, TD: 1%, EAD 1%, MPD 1%, RAE 1%, CL:1%, SAR: 1%, JMR 1%, MFB: 6%

Throughout this chapter I have used the pronoun 'we' to acknowledge the contributions of my coauthors, following the customary etiquette in published manuscripts.

Chapter four: Contrasting microbial taxonomic and functional colonisation patterns in wild populations of the pan-palaeotropical C4 grass, Themeda triandra

Abstract

The interactions between native plants and soil microbiota are not well characterised, despite growing recognition of their contributions to host plant fitness, soil nutrient cycling and ecological functioning. Here, we used shotgun metagenomics to examine the microbial taxonomic and functional colonisation patterns in wild populations of the pan-palaeotropical C4 grass, *Themeda triandra*, across a globally representative aridity gradient (aridity index 0.318–0.903). We investigated these patterns through the two-step selection process. This process involves the movement of microbes from bulk soils into the rhizosphere (soil on the root surface), and then from the rhizosphere into the root interior (the endosphere), using a space for time substitution approach. We focused on how environmental variables – particularly aridity – modulated these colonisation patterns. We provide clear evidence that the two-step selection process progressively filters microbial taxa colonising wild *T. triandra* roots. Surprisingly, microbial functions showed the opposite trend: functional diversity increased from bulk soil to the rhizosphere and endosphere. This likely reflects the wide array of specialised functions performed by the microbial taxa that successfully establish within host roots. Notably, this pattern did not hold for observed (i.e. non-normalised) functional diversity, indicating that the increase was driven by greater evenness in the endosphere, as captured by Shannon's index. Finally, we show that increasing aridity was associated with increasingly homogeneous (i.e., similar), yet highly functionally diverse rhizosphere communities. This association suggests that aridity drives filtering for key ecological functions, favouring either higher functional redundancy under more arid conditions and/or the retention of a critical suite of functional capacities, particularly for stress response genes. Together, these results show that trends of functional microbial colonisation from soil into plant roots follows more complex patterns than taxonomic dynamics, and that these functions can be shaped by climatic factors, including aridity.

4.1 Introduction

Soils are home to 59% of all species (Anthony et al., 2023), including many microbes that form belowground associations with plants. These microbiota have important functional roles, such as supporting the fitness of plants through mutualistic plant-microbe interactions (Bever, 1994). Host plants invest resources that sustain and prioritise specific microbial functions. In return, mutualistic microbes influence plant metabolic processes, provide fitness advantages to their hosts, aid plant resource acquisition, and increase plant tolerance to environmental stressors, including drought-induced water stress (Petipas et al., 2021). Notably, as aridity increases, the beneficial interactions between plants and soil microbiota become stronger, likely due to a greater reliance on mutualistic microbes in these harsher conditions (Jiang et al., 2024). However, the processes that control how these functionally important microbiota colonise, or are recruited into plant roots, are complex and poorly understood in natural ecosystems, especially where plants must navigate variable and changing biotic and abiotic conditions (Bulgarelli et al., 2013).

The two-step selection process is a well-established theoretical framework to view the colonisation of roots by microbes but is poorly studied outside of model species such as *Arabidopsis* (Bulgarelli et al., 2012) and crop species such as tomatoes (Barajas et al., 2020). This process includes the active recruitment of microbes by host plants, first from bulk soils into plant rhizospheres (soil on the surface of roots), and then from rhizospheres into roots (the endosphere) (Bulgarelli et al., 2013, Lundberg et al., 2012). In *Arabidopsis,* for instance, bacterial and fungal communities are well known to shift among the bulk soils, rhizospheres, and endospheres, with a progressive decline in taxonomic alpha diversity as microbiota are filtered into the endosphere (Bulgarelli et al., 2012). Alternative theoretical frameworks include vertical transmissions of microbiota into plant seeds directly from parental plant flowers or via pollinators (Abdelfattah et al., 2023), or internal transport through plant vascular tissue from leaves (phyllosphere microbiota) (Chi et al., 2005). Despite their importance, functional investigations of these colonisation patterns – especially in non-model plant species – remain scarce, limiting our understanding of host-microbe dynamics under natural conditions. It also remains uncertain how environmental change impacts this two-step selection process in plants under natural conditions.

The C4 grass *Themeda triandra* (Forssk.), is a keystone species with a pan-palaeotropical distribution across much of Australia, Asia, and Africa, often dominating grassland ecosystems (Snyman et al., 2013). It provides important ecosystem services by maintaining soil health (e.g., shaping physical structure and microbiota) and supporting native biodiversity (Snyman et al., 2013). While this species is known to be colonised and receive growth benefits from soil microbiota (Hodgson et al., 2024b, Petipas et al., 2017), the different roles provided by their functional genes are unexplored. In this field study, we used a natural experimental design and shotgun metagenomics to investigate the colonisation patterns of microbiota, and their accompanying gene functions, in wild *T. triandra* across a globally-representative aridity gradient. We hypothesised that (1) the microbial taxonomic and functional colonisation patterns in *T. triandra* would align with the two-step selection process (i.e., community and diversity filtering from bulk soil into roots); (2) there will be strong positive correlations between microbial taxonomic and functional colonisation patterns, where we expect that higher bacterial species diversity will be associated with higher functional diversity; and, (3) aridity will modulate both taxonomic and functional colonisation patterns, with higher aridity populations recruiting microbiota linked to water stress tolerance and drought resilience.

4.2 Methods

4.2.1 Study design

We sampled the bulk soils, rhizospheres and endospheres of six randomly selected *T. triandra* plants in eight populations that occurred across a southern Australian aridity gradient (Table S4.1; Figure S4.1). We tested that there was no correlation between the aridity and geographic distances of our sampled populations (Mantel: p =0.489; r =-0.021; Figure S4.1c). Aridity data was collected from the

Atlas of Living Australia (Belbin, 2011, ALA, 2014) via the mean annual aridity index layer (annual precipitation/annual potential evaporation; Figure S4.1b-c). Using this aridity index, low values correspond to more arid conditions (i.e., hotter/drier conditions; hereafter, high aridity) (UNEP, 1992, Middleton and Thomas, 1997). We used shotgun metagenomics to characterise taxonomic and functional patterns in bacterial communities, detailed below, using a space for time substitution experimental approach. We also analysed plant and soil physiochemical conditions, and conducted vegetation surveys to account for host, edaphic, and ecological variation across our populations (see supplementary methods). In this manuscript, we do not experimentally distinguish between plant-driven microbial shifts across compartments (i.e., active recruitment of microbiota) and microbial-dependent changes (i.e., colonisation).

4.2.2 DNA extraction, amplification and sequencing

We collected bulk soils within 30 cm of the north and south of each *T. triandra* plant, and combined these samples for downstream analysis, transporting them on ice from the field, and storing them at -20ºC for later DNA extraction (Figure S4.1e). Microbiota from the rhizospheres were isolated following the protocol from Hodgson et al., (2024b). Briefly, sampled roots were washed in 0.02% Silwet L-77 amended PBS buffer and vortexed before being filtered at 100 μm and centrifuged prior to DNA extraction. We also isolated *T. triandra* endospheres by removing bacteria and DNA from root surfaces, and subsequently extracting DNA directly from these 'cleaned' root tissues as detailed in Hodgson et al. (2024a). Here, roots were sonicated on ice in 0.02% Silwet L-77 amended PBS buffer at 30% amplitude for 5x 30 sec alternating burst/rest periods (5 minutes total) to remove external bacteria and DNA. The roots then underwent a series of 5 additional washes in this sterilised amended PBS buffer solution (5 minutes). To obtain the final endosphere samples, roots were pulverised with metal beads for 1 min in a bead-beating solution (PowerSoil Kit, Qiagen, Hilden, Germany). Sterilisation was validated by plating final wash solutions on Luria-Bertani agar plates (Hodgson et al., 2024b). Following the manufacturer's protocols, DNA extractions were performed on the soil, rhizosphere and endosphere samples using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany).

4.2.3 Shotgun metagenomic data analysis

We performed shotgun metagenomics sequencing on each of the *T. triandra* samples across our aridity gradient. We successfully sequenced 43 endospheres, 22 rhizospheres, and 39 bulk soils (n =104). Libraries were prepared using Accel-NGS® 2S DNA Library Kits from Swift Biosciences Inc. (London, United Kingdom) and sequenced at the South Australian Genomics Centre (Adelaide, Australia). For sample sequencing, an equimolar pool was prepared and denatured for DNA Nanoball (DNB) generation using the MGI DNBSEQ-G400 platform with 300 bp reads. All bioinformatics was done using DeepThought high performance computing (Flinders University, 2021). Data cleaning was done with fastp v0.23.2 (Chen et al., 2018), which included trimming adapter from DNB sequences. Contaminant DNA from *T. triandra* was removed using reference genomes via Bowtie2 v2.4.1 (Langmead and Salzberg, 2012, (NCBI), 2021). Taxonomic IDs were assigned using Kraken2 v2.0.7 (Wood et al., 2019). We used Bracken (Lu J, 2017) to estimate abundances of taxa, then KrakenTools (Lu et al., 2022) to create a taxonomic abundance table for downstream analysis. Gene functions were assigned to reads using SUPER-FOCUS v1.6 (Silva et al., 2015), according to each functional subsystem from the SEED database (Overbeek et al., 2004).

4.2.4 Statistics

Statistics were done in R version 4.2.2 (R Core Team, 2022). We performed relative abundance normalisations on our taxonomic and functional datasets to account for differences in sequencing depth across our samples. We ran detailed downstream analysis on relative functional gene abundances, but we also isolated six different categories at SEED subsystem level 1 to explore in greater detail (Overbeek et al., 2004). These six functional categories included: motility and chemotaxis (movement and sensing), nitrogen metabolism, phosphorus metabolism, regulation and cell signalling (which captures quorum sensing and biofilm production dynamics), stress response functions and, secondary metabolism.

Taxonomic and functional diversity analysis

We calculated the relative abundance of the top bacterial phyla (based on reads classified at the Phylum level) and functional categories (at Subsystem level 1). Alpha diversity was assessed by computing the effective number of species (via reads assigned to species level) and functions, using the exponential of Shannon's diversity index (Jost, 2006). This transformation enhances interpretability by expressing diversity as the number of equally abundant species or functions that would yield the same Shannon index. We used linear mixed-effects models to explore how plant compartments (soil, rhizosphere, endosphere), sampling population, and aridity index (each included as fixed factors) affected alpha diversity metrics (richness, effective no. of species/functions, and Pielou's evenness index). Plant ID was treated as a random factor to account for bias in repeated measures of individual plants across the root/soil compartments.

We used non-metric multidimensional scaling ordination (NMDS) with Bray-Curtis distances to explore differences in taxonomic and functional community composition. We then tested for differences between group centroids via permutational multivariate analysis of variance (PERMANOVA) and for homogeneity of group dispersions by calculating the distance to centroid measures.

To compare compositional (beta diversity) changes across our aridity gradient, we calculated the average Bray-Curtis distance of each sample to every other sample. We tested the effect of the aridity index on these distances by calculating the slope of the linear trend between the aridity index and Bray-Curtis distances. Using bootstrapped (B =2000) 95% CIs, we assessed whether the model slope overlapped zero. See Tables S4.2–S4.3 for a more detailed summary of all statistical tests comparing aridity with alpha and beta diversity, and the relative abundance of taxonomic and functional reads.

For bacterial species, and each subsystem, shared and unique functions were visualised across all populations using petal diagrams.

Differential abundance analysis

We used differential abundance analysis to evaluate differences in bacterial phyla and functions across each of the sampled compartments (i.e., soils, rhizospheres and endospheres) using global and pairwise tests through the *ancombc2* function from the R package ANCOMBC on non-normalised count data (Lin and Peddada, 2020). Differential abundance across the aridity gradient was done by allocating samples to high, medium and low aridity categories (aridity index >0.6 = low; >0.4 but ≤0.6 = medium; and ≤0.4 = high aridity), using low aridity as the reference group.

Canonical correspondence analysis

To predict the environmental drivers of both species-level taxonomic diversity and functional genes across our microbiomes, we ran canonical correspondence analysis (CCA) across all bulk soil, rhizosphere and endosphere samples (explanatory variables included are in Table S4.4). Correlated explanatory variables (r >0.75) were removed. We then performed forward and backward selection of the included explanatory variables using the *ordistep* function in the R package, vegan (Oksanen et al., 2019).

Network analysis

We conducted co-occurrence network analysis of microbial functional processes within low, medium, and high aridity categories (aridity index >0.6, >0.4–0.6, and ≤0.4). This analysis identified hub functions that may perform keystone roles supporting functional pathways. Networks and hub functions were compared across bulk soils, rhizospheres, and endospheres at the lowest functional level, focusing on positive or negative associations. Only functions with >100 sequences were reported. Associations were calculated using SparCC correlations (edge thresholds, $|\text{SparCC}| \geq 0.3$; $p < 0.05$), and significance was estimated via 200 permutations (Friedman and Alm, 2012, Kurtz et al., 2015). Networks were visualized with the R packages igraph (Csardi et al., 2006). Hub functions were identified as the top 20 bacterial functions ranked by node degree and closeness centrality.

4.3 Results

4.3.1 Taxonomic and functional diversity

Across our samples and plant compartments, we generated taxonomic libraries with a total of 26,919,111 reads (~258,838 per sample), with 9,835 unique bacterial species (~7,998 per sample) (Table S4.5). Our functional libraries contained a total of 40,520,567 reads (~385,910 per sample), with 31,167 unique functions (~11,799 per sample) (Table S4.5; see Table S4.6 for proportions of reads across the six functional categories at SEED subsystem level 1).

We identified 13 phyla representing 98% of reads assigned to bacteria with abundance estimates greater than 1.5% (Figure 4.1a). We observed a decreasing effective number of bacterial species from bulk soil into the rhizosphere and the endosphere compartments for our *T. triandra* plants (mean effective no. species ±SD: bulk soils =2,935 ±619, rhizospheres =2,491 ±575, endospheres=1,824 ±400; Table S4.7; Figure 4.1b; LMEM: df =2, $\chi^2 = 95.27$, $p < 0.001$). Additionally, we found that bacterial community compositions were significantly different across each of the compartments (Table S4.8; Figure 4.1c) with aridity index (Table S4.2; Figure 4.1c), and with sampling population (Table S4.8; Figure S4.2). Furthermore, we found that the average distance to centroid values decreased from the rhizospheres into bulk soils, and endospheres (Table S4.9; Figure 4.1d; PermDispersions: $F_{(2,101)} = 4.71$, $p = 0.011$).

There was decreasing richness of observed functions from bulk soils and rhizospheres into the endosphere (mean richness ±SD: bulk soils =12,336 ±3,335, rhizospheres =12,817±2,664, endospheres =10,767 ±2,371) (Table S4.10; Figure 4.2a; LMEM: df =2, χ^2 =10.3, p =0.005). However, the effective number of functions (the exponent of Shannon's diversity index) showed an increase in alpha diversity from bulk soil to rhizospheres, and then to endospheres (bulk soils =3,128 ±356, rhizospheres=3,420 ±503, endospheres =4,204 ±230; Table S4.10; Figure 4.2b; LMEM: df =2, χ^2 =202.35, p<0.001). Pielou's evenness index revealed that functional gene distributions were more even in endospheres than in rhizospheres and bulk soils (Table S4.10; Figure 4.2c; LMEM: df =2, χ^2 =43.45, p <0.001). Together, these results show that when functional diversity is normalised using the Shannon's diversity derived approaches (which account for higher evenness in endosphere), it increases from bulk soil to the endosphere.

The motility and chemotaxis functional subsystems, as well as the stress response functional subsystems showed an increase in effective functional diversity with aridity index, particularly in rhizospheres with higher aridity levels (Figure 4.3a-b; Table S4.2). We also observed the inverse trend for functional diversity of bulk soils in relation to stress response genes, which increased with decreasing aridity levels (Figure 4.3b; Table S4.2). However, responses to aridity varied across functional categories; for example, we did not see evidence of directional trends with the effective number of functions for secondary metabolism (Table S4.2; Figure 4.3c; Figures S4.3-S4.4). Within the root compartments, we found a positive correlation between the effective number of species and functions (Figure S4.5a; Table S4.11; Figure S4.5b; LMEM: $\chi^2_{(1,2)}$ =29.28, p <0.001).

Bulk soils, rhizospheres and endospheres produced distinct functional profiles (Figure 4.2) that were affected by aridity (Figure 4.2d; also see Figures S4.6-S4.11). Furthermore, the beta dispersions for these functional groups also revealed increasingly similar compositions (i.e., tighter groupings) towards the endospheres (Figure 4.2e; see Figures S4.12-S4.14).

Aridity had a compartment-specific effect on the homogeneity of different taxonomic and functional communities based on the mean Bray-Curtis distances. For both the bacterial taxonomic and microbial functions (visualised using the six subsystem categories), the rhizosphere showed the strongest response to aridity, consistently becoming more dissimilar (i.e., heterogeneous) as aridity decreased (i.e., we see increases in the average Bray-Curtis distance with aridity index; Figure 4.4, see Tables S4.2-S4.3 for details). In bulk soils, there was generally a positive correlation with aridity; communities became more similar, taxonomically and functionally, as aridity decreased (Figure 4.4, see Tables S4.2-S4.3 for details). In endospheres, there was no meaningful change in the compositional distances for functions with aridity, although taxonomic compositions became slightly more homogenous as aridity decreased (Figure 4.4; Table S4.2-S4.3).

Overall, we consistently found that more bacterial species and functions in each subsystem were shared (i.e., found in at least one site of every population) than were isolated to each population alone (see Figures S4.15-S4.21).

4.3.2 Differentially abundant taxonomy and functions

We found many differentially abundant bacterial phyla across the soils, rhizospheres and endospheres (Figure 4.1e; Table S4.12). Among those with the highest log fold change in abundance from the endosphere to rhizosphere, and endosphere to bulk soil were Actinomycetota (-1.794 and -2.691, respectively), Myxococcota (-1.02 and -1.984, respectively), and Pseudomonadota (-0.735 and -1.374, respectively) (Figure 4.1a, e). Conversely, phyla that had a positive log fold change in abundances from the endospheres into the rhizospheres and soils were: Bacteroidota (0.929 and 1.231, respectively), Cyanobacteriota (0.712 and 1,234, respectively), Ignavibacteriota (1.438 and 2.033, respectively), Atribacterota (1.151 and 1.915, respectively), and Chlamydiota (1.192 and 2.084, respectively) (among others) (Figure 4.1a, e; Table S4.12).

We focused our analysis on the differences between soils, rhizospheres and endospheres, primarily across three main functional subsystems: secondary metabolism, motility and chemotaxis, and nitrogen metabolism (see Figures S4.22-S4.27 for all differences between soils, rhizosphere and endospheres at SEED subsystem 1). For secondary metabolism, there were more abundances of functional genes associated with plant a variety of growth hormones, defence, and plant-microbial interactions in *T. triandra* endospheres (see Figure S4.25). Within the motility subsystem, *T. triandra* endospheres also had more chemotaxis functions, but lower abundances of functions related to flagellar and non-flagellar movement of bacteria (see Figure S4.22). Finally, we found that the abundances of nitrogen fixation functions decreased in the endospheres, but we found more functions related to nitric oxide synthase (see Figure S4.23). Differential abundance analysis showed changes in a low number of functions across the aridity gradient (see Figures S4.28-S4.32).

4.3.3 Canonical correspondence analysis

Our CCAs revealed variable associations between soil physicochemical parameters and bacterial taxonomic and functional communities within each of the *T. triandra* microbial compartments. After correlated variables were removed (Figure S4.34a), the taxonomic bulk soil communities were associated with pH, aridity, shrub vegetation and *T. triandra* density (Figure S4.34b). In the rhizospheres, bacterial community compositions corresponded with aridity, soil pH, host magnesium, and litter levels (Figure S4.34c). In the endospheres, however, bacterial communities had significant structural associations with aridity, electrical conductivity of the soil, host calcium and copper levels, in addition to soil pH, and graminoid and canopy cover in the vegetation (Figure S4.34d).

The taxonomic and functional CCAs corresponding to the remaining subsystems revealed numerous other environmental associations (Figure S4.35-S4.37; Tables S4.4). The rhizosphere functions associated with motility were significantly structured by pH, host magnesium, and aridity (Figure 4.3d). In the stress responses, we observed important drivers from pH, soil ammonium, electrical conductivity, litter, aridity, and host iron and magnesium levels (Figure 4.3e). Finally, the secondary metabolism functions, which included plant hormone functions (among others), corresponded with ammonium, potassium, phosphorus, electrical conductivity, host zinc and magnesium, and herbaceous plants (Figure 4.3f).

4.3.4 Network analysis

We conducted co-occurrence network analysis across six gene function categories, three plant compartments (soil, rhizosphere, endosphere), and three aridity levels (low, medium, high), focusing on stress response functions (Figure 4.5). Mean edge weights across the remaining five functional subsystems were also analysed (Table S4.13; Figure 4.6). In bulk soil, stress response function networks had higher closeness centrality in high-aridity networks (mean ±SD; $7.2\text{x}10^{-3}$ ±$1.3\text{x}10^{-3}$) compared to low and medium aridity networks ($6.2\text{x}10^{-3}$ ±$0.6\text{x}10^{-3}$ and $4.7\text{x}10^{-3}$ ±$0.5\text{x}10^{-3}$, respectively). Rhizosphere centrality was lower in high aridity ($2.4\text{x}10^{-3}$ ±$0.3\text{x}10^{-3}$) than in the medium ($4.9\text{x}10^{-3}$ ±$0.7\text{x}10^{-3}$) and low ($4.6\text{x}10^{-3}$ ±$0.7\text{x}10^{-3}$) aridity networks (Figure 4.5b). The endosphere centrality remained consistent across the high, medium and low aridity levels ($3.9\text{x}10^{-3}$ ±$0.6\text{x}10^{-3}$, $3.5\text{x}10^{-3}$ ±$0.4\text{x}10^{-3}$, and $3.4\text{x}10^{-3}$ ±$0.4\text{x}10^{-3}$, respectively). In all the bulk soils, rhizospheres and endospheres, mean node degree generally decreased from the medium (61.5 ±34.1, 63.0 ±42.8, and 30 ±26.2, respectively) and low aridity (63.9 ±35.4, 21.7 ±17.1, and 40.8 ±31.2, respectively), into the high aridity (8.8 ±6.1, 12.1±6.8, and 32.5 ±28.2, respectively) networks (Figure 4.5b).

The top 20 hub functions in each aridity and compartment network (Table S4.14) primarily related to heat stress in soils, accounting for 43%, 40%, and 23% of the low, medium, and high aridity networks, respectively (Figure 4.5). Oxidative stress hub functions were also important, comprising 30% in low aridity rhizosphere networks and increasing to 47% and 37% in medium and high aridity networks, respectively. In endosphere networks, oxidative stress functions comprised 33% of the hub functions in low aridity networks, rising to 47% and 37% in medium and high aridity, respectively (Figure 4.5). The proportion of osmotic stress hub functions increased from 7% in soil to 17% in rhizosphere and 33% in endosphere high aridity networks. Comparatively, in the low-aridity networks, osmotic stress comprised 7%, 23%, and 20% of soil, rhizosphere, and endosphere hub functions, respectively, while in medium aridity networks, it comprised 10%, 7%, and 13%, respectively (Figure 4.5).

4.4 Discussion

This study advances our understanding of plant-root colonisation by soil microbiota in wild plant roots by using shotgun metagenomics on soils, rhizospheres, and endospheres of *T. triandra* – a globally important C4 grass species. We report clear evidence that the two-step selection process – the colonisation of microbes first from bulk soils into plant rhizospheres, and then from rhizospheres into endospheres via host plant regulation – was acting on bacterial communities across an aridity gradient. Most endosphere microbiota were a subset of those in the bulk soil, supporting our first hypothesis. Surprisingly, microbial functions showed the opposing trend – which goes against our second hypothesis. Here, functional diversity increased from bulk soil into rhizospheres and endospheres despite the filtering of taxa entering the roots. These results highlight the potential ecological advantage of functional redundancy within endospheres, and the diversity of microbial functions recruited by host plants. In support of our third hypothesis, we also show that increasing aridity modulated taxonomic and functional recruitment by host plants, most noticeably influencing rhizospheres. These communities became more homogeneous and exhibited highly diverse stress response functions. Our findings suggest that *T. triandra* likely benefits from microbiota via higher functional redundancy and/or retention of a diverse suite of microbial functions under more arid conditions (particularly stress response genes). By expanding the two-step selection model to incorporate microbial functions, our study not only advances our knowledge of plant-soil ecology in wild *T. triandra* populations but also provides applications to the restoration of grasslands.

4.4.1 Contrasting taxonomic and functional microbial colonisation

We show that bacterial taxonomic alpha diversity decreased from bulk soils into rhizospheres, then into root endospheres, supporting our first hypothesis and aligning with the two-step selection process (Bulgarelli et al., 2012, Lundberg et al., 2012). However, we observed that microbial functions showed the opposite trend; functional diversity increased from bulk soil to rhizospheres, and into the endospheres. This enriched functional diversity is likely produced by the increased evenness (i.e., via Pielou's evenness index) among different types of functions within the endospheres. The relative abundance weighting in Shannon's diversity index means that greater evenness in functional contributions leads to higher overall diversity. Given the significant changes in microbial profiles observed from rhizospheres into endospheres, this likely reflects substantially different functional requirements of the host plant across these two compartments, and the strong influence of the plant's immune system (Adeleke et al., 2021). While we acknowledge that our functional and taxonomic annotations may be subject to biases, our study contributes valuable new insights into how the two-step selection process operates through a functional ecology lens.

4.4.2 Variation in endosphere functional profiles

We report that endosphere functional and taxonomic profiles converged into more homogeneous communities than in bulk soils and rhizospheres. This suggests that *T. triandra* strongly regulates the entry of microbiota into its endospheres, maintaining a common functional capacity, despite wide geographic distances and varying levels of aridity. We observed converging patterns in endosphere functional communities across almost all functional groupings measured at subsystem level 1 (i.e., motility and chemotaxis, nitrogen metabolism, phosphorus metabolism, regulation and cell signalling, and stress response functions). Only secondary metabolism functions were more heterogeneous in root endospheres compared to rhizospheres and bulk soils (discussed below). The selection pressures for endosphere colonisation are likely driven by the functional needs of *T. triandra* and host traits promoting mutually beneficial interactions (Bulgarelli et al., 2013).

All endosphere samples consistently showed increases in flagellar movement and chemotaxis functions, which are important for the movement and navigation of bacteria (Bulgarelli et al., 2013). Endospheres also became more similar by excluding functions like microbial gliding, which help bacteria travel through biofilms and are commonly found in rhizospheres and soils (Bhattacharyya et al., 2023). Comparable trends occurred for nitrogen metabolism, where we found increased abundances of genes associated with nitric oxide synthases in the endospheres – crucial for signalling between plants and their associated microbiota, and which play key roles in helping plants respond to oxidative and drought stress, although they may also aid microbiota in tolerating the host plant's immune system (Shah et al., 2023). Conversely, nitrogen-fixing functions were less abundant in endospheres, being more prevalent in bulk soils where they are involved in well-described processes within the nitrogen cycle (Stein and Klotz, 2016). These functional roles may differentially affect the fitness of colonising microbiota within the endosphere (e.g., optimized movement and navigation) while contributing to improved host growth and fitness (e.g., enhanced stress responses). Furthermore, the consistent recruitment and exclusion of microbial functions into the endospheres by host plants suggests strong symbiotic community assembly processes.

Unlike motility and nitrogen metabolism, the secondary metabolism functions were more heterogeneous in *T. triandra* endospheres compared to the rhizospheres and soils. Secondary metabolism functions are typically involved with survival adaptations, defence or derived environmental responses, and are not necessarily essential for growth (Khare et al., 2018, Srivastava and Raghuwanshi, 2023). Specifically, we observed changes in functions across biosynthesis and degradation of key metabolites like auxins, flavonoids, and phenylpropanoids, which likely support plant responses to environmental stresses and pathogens, and maintain structural integrity during growth (Kincses et al., 2024). We also suspect that functions associated with clavulanic acid and phenazine metabolites may shape plant-microbe interactions due to their noted associations with antibiotics (Wang et al., 2021). Strong regulation of microbial entry into endospheres is observed in many plant species and can be influenced by factors including growth stage, genotype, and geography (Lumibao et al., 2022). Nevertheless, the high-endosphere variation in secondary metabolism functions provides a unique and novel finding that highlights the diverse ecological needs of different *T. triandra* populations across their natural distribution.

4.4.3 Effect of aridity on rhizospheres

We show that aridity increased with taxonomic and functional diversity of *T. triandra* rhizospheres, but not soils or endospheres. This increased diversity was found across stress responses, motility, and nitrogen metabolism, which included functions related to (but not limited to) oxidative stress, flagellar movement of microbiota, and nitric oxide synthases. Functional redundancy is often associated with ecosystem stability and resilience (Guo et al., 2024). As such, increasing functional diversity in *T. triandra* rhizospheres suggests that there may be greater selection for plants to develop functional redundancy in more arid conditions, which could provide protection for these plants from water stress (Louca et al., 2018). Here, we found that the alpha diversity of the stress response functions was lower in the bulk soils of arid populations. Furthermore, previous work has shown that deterministic processes (i.e., host/environment-driven) more strongly shape microbiomes of drier grasslands over temperate grasslands (Zhong et al., 2022). Our findings highlight the impact *T. triandra* has on its rhizospheres to potentially counter stress from elevated aridity by recruiting high microbial functional diversity around its roots.

We also showed that rhizospheres in lower aridity populations were more heterogeneous compared to those in higher aridity environments (increasing community dissimilarity). Coupled with the higher alpha diversity in arid rhizospheres, this suggests that higher aridity promotes microbial communities that are taxonomically similar yet functionally diverse, a complexity not seen in the general soil environment (Lumibao et al., 2022). Evidence of stronger microbial selection pressures reinforces our third hypothesis that arid conditions alter host plant recruitment and colonisation dynamics. Overall, we provide new evidence of these dynamics in the C4 grass *T. triandra*, with microbe-mediated assistance under arid conditions.

4.4.4 Stress response functions with varying aridity

In our stress response network analysis, the hub functions highlight that the microbiomes across bulk soils, rhizospheres, and endospheres likely have mechanisms to cope with heat shock, oxidative stress, and osmotic stress – especially in high aridity populations. Oxidative stress responses are important mechanisms for plants to thrive in low water conditions by addressing the buildup of reactive oxygen species, which can be toxic to plants and tend to accumulate in tissues when under environmental stress (Berrios and Rentsch, 2022). Furthermore, osmotic stress directly affects cellular water potential responses, which are crucial for sustaining microbial community resilience and functioning under dehydration (Bremer and Krämer, 2019). Ultimately, the hub functions associated with heat shock, oxidative and osmotic stress likely facilitate important connections that help *T. triandra* and its microbiota to thrive in hot, arid environments.

Across the stress response and regulation and cell signalling networks, edge weights were unexpectedly more negative in high aridity populations of the rhizospheres and endospheres, indicating mutual exclusion among functional processes. This could be due to heightened resource competition among microbiota within our gradient, particularly if highly specialised functions are being selected at the expense of others (i.e. strong niche partitioning) (Lin et al., 2021). Only the rhizosphere secondary metabolism genes, and endosphere motility and chemotaxis genes had more positive edge weights in the high aridity networks, compared to low aridity networks. This suggests convergence towards functional niches, or more cooperation across functional processes with stronger mutualistic relationships between microbiota (e.g., possibly involving more complex and interconnected pathways of organic molecule synthesis and/or degradation).

4.4.5 Patterns of community variation

We found that only a small proportion of species and functions were unique to any given sampling population or compartment. This contrasts with previous amplicon-based studies, which have shown more unique taxonomic variation among *T. triandra* populations (Hodgson et al., 2024a). It is possible, however, that these differences might arise due to differences in taxonomic resolution of the two approaches. Despite the high occurrence of functions common to the endospheres, we still observed significant functional community differentiation across populations. While there appear to be commonalities in the availability of microbial functions to host plants, there was still strong community differentiation across endosphere profiles in each population, driven by changes in soils and vegetation (Fitzpatrick et al., 2018).

4.5 Conclusion

Our study reveals key functional differences within *T. triandra* root systems across an aridity gradient. We found that endospheres exhibit higher functional diversity than both rhizospheres and bulk soils. This arose due to expanded functional evenness within endospheres, despite declines in functional richness through the two-step selection process. Furthermore, changes in alpha and beta diversity suggest that in arid populations, rhizospheres foster increasingly homogenous microbiomes with high functional redundancy, which likely bolsters the resilience of *T. triandra* to water-limited environments by supporting key microbial functions under stressful conditions. Ultimately, *T. triandra* actively facilitates symbioses with microbiota in its rhizospheres and endospheres by driving specific functional profiles that impact host metabolism and select for high microbial fitness. These findings advance our understanding of functional plant-microbial dynamics in grasslands and offer new insights for restoration and management of grasslands under climate change.

Acknowledgements

This metagenomics sequencing for this project was supported by the Flinders University Accelerator for Microbiome Exploration. We also received funding from the Holsworth Wildlife Research Endowment with the Ecological Society of Australia, the Conservation Biology Grant 2022 with the Biological Society of South Australia and Nature Conservation Society of South Australia, the Lirabenda Wildlife Research Fund from the Field Naturalists Society of South Australia, and from the Australian Research Council (grant numbers LP190100051, LP190100484) and the New Zealand Ministry of Business Innovation and Employment (grant UOWX2101). We thank Victoria Drysdale, Belinda Martin, Chris Franco, Shawn Peddle, Nicole Fickling, Joel Brame, Carl Watson, and Billy Miller for their assistance in the field. We further acknowledge the traditional custodians of the lands on which we conducted our study: the Kaurna Pangkarra, Peramangk, Ngarrindjeri, Narangga, and Nukunu Peoples.

Figures

Figure 4.1. Alpha and beta diversity metrics for normalised bacterial species abundances associated with plant compartments (i.e., bulk soil, rhizosphere, endosphere). (a) Relative abundances of bacterial phyla across all bulk soil, rhizosphere, endosphere samples. Sample labels

are ordered by aridity index and box colours denote plant compartment. (b) Alpha diversity estimates for bacteria associated within their plant compartments for the effective number of species (see Table S4.7 for statistical output). (c) Bacterial community compositions with plant compartments is shown via NMDS ordinations with Bray-Curtis distances (stress =0.059; Table S4.8) (beta diversity). Point shape and hull colours represent samples from each different plant compartment, and point colour shows the aridity index. (d) Beta dispersion differences are represented by distance to centroid estimates for each compartment (see Table S4.9). (e) Heatmap showing all differentially abundant bacterial phyla across bulk soils, rhizosphere and endosphere at the 0.05 significance level based on log fold change (see Table S4.12 for detail, including full names of abbreviated phyla). Log fold changes are reported as Group1 vs Group2, where Group2 is the reference category (i.e., positive values indicate greater abundance in Group1 relative to Group2).

Figure 4.2. Alpha and beta diversity metrics for normalised functional gene abundances associated with plant compartments (bulk soil, rhizospheres and endospheres). Alpha diversity estimates for microbial genes associated within their plant compartments for (a) functional gene richness, (b) the effective number of functions, and (c) Pielou's evenness index (see Table S4.10). Light grey lines connect the data points with shared plant ID across the bulk soils,

rhizospheres, and endospheres. Beta diversity for estimates across functional gene compositions with plant compartments are shown via (d) NMDS ordinations with Bray-Curtis distances (stress =0.071; Table S4.8). Shape and hulls show the sample compartments, whereas point colour shows the aridity index. (e) Beta dispersion is represented by distance to centroid estimates (see Table S4.9).

Figure 4.3. Changes in the diversity of functions in plant compartments and canonical correspondence analysis (CCA) showing the effect of the environmental variables on microbial functional structure in *T. triandra* rhizospheres. Aridity index is plotted against the alpha diversisty for: (a) motility and chemotaxis, (b) stress response, and (c) secondary metabolism, showing 2000 bootstrapped estimates (see Table S4.2 for all bootstrapped statistical output). CCAs show genes at subsystem level 1 attributed to (d) motility and chemotaxis, (b) stress response, and (f)

secondary metabolism. Vectors represent significant variables associated with gene community compositions. Points are coloured by sampling population. The remaining bulk soil, rhizosphere, and endosphere CCAs are found in Figures S4.35-S4.37, see Table S4.4 for explanation of explanatory variables.

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Figure 4.4. Aridity influences the homogeneity of taxonomic and functional microbiomes differently across plant compartments. (a) Beta diversity of average Bray-Curtis distances of each bacterial taxonomic community, and beta diversity (Bray-Curtis distances) of functions at SEED subsystem level 1: (b) motility and chemotaxis, stress responses, phosphorus metabolism, nitrogen metabolism, regulation and cell signalling, and secondary metabolism. For all comparisons, the mean estimate and 2000 bootstrapped estimates are plotted to give an indication of each relationship (see Tables S4.2-S4.3 for all bootstrapped statistical output).

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Figure 4.5. Network analysis showing the stress response functions across *T. triandra* compartments (bulk soil, rhizosphere, and endosphere), across low, medium and high aridity levels. (a) Each network analysis comprises nodes representing functional processes of stress response genes, coloured at subsystem level 2. Node size shows the relative abundance of each function, connected via positive (blue) or negative (red) edges. Doughnut plots indicate the proportion of functions at subsystem level 2 for each network. (b) Hub functions were chosen as the top rannked functions by node degree, and closeness centrality (Table S4.14). (c) Mean edge weights with upper and lower CIs showing the degree of positive (co-occurrences) versus negative associations (mutual exclusion) between functions in low to high aridity across all plant compartments. For reporting of all statistical output for Kruskal-Wallis tests, see Table S4.13.

Figure 4.6. Network analysis edge weights across six functional levels in *T. triandra* microbial compartments (bulk soil, rhizosphere, and endosphere), across low, medium and high aridity levels. Mean edge weights with upper and lower CIs showing the degree of positive (co-occurrences) versus negative associations (mutual exclusion) in (a) motility and chemotaxis, (b) stress response, (c) phosphorus metabolism, (d) nitrogen metabolism, (e) regulation and cell signalling, and (f) secondary metabolism. For reporting of all statistical output for Kruskal-Wallis tests, see Table S4.13.

References

National Centre for Biotechnology Information (NCBI), N. C. f. B. I. 2021. Assembly: GCA_018135685.1, *Themeda triandra* (kangaroo grass), isolate sf006. National Library of Medicine (US), National Center for Biotechnology Information.

Abdelfattah, A., A. J. M. Tack, C. Lobato, B. Wassermann, and G. Berg. 2023. From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. Trends in Microbiology **31**:346-355.

Adeleke, B. S., O. O. Babalola, and B. R. Glick. 2021. Plant growth-promoting root-colonizing bacterial endophytes. Rhizosphere **20**:100433.

ALA. 2014. Mean annual aridity index. Atlas of Living Australia.

Anthony, M. A., S. F. Bender, and M. G. A. van der Heijden. 2023. Enumerating soil biodiversity. Proceedings of the National Academy of Sciences **120**:e2304663120.

Bates, D., M. Maechler, and M. Jagan. 2023. Matrix: Sparse and Dense Matrix Classes and Methods.

Belbin, L. 2011. The Atlas of Livings Australia's Spatial Portal.*in* In, Proceedings of the Environmental Information Management Conference 2011 (EIM 2011).

Berrios, L., and J. D. Rentsch. 2022. Linking Reactive Oxygen Species (ROS) to Abiotic and Biotic Feedbacks in Plant Microbiomes: The Dose Makes the Poison. International Journal of Molecular Sciences.

Bever, J. D. 1994. Feedback between plants and their soil communities in an old field community. Ecology **75**:1965-1977.

Bhattacharyya, A., O. Mavrodi, N. Bhowmik, D. Weller, L. Thomashow, and D. Mavrodi. 2023. Chapter 1 - Bacterial biofilms as an essential component of rhizosphere plant-microbe interactions. Pages 3-48 *in* V. Gurtler and M. Patrauchan, editors. Methods in Microbiology. Academic Press.

Biggs, C. R., L. A. Yeager, D. G. Bolser, C. Bonsell, A. M. Dichiera, Z. Hou, S. R. Keyser, A. J. Khursigara, K. Lu, A. F. Muth, B. Negrete Jr., and B. E. Erisman. 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. Ecosphere **11**:e03184.

Bremer, E., and R. Krämer. 2019. Responses of Microorganisms to Osmotic Stress. Annual Review of Microbiology **73**:313-334.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature **488**:91-95.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. v. Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology **64**:807-838.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics **34**:i884-i890.

Chi, F., S.-H. Shen, H.-P. Cheng, Y.-X. Jing, G. Yanni Youssef, and B. Dazzo Frank. 2005. Ascending migration of endophytic Rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Applied and environmental microbiology **71**:7271-7278.

Cruz de Carvalho, M. H. 2008. Drought stress and reactive oxygen species: Production, scavenging and signaling. Plant Signal Behav **3**:156-165.

Csardi, G., T. Nepusz, K. Müller, S. Horvát, V. Traag, F. Zanini, and D. Noom. 2006. The igraph software package for complex network research. Complex Systems.

David, A. S., K. B. Thapa-Magar, and M. E. Afkhami. 2018. Microbial mitigation–exacerbation continuum: a novel framework for microbiome effects on hosts in the face of stress. Ecology **99**:517-523.

Debray, R., R. A. Herbert, A. L. Jaffe, A. Crits-Christoph, M. E. Power, and B. Koskella. 2022. Priority effects in microbiome assembly. Nature Reviews Microbiology **20**:109-121.

Dixon, R. A. 2001. Natural products and plant disease resistance. Nature **411**:843-847.

Docherty, K. M., and J. L. M. Gutknecht. 2019. Soil microbial restoration strategies for promoting climate-ready prairie ecosystems. Ecological Applications **29**:e01858.

Dunning, L. T., A.-L. Liabot, J. K. Olofsson, E. K. Smith, M. S. Vorontsova, G. Besnard, K. J. Simpson, M. R. Lundgren, E. Addicott, R. V. Gallagher, Y. Chu, R. T. Pennington, P.-A. Christin, and C. E. R. Lehmann. 2017. The recent and rapid spread of *Themeda triandra*. Botany Letters **164**:327-337.

Egidi, E., D. Bristol, K. Hassan, D. Tissue, I. J. Wright, and U. N. Nielsen. 2024. The influence of plant traits on soil microbial communities varies between arid and mesic grasslands. Plant and Soil.

Escobar Rodríguez, C., B. Mitter, L. Antonielli, F. Trognitz, S. Compant, and A. Sessitsch. 2018. Roots and panicles of the C4 model grasses Setaria viridis (L). and S. pumila jost distinct bacterial assemblages with core taxa conserved across host genotypes and sampling sites. Frontiers in Microbiology **9**.

Farley, G. J., S. M. Bellairs, and S. W. Adkins. 2013. Germination of selected Australian native grass species, with potential for minesite rehabilitation. Australian Journal of Botany **61**:283-290.

Fitzpatrick, C. R., J. Copeland, P. W. Wang, D. S. Guttman, P. M. Kotanen, and M. T. J. Johnson. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. Proceedings of the National Academy of Sciences of the United States of America **115**:E1157-E1165.

Friedman, J., and E. J. Alm. 2012. Inferring Correlation Networks from Genomic Survey Data. Plos Computational Biology **8**:e1002687.

Garden, D., N. Ellis, A. Rab, C. Langford, W. Johnston, C. Shields, T. Murphy, M. Holmberg, K. Dassanayake, and S. Harden. 2003. Fertiliser and grazing effects on production and botanical composition of native grasslands in south-east Australia. Australian Journal of Experimental Agriculture - AUST J EXP AGR **43**.

Guo, J., N. Ling, Y. Li, K. Li, H. Ning, Q. Shen, S. Guo, and P. Vandenkoornhuyse. 2021. Seed-borne, endospheric and rhizospheric core microbiota as predictors of plant functional traits across rice cultivars are dominated by deterministic processes. New Phytologist **230**:2047-2060.

Guo, J., H. Ning, Y. Li, Q. Xu, Q. Shen, N. Ling, and S. Guo. 2024. Assemblages of rhizospheric and root endospheric mycobiota and their ecological associations with functional traits of rice. mBio **15**:e02733-02723.

Hancock, N., and L. Hughes. 2014. Turning up the heat on the provenance debate: Testing the 'local is best' paradigm under heatwave conditions. Austral Ecology **39**:600-611.

Hardoim, P. R., L. S. v. Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring, and A. Sessitsch. 2015. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. Microbiology and Molecular Biology Reviews **79**:293-320.

Hardoim, P. R., L. S. van Overbeek, and J. D. v. Elsas. 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology **16**:463-471.

He, X., D. Wang, Y. Jiang, M. Li, M. Delgado-Baquerizo, C. McLaughlin, C. Marcon, L. Guo, M. Baer, Y. A. T. Moya, N. von Wirén, M. Deichmann, G. Schaaf, H.-P. Piepho, Z. Yang, J. Yang, B. Yim, K. Smalla, S. Goormachtig, F. T. de Vries, H. Hüging, M. Baer, R. J. H. Sawers, J. C. Reif, F. Hochholdinger, X. Chen, and P. Yu. 2024. Heritable microbiome variation is correlated with source environment in locally adapted maize varieties. Nature Plants.

Hodgson, R. J., C. Cando-Dumancela, C. Liddicoat, S. Ramesh, R. A. Edwards, and M. Breed. 2024a. Strong host and environment modulation of rhizosphere-to-endosphere colonisation in the pan-palaeotropical keystone grass species, *Themeda triandra*. Authorea.

Hodgson, R. J., C. Liddicoat, C. Cando-Dumancela, N. W. Fickling, S. D. Peddle, S. Ramesh, and M. F. Breed. 2024b. Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C4 grass, *Themeda triandra*. Applied Soil Ecology **201**:105514.

Jiang, F., J. A. Bennett, K. M. Crawford, J. Heinze, X. C. Pu, A. Luo, and Z. H. Wang. 2024. Global patterns and drivers of plant-soil microbe interactions. Ecology Letters **27**:12.

Johnston-Monje, D., and M. N. Raizada. 2011. Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. Plos One **6**:e20396.

Jost, L. 2006. Entropy and diversity. Oikos **113**:363-375.

Khare, E., J. Mishra, and N. K. Arora. 2018. Multifaceted Interactions Between Endophytes and Plant: Developments and Prospects. Frontiers in Microbiology **9**.

Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, C. R. Fellbaum, G. A. Kowalchuk, M. M. Hart, A. Bago, T. M. Palmer, S. A. West, P. Vandenkoornhuyse, J. Jansa, and H. Bücking. 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science **333**:880-882.

Kincses, A., T. S. A. Ghazal, and J. Hohmann. 2024. Synergistic effect of phenylpropanoids and flavonoids with antibiotics against Gram-positive and Gram-negative bacterial strains. Pharmaceutical Biology **62**:659-665.

Kurtz, Z. D., C. L. Müller, E. R. Miraldi, D. R. Littman, M. J. Blaser, and R. A. Bonneau. 2015. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. Plos Computational Biology **11**:e1004226.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods **9**:357-359.

Lekberg, Y., J. D. Bever, R. A. Bunn, R. M. Callaway, M. M. Hart, S. N. Kivlin, J. Klironomos, B. G. Larkin, J. L. Maron, K. O. Reinhart, M. Remke, and W. H. van der Putten. 2018. Relative importance of competition and plant-soil feedback, their synergy, context dependency and implications for coexistence. Ecology Letters **21**:1268-1281.

Lemanceau, P., M. Blouin, D. Muller, and Y. Moënne-Loccoz. 2017. Let the Core Microbiota Be Functional. Trends in Plant Science **22**:583-595.

Lin, H., and S. D. Peddada. 2020. Analysis of compositions of microbiomes with bias correction. Nature Communications **11**:3514.

Lin, Q., L. Li, J. M. Adams, P. Heděnec, B. Tu, C. Li, T. Li, and X. Li. 2021. Nutrient resource availability mediates niche differentiation and temporal co-occurrence of soil bacterial communities. Applied Soil Ecology **163**:103965.

Louca, S., M. F. Polz, F. Mazel, M. B. N. Albright, J. A. Huber, M. I. O'Connor, M. Ackermann, A. S. Hahn, D. S. Srivastava, S. A. Crowe, M. Doebeli, and L. W. Parfrey. 2018. Function and functional redundancy in microbial systems. Nature Ecology & Evolution **2**:936-943.

Lu J, B. F., Thielen P, Salzberg SL. 2017. Bracken: estimating species abundance in metagenomics data. PeerJ Computer Science **3:e104**.

Lu, J., N. Rincon, D. E. Wood, F. P. Breitwieser, C. Pockrandt, B. Langmead, S. L. Salzberg, and M. Steinegger. 2022. Metagenome analysis using the Kraken software suite. Nature Protocols **17**:2815-2839.

Lumibao, C. Y., E. R. Kimbrough, R. H. Day, W. H. Conner, K. W. Krauss, and S. A. Van Bael. 2020. Divergent biotic and abiotic filtering of root endosphere and rhizosphere soil fungal communities along ecological gradients. Fems Microbiology Ecology **96**.

Lumibao, C. Y., L. Torres Martínez, J. P. Megonigal, S. A. Van Bael, and M. J. Blum. 2022. Microbial mediation of salinity stress response varies by plant genotype and provenance over time. Molecular Ecology **31**:4571-4585.

Lundberg, D. S., S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. d. Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe, and J. L. Dangl. 2012. Defining the core Arabidopsis thaliana root microbiome. Nature **488**:86-90.

McPherson, M. R., P. Wang, E. L. Marsh, R. B. Mitchell, and D. P. Schachtman. 2018. Isolation and Analysis of Microbial Communities in Soil, Rhizosphere, and Roots in Perennial Grass Experiments. JoVE:e57932.

Middleton, N., and D. Thomas. 1997. World atlas of desertification. Arnold: UNEP.

Moroenyane, I., J. Tremblay, and É. Yergeau. 2020. Temporal and spatial interactions modulate the soybean microbiome. Fems Microbiology Ecology **97**.

Naylor, D., and D. Coleman-Derr. 2018. Drought Stress and Root-Associated Bacterial Communities. Frontiers in Plant Science **8**.

Ngumbi, E., and J. Kloepper. 2016. Bacterial-mediated drought tolerance: Current and future prospects. Applied Soil Ecology **105**:109-125.

Ochoa-Hueso, R., S. L. Collins, M. Delgado-Baquerizo, K. Hamonts, W. T. Pockman, R. L. Sinsabaugh, M. D. Smith, A. K. Knapp, and S. A. Power. 2018. Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. Global Change Biology **24**:2818-2827.

Ochoa-Hueso, R., D. J. Eldridge, M. Berdugo, P. Trivedi, B. Sokoya, C. Cano-Díaz, S. Abades, F. Alfaro, A. R. Bamigboye, F. Bastida, J. L. Blanco-Pastor, A. de los Rios, J. Durán, S. Geisen, T. Grebenc, J. G. Illán, Y.-R. Liu, T. P. Makhalanyane, S. Mamet, M. A. Molina-Montenegro, J. L. Moreno, T. U. Nahberger, G. F. Peñaloza-Bojacá, C. Plaza, A. Rey, A. Rodríguez, C. Siebe, B. K. Singh, A. L. Teixido, C. Torres-Díaz, L. Wang, J. Wang, J. Wang, E. Zaady, X. Zhou, X.-Q. Zhou, L. Tedersoo, and M. Delgado-Baquerizo. 2024. Unearthing the soil-borne microbiome of land plants. Global Change Biology **30**:e17295.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, L. P., M. D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. S., Szoecs E., and W. H. 2019. vegan: Community Ecology Package.

Overbeek, R., T. Disz, and R. Stevens. 2004. The SEED: a peer-to-peer environment for genome annotation. Commun. ACM **47**:46–51.

Paradkar, A. 2013. Clavulanic acid production by Streptomyces clavuligerus: biogenesis, regulation and strain improvement. The Journal of Antibiotics **66**:411-420.

Petipas, R. H., M. A. Geber, and J. A. Lau. 2021. Microbe-mediated adaptation in plants. Ecology Letters **00**:1–16.

Petipas, R. H., J. B. González, T. M. Palmer, and A. K. Brody. 2017. Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. Acta Oecologica **78**:71-78.

Philippot, L., and J. C. Germon. 2005. Contribution of Bacteria to Initial Input and Cycling of Nitrogen in Soils. Pages 159-176 *in* A. Varma and F. Buscot, editors. Microorganisms in Soils: Roles in Genesis and Functions. Springer Berlin Heidelberg, Berlin, Heidelberg.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Saleem, A., F. U. Hassan, A. Manaf, and M. S. Ahmedani. 2009. Germination of *Themeda triandra* (Kangaroo grass) as affected by different environmental conditions and storage periods. African Journal of Biotechnology **8**:4094-4099.

Schadt, C., S. Martin, A. Carrell, A. Fortner, D. Hopp, D. Jacobson, D. Klingeman, B. Kristy, J. Phillips, B. Piatkowski, M. A. Miller, M. Smith, S. Patil, M. Flynn, S. Canon, A. Clum, C. J. Mungall, C. Pennacchio, B. Bowen, K. Louie, T. Northen, E. A. Eloe-Fadrosh, M. A. Mayes, W. Muchero, D. J. Weston, J. Mitchell, and M. Doktycz. 2024. An integrated metagenomic, metabolomic and transcriptomic survey of Populus across genotypes and environments. Scientific Data **11**:339.

Shah, S., C. Chen, Y. Sun, D. Wang, T. Nawaz, K. El-Kahtany, and S. Fahad. 2023. Mechanisms of nitric oxide involvement in plant-microbe interaction and its enhancement of stress resistance. Plant Stress **10**:100191.

Silva, G. G. Z., K. T. Green, B. E. Dutilh, and R. A. Edwards. 2015. SUPER-FOCUS: a tool for agile functional analysis of shotgun metagenomic data. Bioinformatics **32**:354-361.

Silva, G. G. Z., F. A. C. Lopes, and R. A. Edwards. 2017. An Agile Functional Analysis of Metagenomic Data Using SUPER-FOCUS. Pages 35-44 *in* D. Kihara, editor. Protein Function Prediction: Methods and Protocols. Springer New York, New York, NY.

Snyman, H. A., L. J. Ingram, and K. P. Kirkman. 2013. *Themeda triandra*: a keystone grass species. African Journal of Range & Forage Science **30**:99-125.

Srivastava, A., and R. Raghuwanshi. 2023. 13 - Endophytes and their secondary metabolites against human pathogenic MDR microbes. Pages 277-303 *in* M. Shah and D. Deka, editors. Endophytic Association: What, Why and How. Academic Press.

Stein, L. Y., and M. G. Klotz. 2016. The nitrogen cycle. Current Biology **26**:R94-R98.

Trivedi, P., J. E. Leach, S. G. Tringe, T. Sa, and B. K. Singh. 2020. Plant–microbiome interactions: from community assembly to plant health. Nature Reviews Microbiology **18**:607-621.

UNEP. 1992. World Atlas of Desertification.

University, F. 2021. Deepthought (HPC).

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

van der Westhuizen, H. C., H. A. Snyman, W. L. J. van Rensburg, and J. H. J. Potgieter. 2001. The quantification of grazing capacity from grazing — and production values for forage species in semi-arid grasslands of southern Africa. African Journal of Range & Forage Science **18**:43-52.

Vanholme, B., I. El Houari, and W. Boerjan. 2019. Bioactivity: phenylpropanoids' best kept secret. Current Opinion in Biotechnology **56**:156-162.

Wang, M., A.-H. Ge, X. Ma, X. Wang, Q. Xie, L. Wang, X. Song, M. Jiang, W. Yang, J. D. Murray, Y. Wang, H. Liu, X. Cao, and E. Wang. 2024. Dynamic root microbiome sustains soybean productivity under unbalanced fertilization. Nature Communications **15**:1668.

Wang, S.-Y., X.-C. Shi, X. Chen, P. Laborda, Y.-Y. Zhao, F.-Q. Liu, and P. Laborda. 2021. Biocontrol ability of phenazine-producing strains for the management of fungal plant pathogens: A review. Biological Control **155**:104548.

Wood, D. E., J. Lu, and B. Langmead. 2019. Improved metagenomic analysis with Kraken 2. Genome Biology **20**:257.

Wood, D. E., and S. L. Salzberg. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biology **15**:R46.

Zhong, Y., P. O. Sorensen, G. Zhu, X. Jia, J. Liu, Z. Shangguan, R. Wang, and W. Yan. 2022. Differential microbial assembly processes and co-occurrence networks in the soil-root continuum along an environmental gradient. iMeta **n/a**:e18.

Zhuang, W., X. Yu, R. Hu, Z. Luo, X. Liu, X. Zheng, F. Xiao, Y. Peng, Q. He, Y. Tian, T. Yang, S. Wang, L. Shu, Q. Yan, C. Wang, and Z. He. 2020. Diversity, function and assembly of mangrove root-associated microbial communities at a continuous fine-scale. npj Biofilms and Microbiomes **6**:52.

Zou, H., N. Chen, M. Shi, M. Xian, Y. Song, and J. Liu. 2016. The metabolism and biotechnological application of betaine in microorganism. Applied Microbiology and Biotechnology **100**:3865-3876.

Chapter five: Arid soil bacteria legacies improve drought resilience of a keystone grass

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Percentage contribution: **RJH: 85%,** CCD: 1%, TD: 1%, VD 1%, NWF 1%, CL 1%, SDP 1%, SAR: 1%, DS 1%, AT 1%, MFB: 6%

Throughout this chapter I have used the pronoun ‘we’ to acknowledge the contributions of my coauthors, following the customary etiquette in published manuscripts.

Abstract

Plant-microbe interactions are critical to ecosystem functioning and result in soil legacies, where plants influence the soil in which they grow affecting the fitness of future generations. Soil legacies are driven in part by the two-step selection process, where soil microbes are recruited from bulk soil into rhizospheres (space around roots) and then into endospheres (within plant roots). However, the potential of these soil legacies to provide host plant drought tolerance is poorly understood. In a drought stress greenhouse trial, we show that arid-associated soil legacies increased the biomass under both drought and control conditions of the keystone grass *Themeda triandra*. We report strong positive associations between *T. triandra* biomass and bacterial alpha diversity across soils, rhizospheres and endospheres. These findings show that bacterial soil legacies have an important but underappreciated role in grassland resilience to drought, and could be better harnessed to support resilient grassland restoration efforts.

5.1 Introduction

Grasslands are in significant decline globally (Bardgett et al. 2021). The productivity, diversity, and resilience of these ecosystems is heavily shaped by their soil microbiota (Koziol and Bever 2017, Wang et al. 2019, Liu et al. 2022). Despite strong plant-soil interactions in grasslands (i.e., plant-soil feedbacks), these interactions are under acknowledged and underutilised in conservation and restoration efforts (Robinson et al. 2023, Peddle et al. 2024). As climate change and land-use pressures intensify, understanding how soil microbiota support grassland productivity and stress tolerance is increasingly important to aid conservation and restoration efforts (Trivedi et al. 2022, Fadiji et al. 2023).

Carbon and nutrient cycling are among the many microbial-driven processes in soil that can shape plant communities (Bever et al. 2010, Wagg et al. 2014). Plants also form direct symbioses with soil microbiota in their rhizospheres (areas around plant roots) and endospheres (inside plant roots)

(Bulgarelli et al. 2013). The colonisation of these plant compartments by soil microbiota is described by the two-step selection process (Bulgarelli et al. 2012, Lundberg et al. 2012, Bulgarelli et al. 2013). This process involves initial resource provision through plant roots which support microbial assemblages from the bulk soil to colonise host rhizospheres (step 1). Microbiota are then filtered into the endosphere with plant immune system regulation (step 2) (Bulgarelli et al. 2013). These rhizosphere and endosphere microbiota aid in plant nutrient acquisition and metabolic processes, but we currently lack a clear understanding of how recruitment is affected by plants growing under stressful conditions, such as drought. We also lack knowledge of how plant recruitment of these microbiota is affected by ecological contexts (e.g., high vs low aridity) (Ling et al. 2022, Santoyo 2022).

Harnessing soil biodiversity is increasingly recognised for its potential to enhance plant growth in applied ecology contexts (Mariotte et al. 2018, Porter and Sachs 2020, Peddle et al. 2024). One promising method to do this is through whole soil inoculations via the translocation of soil, including their microbiota, into new areas (Gebhardt et al. 2017, Wolfsdorf et al. 2021, Han et al. 2022). This approach leverages positive soil legacies where plant populations naturally cultivate soil microbiota that support the offspring of these plants (Kaisermann et al. 2017, Pineda et al. 2017, Buchenau et al. 2022). Positive soil legacies can improve plant tolerance to water stress and herbivory (Kaisermann et al. 2017, Hannula et al. 2021), but we lack theoretical understanding of the colonisation mechanisms within soil and plant compartments. Experimental testing of how different soils and their microbiota influence plant growth along with comprehensive characterisation of bacterial colonisation patterns can address these knowledge gaps, especially when accounting for stress scenarios.

Themeda triandra (Forssk.) is a globally important keystone C4 grass species with a pan-palaeotropical distribution (Snyman et al. 2013, Dunning et al. 2017, Pascoe 2018). Currently, the processes by which microbiota colonise and influence the growth of *T. triandra* remain poorly understood. To address this, we conducted a greenhouse experiment on how soil microbiota from high and low aridity--associated regions affected the germination and growth of *T. triandra* under both water-available and drought-like (i.e., water stress) conditions. We used 16S rRNA amplicon sequencing to characterise the *T. triandra*-associated microbiota of high and low aridity-associated soils under live versus sterilised, and water stress treatment conditions, plus the recruitment patterns of these microbiota from the bulk soils into *T. triandra* rhizospheres and endospheres. We hypothesised that: (1) soil microbiota sourced from arid-associated sites would enhance *T. triandra* growth under stress conditions by providing mutualistic microbiota that support growth under drought-like conditions; (2) distinct microbial communities would be recruited into the rhizosphere and endosphere under each water treatment, reflecting shifts in host plant requirements; and (3) the presence of *T. triandra* plants would alter the bacterial community in soil due to a cumulative influence of microbe-root interactions. By assessing how microbiota impact the drought responses of this important grass, and monitoring their recruitment across root compartments, we can better understand the value of soil biodiversity as a tool for improving the resilience of grassland ecosystems.

5.2 Methods

5.2.1 Experimental design

We prepared a germination and five-month growth trial to test the influence of microbiota in soils collected from either high or low aridity-associated locations by growing *T. triandra* under sterilised and live microbiota conditions. We also assessed the germination and growth of *T. triandra* plants in these soils under water-available versus water-stress conditions (mimicking a drought). Each of the eight treatments (i.e., 2 x aridity-associated soil levels, 2 x sterilisation treatments, 2 x water availability) had 10 replicate pots, making 80 pots in total (see Figure S5.1a-b). Each pot received an equal 1,190 g dry weight of its assigned soil. We calculated the relative soil water content for each soil treatment to give a standardised measure of moisture, with 0% corresponding to oven-dry soil and

100% to maximum water-holding capacity. For our control treatment, we watered each pot to 100% relative soil water content, while we kept the water stress treatment to 40% relative soil water content by regularly weighing and watering according to methods described by Earl (2003). Relative soil water content generally sat between 75-88% for the control (no-stress) treatment, and 35-38% for the water stress treatment (Figure S5.2). We included an additional 24 soil-only pots to account for changes in microbiota across each treatment in the absence of *T. triandra* (Figure S5.1c).

To capture naturally occurring soil microbiota associated with *T. triandra*, bulk soil was collected from around the roots of *T. triandra* plants in two undisturbed remnant sites (Figure 5.1a) associated with different levels of aridity: Kuitpo Forest Reserve at 35.2279ºS, 138.7199ºE (the mesic, low aridity site; aridity index = 0.658 – henceforth low aridity soil) and Quorn Floral Reserve at 32.3434ºS, 138.0182ºE (the semi-arid, high aridity site; aridity index = 0.227 – hereafter high aridity soil) on 14 and 16 November 2023, respectively (Table S5.1). Seeds were collected from the remnant *T. triandra* in Kuitpo Forest Reserve in December 2020. After collection, soils were sieved at 5 mm to remove large stones and litter and then stored at 4ºC for one month prior to setting up the growth trial. Half of the soil volumes were sterilised by autoclaving them twice at 121ºC, two days apart.

5.2.2 Germination and growth trial

The greenhouse was set at 16 h – 8 h day-night cycle, with temperatures set to 30ºC and 18ºC, respectively. In each pot (14 cm diameter, 2 L pots), we sowed eight *T. triandra* seeds and recorded their weights before placing them in identifiable wells in each pot on 13 December 2023 (Figure S5.3; 80 seeds per treatment, 640 seeds total). We monitored seedling emergence rates across each treatment. After 8 weeks, seedlings were randomly thinned to one plant per pot to avoid intra pot competition for space and soil resources such as water and nutrients (6 February 2024). Following this, water stress conditions were imposed at 10 weeks (21 February 2024). At the conclusion of the experiment (23 weeks; 21 May 2024), we recorded aboveground and belowground biomass, root-mass fraction (Pérez-Harguindeguy et al. 2013), and plant-soil feedback ratios (described below). Soils were collected immediately post-harvest for both physicochemical and bacterial community profiling in 40 pots (5 pots per treatment). We also collected rhizosphere and endosphere samples from 40 pots for microbial profiling (described below).

5.2.3 Soil physicochemical analysis

We analysed the following soil physicochemical conditions from each sampling site before and from pots after the growth trial at CSBP Laboratories (Bibra Lake, Australia): phosphorus and potassium (Colwell 1965), sulphur (KCl 40) (Blair et al. 1991), organic carbon (Walkley and Armstrong 1934), nitrate, ammonium, electrical conductivity and pH ($CaCl_2$).

5.2.4 DNA extraction, sequencing and bioinformatics

We prepared DNA extractions from *T. triandra* root endospheres following methods outlined in Hodgson et al. (2024b). This involved cleaning the exterior of plant root surfaces by sonication at five 30 s on/off burst cycles in 0.02% Silwet L-77 supplemented PBS buffer (pH = 6.5) for 5 min, followed by five 5 min washes in sterilised, distilled water. These methods underwent prior validation described in Hodgson et al. (2024b). To extract microbial DNA from rhizospheres, we followed the protocol from McPherson et al. (2018). Briefly, root samples were washed in 0.02% Silwet L-77 supplemented PBS buffer, vortexed for 45 min and then filtered using 100 µm sieves (Thermo Fisher Scientific, Waltham, USA) and centrifuged at 1000 RPM. Soils samples taken from the plant plots at either the start or end of the trial were stored at -20 °C after collection. DNA from soil, rhizosphere and endosphere samples was extracted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany), as per manufacturer’s instructions.

Amplicon libraries of the 16S rRNA V3-4 gene region were generated by the Australian Genome Research Facility (Brisbane, Australia). Sequences were generated using the 300 base pair paired end run of the Illumina NextSeq 2000 platform. The DADA2 bioinformatics pipeline was used to infer amplicon sequence variants (ASVs), with monotonicity enforced during error estimation.

Additionally, loess smoothing was applied with specified weights, span, and degree to improve error rate modelling. Qiime2 was used to identity profiles from amplicon sequence data from the SILVA database (v138.1) (Wang et al. 2007, Quast et al. 2013), using a naïve Bayesian classifier (Wang et al. 2007, Callahan et al. 2016, Bokulich et al. 2018). Taxa that were not assigned as Bacteria, unassigned at the Phylum level, and associated to mitochondria or chloroplasts were removed. We also removed remove taxa that did not occur in at least two samples, that is, we avoided including unrepresentative taxa which were only present in a single sample. We were unable to extract and sequence viable DNA concentrations from sterilised low aridity conditions at the beginning of the experiment, possibly due to the sterilising effects of autoclaving on microbiota and their DNA.

5.2.5 Statistical analysis

All analyses were performed using R version 4.0.2 (R Core Team 2022).

Germination analysis

Seed germination across treatments was compared using generalised linear mixed effects models with a binomial link function with the R package lme4 (Bates et al. 2015). Soil source, sterilisation and seed mass were used as fixed effects, and pot ID was included as a random effect. The random effect was included in the event that germination across multiple seeds in a shared pot environment could be influence by shared soil characteristics. We decided to include seed mass as a fixed effect in addition to our two main treatment variables (Soil source, sterilisation) to account for the potential influence of variation in seed maturity on our germination outcomes (see Table S5.2 for full model details).

Plant functional trait analysis

To compare the differences in total biomass, aboveground biomass, belowground biomass, and root-mass fractions of *T. triandra*, we used randomised linear mixed-effects models. Across our models, we included soil source, sterilisation and water stress as fixed effects, with interaction terms in different combinations, and random effects to account for within-group variation. This was done to better focus on different hypotheses (i.e., the impacts of our sterilisation, water stress etc. on plant responses), given our fully factorial experimental design.

For each response variable, we first constructed a model with sterilisation and water stress as fixed effects, including their interaction term, and treated aridity as a random effect. We then tested an alternative model in which aridity, sterilisation, and water stress were treated as fixed effects with all interaction terms, while sterilisation and water stress were also included as random effects to account for variance not captured by the fixed components (for full details, see Table S5.3-S5.4). Model significance was assessed using 10,000 permutations, comparing observed test statistics to the simulated null distributions.

For root-mass fractions, we separated samples from low- and high-aridity sites and tested models with sterilisation as a fixed effect and water stress as a random effect. We also ran general linear models on water-stress and water-stress control subsets, testing aridity, sterilisation, and their interaction. A final model was fitted using the full dataset, with aridity, sterilisation, and water stress included as fixed effects, and sterilisation and water stress included as random effects. As our design was factorial and strong interactions were present, this approach allowed us to better account for non-independence and to estimate treatment-level variance more accurately (for full details, see Table S5.3-S5.4).

We assessed plant-soil feedback (PSF) ratios for each plant trait across the different aridity soils and water stress treatment groups. For each treatment group, we calculated the average plant response under live and sterilised conditions, using the following formula, where $\bar{\boldsymbol{x}}$ represents average plant biomass from the live or sterile treatment groups:

$$PSF\ ratio = \frac{\left(\ \bar{x}\,Live - \ \bar{x}\,Sterile\right)}{\bar{X}\,Sterile}$$

Using the R package boot, we generated distributions of plant-soil feedback ratios by calculating 95% bias-corrected and accelerated (BCa) bootstrapped confidence intervals from 10,000 repetitions. Significant differences were found when there was no overlap between the 95% confidence intervals with the mean PSF ratios of other treatments.

Bacterial diversity analysis

Samples were rarefied to 18,738 reads to normalise variation in library sizes across samples of the soil, rhizosphere and endosphere samples (Cameron et al. 2021) (Figure S5.4). We also visualised the relative abundance of major phyla, and used differential abundance analysis to evaluate differences across each treatment using the *ancombc2* function in the R package ANCOMBC using non-rarified data (Lin and Peddada 2020).

To calculate alpha diversity across plant compartments and treatments, we estimated the effective number of ASVs by taking the exponential transformation of Shannon's diversity (Jost 2006). Comparisons in alpha diversity levels across treatments were conducted using permuted linear mixed effects models, and permuted analysis of variance (ANOVAs). Here, treatments were included as fixed an random effects as per our plant functional traits analyses. For instance, for each alpha diversity response variable, we first constructed a model with sterilisation and water stress as fixed effects, including their interaction term, and treated aridity as a random effect. We then tested an alternative model in which aridity, sterilisation, and water stress were treated as fixed effects with all interaction terms, while sterilisation and water stress were also included as random effects to account for variance not captured by the fixed components (for full details, see Table S5.6). Model significance was assessed using 10,000 permutations, comparing observed test statistics to the simulated null distributions.

Bacterial communities were visualised using non-metric multidimensional scaling (NMDS) and principal coordinates analysis (PCoA) ordinations based on Bray–Curtis distances. The effects of treatments on bacterial communities were assessed using permutational multivariate analysis of variance (PERMANOVA) via the adonis2 function in vegan (Oksanen et al. 2019). We first fitted an overarching model to test how bacterial communities were shaped by plant compartment. We then ran separate models within each compartment dataset, testing the effects of sterilisation, aridity, and water stress, individually, to ensure independence of data points in our statistical analysis (see Table S5.7, for full details of our statistical models).

5.3 Results

5.3.1 Germination

We found no effects of high/low aridity-associated soils (hereafter referred to as soil aridity) (GLMM; estimate =0.09, z =0.422, p =0.67) or sterilisation treatments (GLMM; estimate =-0.09, z =-0.426, p =0.67) on germination rates (Figure S5.5a), however larger seeds germinated faster (GLMM; estimate =397.87, z =9.97, p <0.001).

A generalised linear mixed model (GLMM) with a binomial distribution revealed no significant effects of soil aridity (estimate = 0.09 ± 0.21 SE, z = 0.42, p = 0.67) or sterilisation (estimate = −0.09 ± 0.21 SE, z = −0.43, p = 0.67) on germination probability. In contrast, seed weight had a strong positive effect (estimate = 397.87 ± 39.91 SE, z = 9.97, p < 0.001). The model included random intercepts for pot ID (variance = 0.195), capturing between-pot variation (see Table S5.2). The marginal R^2 (variance explained by fixed effects) was 0.241, and the conditional R^2 (variance explained by both fixed and random effects) was 0.284. We also produced a predicted effects plot showing how germination changes with seed weight (Figure S5.5b).

5.3.2 Plant biomass and stress responses

Water stress and soil sterilisation treatments significantly reduced the total *T. triandra* biomass recorded compared to control (no-stress) and live soil conditions (both p<0.001; Figure 5.1b; Table

S5.3-S5.4). The water stress-sterilisation interaction was significant, with the most notable difference being increased biomass in the live control (no-stress) soil treatment ($p<0.001$; Table S5.3-S5.4; Figure S5.6). Plants in the live high aridity soils had greater biomass than those in live low aridity soils ($p<0.001$; Figure 5.1b; Table S5.3-S5.4), and the soil aridity-sterilisation interaction was also significant, and showed plant biomass was higher under high aridity soil conditions ($p=0.022$; Table S5.3-S5.4; Figure S5.6).

The aboveground biomass of *T. triandra* plants was lower in the water stress ($p<0.001$; Figure 5.1c; Table S5.3-S5.4) and sterilisation treatments ($p<0.001$; Figure 5.1c; Table S5.3-S5.4). A water stress-sterilisation interaction was also present, where aboveground plant biomass was greater under live soil, with control water conditions, while unaffected by sterile soil conditions under both water availabilities ($p<0.001$; Table S5.3-S5.4; Figure S5.7). Like total biomass, we found higher *T. triandra* aboveground biomass for plants grown under high compared to low aridity soil conditions ($p<0.001$; Figure 5.1c; Table S5.3-S5.4). We also found significant interactions between soil aridity-sterilisation ($p=0.032$; Figure S5.7; Table S5.3-S5.4), soil aridity-water stress ($p<0.001$; Table S5.3-S5.4; Figure S5.7), and soil aridity-sterilisation-water stress ($p=0.046$; Table S5.3-S5.4; Figure S5.7). Here, there was a stronger increase in aboveground biomass in the live high aridity soils compared to the sterile high aridity soils. We also found that the aboveground biomass increase was greater between the water stress and the control treatments in high aridity soils than in the low aridity soils (Figure S5.7).

We found that belowground biomass decreased when under water stress ($p<0.001$; Figure 5.1d; Table S5.3-S5.4) and sterilisation treatments ($p<0.001$; Figure 5.1c; Figure 5.1d; Table S5.3-S5.4). High aridity soils also led to increased belowground biomass than low aridity soils ($p<0.001$; Figure 5.1d; Table S5.3-S5.4). Belowground biomass was also affected by a water stress-sterilisation interaction ($p= 0.014$; Figure 5.1d; Table S5.3-S5.4; Figure S5.8). Here, sterilisation reduced belowground biomass more under low aridity soil conditions than under high aridity soil conditions.

There was no effect of water stress on root-mass fraction. However, sterilisation of low aridity soils increased the root-mass fractions (p=0.003; Figure 5.1e; Table S5.3-S5.4) and sterilisation of high aridity soils reduced the root-mass fraction (p=0.002; Figure 5.1e; Table S5.3-S5.4; see Figure S5.9).

All plant soil feedback ratios were positive in each treatment, though we found significantly higher plant soil feedback ratios in the low aridity soils compared to high aridity soils for total, aboveground, and belowground biomass and root mass fractions (see Table S5.5; Figure S5.10). The elevated plant soil feedback ratios in low aridity soils appear to be driven by the very low biomass outcomes when these soils were sterilised (Figure 5.1b-d). In the low aridity soils, the plant soil feedback ratios were higher in the control treatments compared to water stress treatments for total, aboveground, and belowground biomass (Table S5.5; Figure S5.10a-c).

5.3.3 Bacterial diversity across belowground compartments

We observed 11 bacterial phyla across all samples, which represented 94.8% of reads and had abundance estimates of >10% across all plant compartments, treatments and timepoints throughout this experiment (Figure 5.2a). The soil-only pots had 8 bacterial phyla, which represented 96.9% of reads and had abundance estimates of >2.5% (Figure S5.11).

Alpha diversity levels across the soils and rhizospheres were both higher than the *T. triandra* endospheres in the live (permutedANOVA: $F_{(3,75)} = 14.26$, p <0.001; Figure 5.3a) and sterilised treatments (permutedANOVA: $F_{(3,63)} = 5.824$, p = 0.003). Alpha diversity was also higher for soil-only pots (in all treatments) at the beginning of the trial than at harvest (permutedANOVA: $F_{(2,22)} = 7.932$, p = 0.01), though there were no differences between soils in the sterilised soil-only pots over time (permutedANOVA: $F_{(1,14)} = 0.313$, p = 0.59).

Sterilisation reduced alpha diversity of all soils at the beginning of the experiment (p<0.001; Table S5.6; Figure S5.12a) and these differences persisted until harvest (p<0.001; Table S5.6; Figure S5.12).

We saw no effect of soil aridity (live $p = 0.875$; sterile $p = 0.086$; Table S5.6; Figure S12) or water-stress treatment (live $p = 0.312$, sterile $p = 0.840$; Table S5.6; Figure S5.12) on soil alpha diversity. The soil-only pots also did not vary in alpha diversity between low and high aridity soil conditions or water availability treatment groups (live permutedLMEM: t-value -0.567, $p = 0.584$, sterile permutedLMEM: t-value 1.159, $p = 0.255$).

Bacterial communities significantly varied by compartment (i.e., soils, rhizospheres, endospheres) across all treatments (PERMANOVA: $F_{(2,183)} = 7.465$, $R^2 = 0.075$, $p < 0.001$; Figure 5.3b; Figure S5.13). Sterilisation ($p < 0.001$, $p < 0.001$, and $p = 0.013$, respectively; Figure 5.4a; Table S5.6), soil aridity ($p < 0.001$, $p < 0.001$, and $p = 0.023$, respectively; Figure 5.4a; Table S5.6), and water stress treatments ($p < 0.001$, $p < 0.001$, and $p = 0.033$, respectively; Figure 5.4a; Table S5.6) each affected soil, rhizosphere and endosphere bacterial community composition.

There was no detectable difference between the bacterial community compositions between the soil-only pots to the plant-present pots (PERMANOVA: $F_{(1, 103)} = 0.733$, $R^2 = 0.007$, $p = 0.755$; Figure 5.4b), but we did observe a difference in communities from the initial sampling to the harvest (PERMANOVA: $F_{(1, 103)} = 7.354$, $R^2 = 0.066$, $p < 0.001$; Figure 5.4b).

Soil aridity, sterilisation, and water-stress treatments had effects on differential abundance of bacterial phyla across the soils, rhizospheres and endospheres (Figure 5.1b; Tables S5.8-S5.9).

Bacterial alpha diversity in soils, rhizospheres, and endospheres correlated positively and strongly with plant biomass (LMEM: t-value = 10.857, $p < 0.001$; Figure 5.5). Biomass increased more with bacterial alpha diversity in high aridity soils (Figure 5.5).

5.3.4 Soil physicochemical conditions

Organic carbon and pH were both higher in low aridity soils compared with high aridity soils. Sterilisation increased ammonium levels in high aridity soils, and potassium for low aridity soils (Figure S5.14). Nitrate, phosphorus, sulphur and electrical conductivity did not statistically differ across treatments.

At harvest, most soil physicochemical variables showed differences across treatments, except for ammonium, nitrate and electrical conductivity, which did not differ (Figure S5.14). The greatest differences were increases in phosphorus in sterilised soils, higher potassium in the high aridity soils, higher organic carbon in the low aridity soils, and higher pH in the low aridity soils (Figure S5.14).

5.4 Discussion

We experimentally assessed the effects of high and low aridity-associated soil legacies on the growth of the keystone grass species, *Themeda triandra*, under drought conditions. We show that microbiota from high aridity-associated soil supported increased growth of this grass species under both drought-like, water stress and control treatments, highlighting the powerful impact of soil legacies and supporting our first hypothesis. We also show that bacterial alpha diversity was positively correlated with *T. triandra* biomass, and that each of our treatments (i.e., aridity-associated soils, sterilisation, and water stress) led to distinct bacterial assemblages in soils, rhizospheres and endospheres. While site differences likely reflect aridity, they may also capture other environmental variables (e.g., soil P or C), supporting our second hypothesis that *T. triandra* forms context-dependent relationships with its bacterial communities. Finally, we did not see meaningful differences across the bacterial communities of our soil-only versus plant-present pots, which goes against the expectations of our third hypothesis. Our findings highlight the importance of soil microbiota for host plant growth and fitness under climate change. Our study underscores the importance of protecting diverse soil communities to support grassland health, and highlights the potential of harnessing these communities to increase grassland restoration that is more resilient to climate change.

5.4.1 Microbially mediated effects on biomass

We show that live soil communities enhanced plant growth in high and low aridity-associated soils, and under control and water-stress conditions. Additionally, bacterial alpha diversity across the soils, rhizospheres and endospheres were positively correlated with *T. triandra* biomass, suggesting that a greater variety of unique bacteria, either naturally present in the soil or recruited into the rhizospheres and endospheres, leads to greater plant growth. Alpha diversity is a well-known driver of plant productivity and is associated with greater ecosystem functionality (Schnitzer et al. 2011, Byrnes et al. 2014, Wang et al. 2019). Our findings support previous research which shows that host-benefiting microbial functions are present within *T. triandra* soils, rhizospheres and endospheres (Hodgson et al. 2024a), and the importance of the habitat source of microbes (e.g., arbuscular mycorrhizal fungi) for *T. triandra* drought response (Petipas et al. 2017). As such, there is now a strong body of evidence to suggest that soil microbiota support *T. triandra* growth across diverse ecosystems, under both stress and non-stress conditions.

Our *T. triandra* plants developed larger root-mass fractions in the sterilised high aridity-associated soils, compared to the live high aridity-associated soils. This shows that a higher proportion of plant resources were allocated to the development of roots under sterilised soil conditions, perhaps in response to an absence of microbiota which typically aid the acquisition or unblocking of nutrient resources in the soil (Pérez-Harguindeguy et al. 2013, Bai et al. 2022, Wang et al. 2024). Interestingly, we observed the opposite trend in the low aridity-associated soils, where greater root investment occurred in the live low aridity-associated soils compared to those grown in sterilised low aridity-associated soils. As the bacterial communities were distinct, the low aridity-associated soil microbiota may not provide the same functional benefits as those found in the high aridity-associated soils – where different soil conditions, like available moisture or organic matter, could create different host needs (Hodgson et al. 2024a). Plants growing in the low aridity-associated soils may not typically produce such strong microbial-root interactions, given the potential absence of these stress-tolerance benefits by the microbiota (Comas et al. 2013, Hodgson et al. 2024a). The importance of microbiota for plant growth, and the strength of their interactions, may therefore depend on the aridity of the soil and broader environmental conditions, which involve a complex interplay of factors such as soil nutrients, moisture, and structure (De Long et al. 2019).

5.4.2 Treatment effects on the two-step selection process

We show a decrease in bacterial diversity in the rhizosphere and endosphere versus bulk soils, which is consistent with previous findings expected under the two-step selection process (Bulgarelli et al. 2012, Lundberg et al. 2012, Urbina et al. 2018). *T. triandra* plants recruited different communities of bacteria from the soil into their rhizospheres and endospheres depending on whether they underwent soil sterilisation or water-stress treatments. These findings show that the plant's growth environment alters the recruitment dynamics of soil bacteria. It also shows that *T. triandra* plants under stress appear to alter their entry screening strategies of soil bacteria when growing under drought-like conditions.

Endosphere recruitment dynamics were most sensitive to the long-term effects of soil sterilisation, compared to aridity-associated soil or water-stress treatments. In all sterilised treatments, endosphere diversity was lower and bacterial communities were differently structured to the unsterilised soils. However, it remains unclear how bacteria from sterilised soils were selectively recruited into the endospheres – whether they originated from the seed microbiome or were microbiota that were not entirely removed from the soils during sterilisation (Kim et al. 2022, Ling et al. 2022, Abdelfattah et al. 2023, He et al. 2024). Given the reduced *T. triandra* growth rates (biomass) in the low aridity-associated sterilised soils (which was much lower than in high aridity-associated sterilised soil treatments), we suspect that this grass may also be more susceptible to colonisation by microbial pathogens that possible thrive under the low competition environment created by soil sterilisation (Mallon et al. 2015, Mawarda et al. 2022). Furthermore, seeds were not sterilised at the start of the germination trial, and so may be responsible for introducing some initial cohort of microbiota from their exterior surfaces that may shaped resultant microbiota across all treatments. Shotgun metagenomic analysis could help identify properties of microbial endosphere colonisation, such as the acquisition of growth-promoting functions. Alternatively, it could reveal whether colonisation dynamics are being hijacked by pathogenic or opportunistic microbes (i.e., 'cheater' organisms) that do not provide the same host plant services, despite other shared traits (Kiers et al. 2002, Kiers et al. 2011). The consequences of these interactions could help inform the vulnerability of *T. triandra* to soil degradation, making this an important avenue for future research.

5.5 Conclusion

In our study, we sought to determine whether the widespread keystone grass*, Themada triandra*, relied on soil microbiota from arid-associated locations to gain growth advantages when grown under drought conditions. We report that soil microbiota from more arid-associated sources had strong positive effects on plant growth under drought conditions. We also show that aridity-associated soil, water stress, and sterilisation treatments shaped both plant growth and the soil-to-endosphere recruitment as described by the two-step selection process. Finally, soil physicochemical variables associated with our stress and sterilisation conditions influenced the composition of bacterial communities far more strongly that the presence of *T. triandra* plants. Together, these results suggest that grassland decline driven by increased aridity or other suboptimal physicochemical soil conditions, arising from climate and land cover change, may be partly mitigated by the beneficial effects of healthy soil microbiota on keystone grass species. To validate these findings, further studies are needed that replicate a broader range of aridity conditions and assess their influence on the soil environment, which we were unable to fully capture in this experiment. Based on our results, we anticipate that soil-based interventions aimed at enhancing microbiota could play an increasingly important role in the restoration of climate-resilient grasslands, pending further investigation.

Acknowledgements

This work was supported by funding from the Ecological Society of Australia (Holsworth Wildlife Research Endowment). We thank James Stangoulis and Van Lam Nguyen for their advice on experimental design. Additionally, we would like to thank Shaun Kennedy and Seeding Natives for supplying seed for this project. We further acknowledge the Kaurna and Nukunu peoples, the traditional owners of the lands on which we conducted our study.

Figures

Figure 5.1. High and low aridity sampling sites, and *T. triandra* plant trait responses to treatment effects. (a) High and low aridity sampling sites for the collection of soil microbiota for experimental manipulation (yellow points). Mean annual aridity index data layer (ADM) is sourced from the Soil and Landscape Grid of Australia (Searle et al. 2022), where aridity index is calculated via annual precipitation/annual potential evaporation. *T. triandra* plant growth responses to soil aridity, sterilisation treatments, and water stress, showing: *T. triandra* (b) total biomass, (c) aboveground biomass, (d) belowground biomass, and (e) root-mass fraction differences.

Figure 5.2. Mean relative abundance of major bacterial phyla across plant-present pots within *T. triandra* compartments over time. (a) Compartment and timpeoint included were the initial soil sampling period, soils at plant harvest, *T. triandra* rhizospheres at plant harvest, and *T. triandra* endospheres at plant harvest. Treatments include sterilisation (live, sterile), soil aridity (high, low aridity soils), and watering regime (water-stress as red text labels, control as blue text labels). Note: we did not sequence viable DNA from sterilised low aridity soils. (b) Differential abundance analysis comparing changes in phyla within each timpoint and compartment across treatments. Each category compares differences to a reference group (the high aridity, live, control

soil treatment). Log fold changes for the reference groups identify differences from the grandmean of each phyla.

Figure 5.3. Bacterial community differences across *T. triandra* compartments and timepoints. (a) Alpha diversity (effective number of ASVs) across treatments, time, and plant-present versus soil-only pots. (b) Non metric multidimenional scaling (NMDS) plot showing

bacterial community composition differences for each sampling treatment. Each point represents a sample, and closer points have more similar communities. Sample library sizes were rarified to 18,738 reads.

Figure 5.4. Bacterial community differences across each experimental treatment, and comparisions to soil-only pots. Non metric multidimensional scaling (NMDS) plot showing bacterial community composition differences across treatments in (a) sample types from plant-present pots, and (b) soil-only containing low versus high aridity soils. NMDS ordinations are based on Bray-Curtis distances (sample library sizes were rarified to 18,738 reads). Each point represents a sample, and closer points have more similar communities.

Figure 5.5. Bacterial diversity is correlated with *T. triandra* total biomass. Alpha diversity (effective number of ASVs) is positively correlated with post harvest *T. triandra* biomass across all plant compartments, and watering treatments. Soil aridity is denoted by colour (red = high aridity soils, blue = low aridity soils), and soils exposed to sterilisation at the beginning of the trial are shown with point shape (sterilisation = triangles, live = circles).

References

Abdelfattah, A., A. J. M. Tack, C. Lobato, B. Wassermann, and G. Berg. 2023. From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. Trends in Microbiology **31**:346-355.

Bai, B., W. Liu, X. Qiu, J. Zhang, J. Zhang, and Y. Bai. 2022. The root microbiome: Community assembly and its contributions to plant fitness. Journal of Integrative Plant Biology **64**:230-243.

Bardgett, R. D., J. M. Bullock, S. Lavorel, P. Manning, U. Schaffner, N. Ostle, M. Chomel, G. Durigan, E. L. Fry, D. Johnson, J. M. Lavallee, G. Le Provost, S. Luo, K. Png, M. Sankaran, X. Hou, H. Zhou, L. Ma, W. Ren, X. Li, Y. Ding, Y. Li, and H. Shi. 2021. Combatting global grassland degradation. Nature Reviews Earth & Environment **2**:720-735.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1-48.

Bever, J., I. Dickie, E. Facelli, J. Facelli, J. Klironomos, M. Moora, M. Rillig, W. Stock, M. Tibbett, and M. Zobel. 2010. Rooting Theories of Plant Community Ecology in Microbial Interactions. Trends in Ecology & Evolution **25**:468-478.

Blair, G., N. Chinoim, R. Lefroy, G. Anderson, and G. Crocker. 1991. A soil sulfur test for pastures and crops. Soil Research **29**:619-626.

Bokulich, N. A., M. R. Dillon, Y. Zhang, J. R. Rideout, E. Bolyen, H. Li, P. S. Albert, and J. G. Caporaso. 2018. q2-longitudinal: Longitudinal and paired-sample analyses of microbiome data. mSystems **3**:e00219-00218.

Buchenau, N., M. van Kleunen, and R. A. Wilschut. 2022. Direct and legacy-mediated drought effects on plant performance are species-specific and depend on soil community composition. Oikos **2022**:e08959.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature **488**:91-95.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. v. Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology **64**:807-838.

Byrnes, J. E. K., L. Gamfeldt, F. Isbell, J. S. Lefcheck, J. N. Griffin, A. Hector, B. J. Cardinale, D. U. Hooper, L. E. Dee, and J. Emmett Duffy. 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods in Ecology and Evolution **5**:111-124.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods **13**:581-583.

Cameron, E. S., P. J. Schmidt, B. J. M. Tremblay, M. B. Emelko, and K. M. Müller. 2021. Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. Scientific Reports **11**:22302.

Colwell, J. D. 1965. An automatic procedure for the determination of Phosphorus in sodium hydrogen carbonate extracts of soils. Chemistry Industry **22**:893-895.

Comas, L., S. Becker, V. M. V. Cruz, P. F. Byrne, and D. A. Dierig. 2013. Root traits contributing to plant productivity under drought. Frontiers in Plant Science **4**.

De Long, J. R., E. L. Fry, G. F. Veen, and P. Kardol. 2019. Why are plant–soil feedbacks so unpredictable, and what to do about it? Functional Ecology **33**:118-128.

Dunning, L. T., A.-L. Liabot, J. K. Olofsson, E. K. Smith, M. S. Vorontsova, G. Besnard, K. J. Simpson, M. R. Lundgren, E. Addicott, R. V. Gallagher, Y. Chu, R. T. Pennington, P.-A. Christin, and C. E. R. Lehmann. 2017. The recent and rapid spread of *Themeda triandra*. Botany Letters **164**:327-337.

Earl, H. J. 2003. A precise gravimetric method for simulating drought experiments. Crop Science **43**:1868-1873.

Fadiji, A. E., A. N. Yadav, G. Santoyo, and O. O. Babalola. 2023. Understanding the plant-microbe interactions in environments exposed to abiotic stresses: An overview. Microbiological Research **271**:127368.

Gebhardt, M., J. S. Fehmi, C. Rasmussen, and R. E. Gallery. 2017. Soil amendments alter plant biomass and soil microbial activity in a semi-desert grassland. Plant and Soil **419**:53-70.

Han, X., Y. Li, Y. Li, X. Du, B. Li, Q. Li, and T. M. Bezemer. 2022. Soil inoculum identity and rate jointly steer microbiomes and plant communities in the field. ISME Communications **2**:59.

Hannula, S. E., R. Heinen, M. Huberty, K. Steinauer, J. R. De Long, R. Jongen, and T. M. Bezemer. 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. Nature Communications **12**:5686.

He, X., D. Wang, Y. Jiang, M. Li, M. Delgado-Baquerizo, C. McLaughlin, C. Marcon, L. Guo, M. Baer, Y. A. T. Moya, N. von Wirén, M. Deichmann, G. Schaaf, H.-P. Piepho, Z. Yang, J. Yang, B. Yim, K. Smalla, S. Goormachtig, F. T. de Vries, H. Hüging, M. Baer, R. J. H. Sawers, J. C. Reif, F. Hochholdinger, X. Chen, and P. Yu. 2024. Heritable microbiome variation is correlated with source environment in locally adapted maize varieties. Nature Plants.

Hodgson, R. J., C. Cando-Dumancela, T. Davies, E. A. Dinsdale, M. P. Doane, R. A. Edwards, C. Liddicoat, S. Ramesh, J. A. Robinson, and M. Breed. 2024a. Contrasting microbial taxonomic and functional colonisation patterns in wild populations of the pan-palaeotropical C4 grass, *Themeda triandra*. Authorea.

Hodgson, R. J., C. Cando-Dumancela, C. Liddicoat, S. Ramesh, R. A. Edwards, and M. Breed. 2024b. Strong host and environment modulation of rhizosphere-to-endosphere colonisation in the pan-palaeotropical keystone grass species, *Themeda triandra*. Authorea.

Jost, L. 2006. Entropy and diversity. Oikos **113**:363-375.

Kaisermann, A., F. T. de Vries, R. I. Griffiths, and R. D. Bardgett. 2017. Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. New Phytologist **215**:1413-1424.

Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, C. R. Fellbaum, G. A. Kowalchuk, M. M. Hart, A. Bago, T. M. Palmer, S. A. West, P. Vandenkoornhuyse, J. Jansa, and H. Bücking. 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science **333**:880-882.

Kiers, E. T., S. A. West, and R. F. Denison. 2002. Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. Journal of Applied Ecology **39**:745-754.

Kim, H., J. Jeon, K. K. Lee, and Y.-H. Lee. 2022. Longitudinal transmission of bacterial and fungal communities from seed to seed in rice. Communications Biology **5**:772.

Koziol, L., and J. D. Bever. 2017. The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology **54**:1301-1309.

Lin, H., and S. D. Peddada. 2020. Analysis of compositions of microbiomes with bias correction. Nature Communications **11**:3514.

Ling, N., T. Wang, and Y. Kuzyakov. 2022. Rhizosphere bacteriome structure and functions. Nature Communications **13**:836.

Liu, N., H. Hu, W. Ma, Y. Deng, D. Dimitrov, Q. Wang, N. Shrestha, X. Su, K. Feng, Y. Liu, B. Hao, X. Zhang, X. Feng, and Z. Wang. 2022. Relationships Between Soil Microbial Diversities Across an Aridity Gradient in Temperate Grasslands. Microbial Ecology.

Lundberg, D. S., S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. d. Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe, and J. L. Dangl. 2012. Defining the core Arabidopsis thaliana root microbiome. Nature **488**:86-90.

Mallon, C. A., F. Poly, X. Le Roux, I. Marring, J. D. van Elsas, and J. F. Salles. 2015. Resource pulses can alleviate the biodiversity–invasion relationship in soil microbial communities. Ecology **96**:915-926.

Mariotte, P., Z. Mehrabi, T. m. Bezemer, G. B. Deyn, A. Kulmatiski, B. Drigo, C. Veen, M. Van der Heijden, and P. Kardol. 2018. Plant–Soil Feedback: Bridging Natural and Agricultural Sciences. Trends in Ecology & Evolution **33**:129-142.

Mawarda, P. C., C. A. Mallon, X. Le Roux, J. D. van Elsas, and J. F. Salles. 2022. Interactions between Bacterial Inoculants and Native Soil Bacterial Community: the Case of Spore-forming Bacillus spp. Fems Microbiology Ecology **98**.

McPherson, M. R., P. Wang, E. L. Marsh, R. B. Mitchell, and D. P. Schachtman. 2018. Isolation and Analysis of Microbial Communities in Soil, Rhizosphere, and Roots in Perennial Grass Experiments. JoVE:e57932.

Oksanen J., Blanchet F. G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. S., Szoecs E., and W. H. 2019. vegan: Community Ecology Package.

Pascoe, B. 2018. Dark Emu: Aboriginal Australia and the Birth of Agriculture. Magabala Books.

Peddle, S. D., R. J. Hodgson, R. J. Borrett, S. Brachmann, T. C. Davies, T. E. Erickson, C. Liddicoat, M. Muñoz-Rojas, J. M. Robinson, C. D. Watson, S. L. Krauss, and M. F. Breed. 2024. Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions. Biological Reviews **100**: 1-18

Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, W. K. Cornwell, J. M. Craine, D. E. Gurvich, C. Urcelay, E. J. Veneklaas, P. B. Reich, L. Poorter, I. J. Wright, P. Ray, L. Enrico, J. G. Pausas, A. C. de Vos, N. Buchmann, G. Funes, F. Quétier, J. G. Hodgson, K. Thompson, H. D. Morgan, H. ter Steege, L. Sack, B. Blonder, P. Poschlod, M. V. Vaieretti, G. Conti, A. C. Staver, S. Aquino, and J. H. C. Cornelissen. 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany **61**:167-234.

Petipas, R. H., J. B. González, T. M. Palmer, and A. K. Brody. 2017. Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. Acta Oecologica **78**:71-78.

Pineda, A., I. Kaplan, and T. M. Bezemer. 2017. Steering Soil Microbiomes to Suppress Aboveground Insect Pests. Trends in Plant Science **22**:770-778.

Porter, S. S., and J. L. Sachs. 2020. Agriculture and the Disruption of Plant–Microbial Symbiosis. Trends in Ecology & Evolution.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research **41**:D590-D596.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Robinson, J. M., R. Hodgson, S. L. Krauss, C. Liddicoat, A. A. Malik, B. C. Martin, J. J. Mohr, D. Moreno-Mateos, M. Muñoz-Rojas, S. D. Peddle, and M. F. Breed. 2023. Opportunities and challenges for microbiomics in ecosystem restoration. Trends in Ecology & Evolution.

Santoyo, G. 2022. How plants recruit their microbiome? New insights into beneficial interactions. Journal of Advanced Research **40**:45-58.

Schnitzer, S. A., J. N. Klironomos, J. HilleRisLambers, L. L. Kinkel, P. B. Reich, K. Xiao, M. C. Rillig, B. A. Sikes, R. M. Callaway, S. A. Mangan, E. H. van Nes, and M. Scheffer. 2011. Soil microbes drive the classic plant diversity-productivity pattern. Ecology **92**:296-303.

Searle, R., B. Malone, J. Wilford, J. Austin, C. Ware, M. Webb, M. Roman Dobarco, and T. Van Niel. 2022. TERN Digital Soil Mapping Raster Covariate Stacks. CSIRO.

Snyman, H. A., L. J. Ingram, and K. P. Kirkman. 2013. *Themeda triandra*: a keystone grass species. African Journal of Range & Forage Science **30**:99-125.

Trivedi, P., B. D. Batista, K. E. Bazany, and B. K. Singh. 2022. Plant–microbiome interactions under a changing world: responses, consequences and perspectives. New Phytologist **234**:1951-1959.

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

Wagg, C., S. F. Bender, F. Widmer, and M. G. A. van der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences **111**:5266-5270.

Walkley, A., and B. I. Armstrong. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science **37**:29-38.

Wang, G. Z., P. Schultz, A. Tipton, J. L. Zhang, F. S. Zhang, and J. D. Bever. 2019. Soil microbiome mediates positive plant diversity-productivity relationships in late successional grassland species. Ecology Letters **22**:1221-1232.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology **73**:5261-5267.

Wang, X., Y. Chi, and S. Song. 2024. Important soil microbiota's effects on plants and soils: a comprehensive 30-year systematic literature review. Frontiers in Microbiology **15**.

Wolfsdorf, G., A. Abrahão, A. M. D'Angioli, M. de Sá Dechoum, S. T. Meirelles, L. F. L. Pecoral, L. Rowland, L. da Silveira Verona, I. B. Schmidt, A. B. Sampaio, and R. S. Oliveira. 2021. Inoculum origin and soil legacy can shape plant–soil feedback outcomes for tropical grassland restoration. Restoration Ecology **n/a**:e13455.

Chapter six: Thesis discussion

This thesis explored the interactions between the pan-palaeotropical, keystone C4 grass species, *Themeda triandra,* and its soil microbial communities across an aridity gradient. As climate change pressures intensify, the way in which environmental conditions alter plant-microbe interactions under stress is increasingly important for understanding and potentially enhancing grassland resilience. My data chapters investigated the changing host plant recruitment of microbial communities found across bulk soils, rhizospheres, and endospheres under varying aridity conditions and highlighted the importance of local host-microbiota interactions. By understanding these dynamics, we can make more well-informed decisions on ecological interventions that improve grassland plant establishment via the soil microbiome.

6.1 Data chapter synthesis, limitations and future research recommendations

The findings of my thesis provide a broad foundation of evidence for the aridity-modulated interactions between *T. triandra* and its bacterial communities, but there are still important knowledge gaps. Below, I discuss the primary findings of each thesis chapter and evaluate their implications with respect to my thesis aims and outline further research directions that could advance our understanding of *T. triandra*-microbiome interactions. Finally, I consider approaches for addressing potential limitations of my thesis chapters, and raise research questions that stem from my thesis outcomes which could help improve grassland restoration practices.

6.1.1 Geographic variation in bulk soil and rhizosphere bacterial communities

In *Chapter two*, I showed changing bacterial communities within the rhizospheres and associated bulk soils of *T. triandra* across an aridity gradient, using a space-for-time substitution design. I found that aridity altered the abundances of core *T. triandra* rhizosphere bacteria, identifying a suite of host-selected, arid bacterial taxa (aim 1). Additionally, *T. triandra* rhizosphere bacterial communities were distinct from those of bulk soils, but there was little difference between the bacterial communities found in bulk soil beyond the rhizosphere, i.e., at 2 m and 30 cm from host plants (aim 2). Consequently, *T. triandra* plants are unlikely to produce strong bacterial community turnover in soils outside the zone of rhizosphere influence. Ultimately, I concluded from *Chapter two* that aridity strengthens the core microbiome associations in *T. triandra* rhizospheres, and this builds on previous research that shows how plant-microbiome associations are strongly shaped by aridity (Chen et al. 2021, Dadzie et al. 2022, Zhong et al. 2022).

Characterising soil and rhizosphere community complexity

While *Chapter two* only characterises bacterial interactions, it is known that bulk soils and rhizospheres also contain a diverse array of different microbiota, including fungi, eukaryotes, and archaea (Anthony et al. 2023). Previous research has considered how arbuscular mycorrhizal fungi interact with *T. triandra* (Petipas et al. 2017, Gonzalez et al. 2018, Tang et al. 2021). Fungal organisms can be profiled using amplicon sequencing (i.e., via the ITS gene region) or fungi enriched shotgun metagenomic techniques (Cuadros-Orellana et al. 2013); and fungal characterisation using these approaches could detect taxonomic patterns and indicate functional roles. For instance, functional roles for fungi can be obtained via taxonomy-based functional annotation using the FungalTraits database on amplicon data (Põlme et al. 2020), or via annotation of metagenomic sequences (e.g., using the KEGG orthology database) (Kanehisa et al. 2016). This could reveal patterns in *T. triandra* root and soil structures, not provided by bacterial communities.

Future analysis of root fungal communities could identify trends and benefits and/or impediments of long-term soil legacies provided by slow-forming fungal mycelial networks which take longer to recover following disturbance (Koziol and Bever 2017, Sun et al. 2017, Watson et al. 2022). This could identify key fungal interactions which are critical to the stability and success of *T. triandra* grassland communities.

Below, I propose a limited set of 'next step' research questions to advance our understanding of *T. triandra*-microbe interactions:

- Do bacterial and fungal interactions (i.e., inter-kingdom interactions) influence *T. triandra*-soil feedbacks?
- What are the long-term effects of plant-microbe interactions on the successional dynamics of grassland ecosystems dominated by *T. triandra*?

6.1.2 Rhizosphere-to-endosphere bacterial colonisation trends

In *Chapter three*, I explored how soil bacteria colonise *T. triandra* root endospheres from their rhizospheres, according to the two-step selection process posed by Bulgarelli et al. (2012). Building on the spatial patterns explored in *Chapter two*, I examined how *T. triandra* plants differentially recruit endosphere microbiota across an aridity gradient (Aim 2), using a space-for-time substitution. Bacterial community change patterns were consistent with the expected trends described by the two-step selection process, with decreasing bacterial taxonomic diversity from rhizospheres into the endospheres (Bulgarelli et al. 2012, Lundberg et al. 2012, Urbina et al. 2018). Despite local variation across endosphere microbial communities, bacterial community compositions converged from bulk soils into the endospheres for all sites. Across the aridity gradient, I found that most bacterial community variation was reduced from rhizosphere samples into the endosphere samples. Furthermore, I provide evidence that the *T. triandra* endospheres were assembled by deterministic processes with greater homogeneous selection (i.e., selection for more phylogenetically similar species) compared to rhizospheres; a process that was likely imposed by the host plant (Stegen et al. 2012, Stegen et al. 2013). As such, despite good evidence of broad selective pressure across all *T. triandra* endospheres, bacterial recruitment strategies differed across sites, showing the impact of local conditions on assembly processes (Lumibao et al. 2020, Moroenyane et al. 2020, He et al. 2024).

Representative sampling of bacterial colonisation sources

My data chapters, *two* and *three,* together assessed the colonisation of bacterial communities from soil into rhizospheres, and from rhizospheres into endospheres, respectively. This involved collecting soil and plant samples (including across each plant compartment: soils, rhizospheres or endospheres) across an aridity gradient in a singular sampling period, representing a cross-sectional (or one off) observation. However, a more comprehensive explanation of bacterial colonisation processes could be provided by expanding sampling and amplicon sequencing to consider repeated soil sampling across different seasons (i.e., those not captured during my sampling by chance), or potential non-soil sources (i.e., flowers, seeds, pollinators). As such, repeated sampling of soils and plant compartments would better reveal the full breadth of bacterial colonisation in *T. triandra* plants, and broad environmental influences on microbial community compositions. This would build a more complete representation of *T. triandra* microbiomes than I can provide with my current research.

Plant-soil interactions change seasonally and across a plant host's lifecycle in response to changing biotic and abiotic conditions (Casper and Castelli 2007, Hawkes et al. 2013). As such, exploring shifts in host-microbiome recruitment processes across key lifecycle stages, including early growth, mature growth, and during flowering and seed production would expand on the findings of this thesis, and identify complexity in symbiotic relationships.

The following research questions represent key additional steps that would build on these thesis outcomes, and address remaining knowledge gaps raised above:

- Are microbiota inherited from parent plants, during seed development, and/or are they transferred across different aboveground compartments (e.g., to and from phyllosphere microbiota)?
- Do *T. triandra* plant-soil interactions change across different developmental stages of its lifecycle (e.g., as seedlings, mature plants, and during reproduction)?

- How does seasonal variations affect the colonisation of different soil microbiota into *T. triandra* rhizospheres and endospheres?
- Do spatial trends in microbial community composition across soils, rhizospheres and/or endospheres reflect those observed in temporal colonisation studies?

6.1.3 Functional microbiomes of *T. triandra* under changing aridity conditions

In *Chapter four* I used shotgun metagenomics to analyse the functional roles of microbiota spatially in bulk soils, rhizospheres, and endospheres across the same aridity gradient (aims 2 and 3). I also examined plant recruitment of microbiota based on specific functional properties, contributing a new perspective on the two-step selection process. Unlike taxonomic diversity, which decreased from bulk soils into rhizospheres, and then endospheres – a common pattern found in the literature (Bulgarelli et al. 2012, Lundberg et al. 2012, Urbina et al. 2018), and found in chapters *two* and *three* – I discovered that functional diversity increased from bulk soils, rhizospheres, and into endospheres. Specifically, this was achieved through increasing levels of functional evenness, which overcame the pattern of taxonomic filtering. This meant that the functional diversity measure based on Shannon's index – reflecting both richness (which declined with taxonomic filtering) and evenness – ultimately increased within endospheres compared to other plant compartments. This outcome highlights the many specialised roles performed by microbes within host plant roots, and provides a novel contribution to our understanding of plant-microbiota recruitment dynamics.

Rhizosphere functional diversity was positively correlated with higher aridity, and community compositions became more heterogeneous as aridity conditions decreased. Specific functional changes included the increasing diversity and abundance of microbial functions related to plant growth and metabolism in *T. triandra* endospheres compared to rhizospheres. While under high aridity conditions, I found increased importance of osmotic and oxidative stress functions in the rhizospheres and endosphere – important for plants under water-stress (Cruz de Carvalho 2008, Bremer and Krämer 2019). These findings underscore the influence of aridity on microbiota and supports their role in promoting host plant growth under changing climatic conditions. They also show that the roles of microbiota change with climatic variation, as does the likely importance of specific components of soil, rhizosphere and endosphere microbiomes to *T. triandra*.

Better understanding functional links between plant-soil compartments

While this chapter identifies trends in soils, rhizospheres, and endospheres that describe changing bacterial compositions and functional roles, different methods could have provided direct functional activity occurring between plants and microbes within each of these compartments. Characterising exudates that are released by *T. triandra* plants in rhizospheres could indicate the degree of resource investment occurring, and highlight functional properties of specific microbiota promoted by the plant. Additionally, considering the meta-transcriptomes of soils and host plant roots would further highlight the direct shifts in microbial activity and/or plant investment as needs and external stimuli change (Carvalhais et al. 2012, Martinez et al. 2016, Yates et al. 2021) (see *Chapter one, 1.3.2 Tools and techniques for microbial community profiling*). Together, these approaches would provide stronger causal links to the nutrient cycling roles and host-metabolic processes in *T. triandra* belowground compartments.

Below, I provide additional research questions that continue to explore the functional interactions between *T. triandra* and its bulk soils, rhizospheres and endospheres in greater detail. Expanding the analysis of my data chapters will help define causal links between host and microbial functional processes:

- How does aridity alter the specific signalling pathways, and exudate release in *T. triandra* roots and soil microbes?
- Are there specific genetic traits in *T. triandra* that influence its ability to form symbioses with soil microbes?

6.1.4 Soil microbial communities alter *T. triandra* biomass under drought-stress

In *Chapters two, three*, and *four*, I present strong evidence that aridity influences plant-microbe interactions, based on observational studies using space-for-time substitution. In *Chapter five*, I used a greenhouse experiment to verify the influence of a high and low aridity soil bacterial community on the growth and drought tolerance of *T. triandra* (aim 4). By manipulating soil conditions (aridity-associated soil, sterilisation and drought stress), I was able to investigate the direct effects of microbiota on plant growth and how these soil manipulations influenced bacterial bulk soil-to-endosphere colonisation dynamics (aim 2).

I found that arid *T. triandra* associated soil legacies lead to greater *T. triandra* biomass under both drought-stress and control conditions. I also found that bacterial alpha diversity was positively correlated with *T. triandra* biomass, with improved growth of plants under sterilised conditions for all treatment groups (autoclaving is discussed further below). These results expose the important causal influence of microbiota on *T. triandra* fitness, especially in arid and drought conditions – indeed, they are in direct support of the observational findings in earlier chapters plus previous research findings that arbuscular mycorrhizal fungi improve *T. triandra* growth under drought stress (Petipas et al. 2017). In *Chapters two* and *four*, I showed that local arid microbiota are likely important to host fitness, and recruitment patterns are shaped by host plant needs. In *Chapter three*, I also found that local soil conditions were important factors for bacterial recruitment in both rhizospheres, and endospheres. In *Chapter five* I then showed that soil manipulations influenced the bacteria that were found in *T. triandra* root compartments. As such, there is strong evidence that *T. triandra* is dependent on available local soil microbiota to shape its bacterial recruitment. Based on these findings, it will be important to differentiate the general fitness improving microbes (i.e., 'core microbiota', important across all populations), from the microbiota that provide specific host benefits under environmental stress conditions (i.e., conditionally important microbes).

Improving experimental designs: from glasshouse to field

Different implementation of greenhouse trial designs can influence project outcomes, however, there are often experimental trade-offs that need to be considered. Autoclaving, as used in *Chapter five*, has been found to release micronutrients into soils that can confound the effects of sterilisation in greenhouse experiments (Berns et al. 2008). Additional analyses could consider sterilising soils using other techniques (e.g., gamma irradiation) to minimise confounding and ensure reproducibility. Furthermore, my approach in *Chapter five* had a greater risk of soil physicochemical confounders due to the use of different high and low aridity soils. To address this, many greenhouse experiments will inoculate microbiota into standardised, and often artificially constructed common soils to explore microbial impacts on plant growth under standardised soil physicochemical conditions. However, these methods assume that inoculation will not alter microbial community composition and function from its original state – for example, based on effects the new physicochemical soil environment (like pH, and soil aggregate stability) and/or inoculation technique (like intact vs. disturbed whole-soil transfer) (see *Chapter one, 1.3.3 Applications of whole-soil translocations of microbiota for ecosystem interventions*). As such, further studies should incorporate different soil manipulations and treatments that account for these experimental limitations to form a complete picture of microbial community dynamics.

While controlled greenhouse experiments are important to isolate specific predictor-response relationships in highly controlled environments, they do not capture the complexity of natural field conditions (Forero et al. 2019). As such, my greenhouse experiment has a limited capacity to predict real world plant-microbe dynamics, which need to be considered in a context of greater abiotic and biotic complexity. Field experiments that include the impacts of other plant species, and their associated microbiota on *T. triandra* growth would strengthen the findings of this thesis (Bever et al. 2010). This could be done using reciprocal transplant experiments that expand on outcomes from this thesis and investigate how they perform under natural conditions. These methodologies could reveal the impacts of competitive native or exotic plants on *T. triandra* which may constitute microbial barriers to ecosystem restoration (Robinson et al. 2023, Peddle et al. 2024).

The following research questions can address the methodological constraints in my thesis chapters. These reflect experimental limitations that can deepen our understanding of the outcomes from this thesis:

- Do we find that soil microbiota under drought stress provide the same host-benefits, and microbial colonisation patterns in different soil types, or under different soil manipulations (autoclaving, and/or imposed drought stress duration)?
- Can we restore the local ecosystems condition to historic states to build resilient ecosystems with native grass community assemblages?
- How dependent are local soil microbiota on soil physicochemical characteristics (i.e., pH, soil structure, nutrient availability)?
- Are there effective methods to transfer microbial biodiversity and functional properties across local and regional scales?
- What are the mechanisms by which natural stress events shape root colonisation dynamics, and alter root-microbiome functioning?
- How do root endosphere microbiota differ from microbiota naturally occurring on seeds or within seed tissues prior to germination?

6.2 Thesis conclusions

In this thesis, I identify changing patterns of bacterial communities across an aridity gradient within *T. triandra* bulk soils, rhizospheres, and endospheres. I explore the regional taxonomic recruitment trends and examine which host and environmental community assembly processes have shaped them. I also show specific functional properties of *T. triandra* microbiomes that improve host fitness under high and/or low aridity-associated conditions. Finally, I directly show that *T. triandra* microbiota impact host plant growth and drought stress responses via a glasshouse experiment, identifying advantages of arid-associated soil microbial and physicochemical legacies. These findings not only highlight important species-specific processes, but contribute to our understanding of broader soil-to-endosphere colonisation processes, furthering our knowledge of the two-step selection process.

References

Anthony, M. A., S. F. Bender, and M. G. A. van der Heijden. 2023. Enumerating soil biodiversity. Proceedings of the National Academy of Sciences **120**:e2304663120.

Berns, A. E., H. Philipp, H.-D. Narres, P. Burauel, H. Vereecken, and W. Tappe. 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. European Journal of Soil Science **59**:540-550.

Bever, J., I. Dickie, E. Facelli, J. Facelli, J. Klironomos, M. Moora, M. Rillig, W. Stock, M. Tibbett, and M. Zobel. 2010. Rooting Theories of Plant Community Ecology in Microbial Interactions. Trends in Ecology & Evolution **25**:468-478.

Bremer, E., and R. Krämer. 2019. Responses of Microorganisms to Osmotic Stress. Annual Review of Microbiology **73**:313-334.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature **488**:91-95.

Carvalhais, L. C., P. G. Dennis, G. W. Tyson, and P. M. Schenk. 2012. Application of metatranscriptomics to soil environments. Journal of Microbiological Methods **91**:246-251.

Casper, B. B., and J. P. Castelli. 2007. Evaluating plant–soil feedback together with competition in a serpentine grassland. Ecology Letters **10**:394-400.

Chen, Y., J. W. Neilson, P. Kushwaha, R. M. Maier, and A. Barberán. 2021. Life-history strategies of soil microbial communities in an arid ecosystem. The ISME Journal **15**:649-657.

Cruz de Carvalho, M. H. 2008. Drought stress and reactive oxygen species: Production, scavenging and signaling. Plant Signal Behav **3**:156-165.

Cuadros-Orellana, S., L. R. Leite, A. Smith, J. D. Medeiros, F. Badotti, P. L. Fonseca, A. B. Vaz, G. Oliveira, and A. Góes-Neto. 2013. Assessment of fungal diversity in the environment using metagenomics: a decade in review. Fungal Genomics & Biology **3**:1.

Dadzie, F. A., A. T. Moles, T. E. Erickson, E. Slavich, and M. Muñoz-Rojas. 2022. Native bacteria and cyanobacteria can influence seedling emergence and growth of native plants used in dryland restoration. Journal of Applied Ecology **59**:2983-2992.

Forero, L. E., J. Grenzer, J. Heinze, C. Schittko, and A. Kulmatiski. 2019. Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated. Frontiers in Environmental Science **7**.

Gonzalez, J. B., R. H. Petipas, O. Franken, E. T. Kiers, K. E. Veblen, and A. K. Brody. 2018. Herbivore removal reduces influence of arbuscular mycorrhizal fungi on plant growth and tolerance in an East African savanna. Oecologia **187**:123-133.

Hawkes, C. V., S. N. Kivlin, J. Du, and V. T. Eviner. 2013. The temporal development and additivity of plant-soil feedback in perennial grasses. Plant and Soil **369**:141-150.

He, X., D. Wang, Y. Jiang, M. Li, M. Delgado-Baquerizo, C. McLaughlin, C. Marcon, L. Guo, M. Baer, Y. A. T. Moya, N. von Wirén, M. Deichmann, G. Schaaf, H.-P. Piepho, Z. Yang, J. Yang, B. Yim, K. Smalla, S. Goormachtig, F. T. de Vries, H. Hüging, M. Baer, R. J. H. Sawers, J. C. Reif, F. Hochholdinger, X. Chen, and P. Yu. 2024. Heritable microbiome variation is correlated with source environment in locally adapted maize varieties. Nature Plants.

Kanehisa, M., Y. Sato, M. Kawashima, M. Furumichi, and M. Tanabe. 2016. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Research **44**:D457-D462.

Koziol, L., and J. D. Bever. 2017. The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology **54**:1301-1309.

Lumibao, C. Y., E. R. Kimbrough, R. H. Day, W. H. Conner, K. W. Krauss, and S. A. Van Bael. 2020. Divergent biotic and abiotic filtering of root endosphere and rhizosphere soil fungal communities along ecological gradients. Fems Microbiology Ecology **96**.

Lundberg, D. S., S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. d. Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe, and J. L. Dangl. 2012. Defining the core Arabidopsis thaliana root microbiome. Nature **488**:86-90.

Martinez, X., M. Pozuelo, V. Pascal, D. Campos, I. Gut, M. Gut, F. Azpiroz, F. Guarner, and C. Manichanh. 2016. MetaTrans: an open-source pipeline for metatranscriptomics. Scientific Reports **6**:26447.

Moroenyane, I., J. Tremblay, and É. Yergeau. 2020. Temporal and spatial interactions modulate the soybean microbiome. Fems Microbiology Ecology **97**.

Peddle, S. D., R. J. Hodgson, R. J. Borrett, S. Brachmann, T. C. Davies, T. E. Erickson, C. Liddicoat, M. Muñoz-Rojas, J. M. Robinson, C. D. Watson, S. L. Krauss, and M. F. Breed. 2024. Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions. Biological Reviews **100**, 1-18.

Petipas, R. H., J. B. González, T. M. Palmer, and A. K. Brody. 2017. Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. Acta Oecologica **78**:71-78.

Põlme, S., K. Abarenkov, R. Henrik Nilsson, B. D. Lindahl, K. E. Clemmensen, H. Kauserud, N. Nguyen, R. Kjøller, S. T. Bates, P. Baldrian, T. G. Frøslev, K. Adojaan, A. Vizzini, A. Suija, D. Pfister, H.-O. Baral, H. Järv, H. Madrid, J. Nordén, J.-K. Liu, J. Pawlowska, K. Põldmaa, K. Pärtel, K. Runnel, K. Hansen, K.-H. Larsson, K. D. Hyde, M. Sandoval-Denis, M. E. Smith, M. Toome-Heller, N. N. Wijayawardene, N. Menolli, N. K. Reynolds, R. Drenkhan, S. S. N. Maharachchikumbura, T. B. Gibertoni, T. Læssøe, W. Davis, Y. Tokarev, A. Corrales, A. M. Soares, A. Agan, A. R. Machado, A. Argüelles-Moyao, A. Detheridge, A. de Meiras-Ottoni, A. Verbeken, A. K. Dutta, B.-K. Cui, C. K. Pradeep, C. Marín, D. Stanton, D. Gohar, D. N. Wanasinghe, E. Otsing, F. Aslani, G. W. Griffith, T. H. Lumbsch, H.-P. Grossart, H. Masigol, I. Timling, I. Hiiesalu, J. Oja, J. Y. Kupagme, J. Geml, J. Alvarez-Manjarrez, K. Ilves, K. Loit, K. Adamson, K. Nara, K. Küngas, K. Rojas-Jimenez, K. Bitenieks, L. Irinyi, L. G. Nagy, L. Soonvald, L.-W. Zhou, L. Wagner, M. C. Aime, M. Öpik, M. I. Mujica, M. Metsoja, M. Ryberg,

M. Vasar, M. Murata, M. P. Nelsen, M. Cleary, M. C. Samarakoon, M. Doilom, M. Bahram, N. Hagh-Doust, O. Dulya, P. Johnston, P. Kohout, Q. Chen, Q. Tian, R. Nandi, R. Amiri, R. H. Perera, R. dos Santos Chikowski, R. L. Mendes-Alvarenga, R. Garibay-Orijel, R. Gielen, R. Phookamsak, R. S. Jayawardena, S. Rahimlou, S. C. Karunarathna, S. Tibpromma, S. P. Brown, S.-K. Sepp, S. Mundra, Z.-H. Luo, T. Bose, T. Vahter, T. Netherway, T. Yang, T. May, T. Varga, W. Li, V. R. M. Coimbra, V. R. T. de Oliveira, V. X. de Lima, V. S. Mikryukov, Y. Lu, Y. Matsuda, Y. Miyamoto, U. Kõljalg, and L. Tedersoo. 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Diversity **105**:1-16.

Robinson, J. M., R. Hodgson, S. L. Krauss, C. Liddicoat, A. A. Malik, B. C. Martin, J. J. Mohr, D. Moreno-Mateos, M. Muñoz-Rojas, S. D. Peddle, and M. F. Breed. 2023. Opportunities and challenges for microbiomics in ecosystem restoration. Trends in Ecology & Evolution.

Stegen, J. C., X. Lin, J. K. Fredrickson, X. Chen, D. W. Kennedy, C. J. Murray, M. L. Rockhold, and A. Konopka. 2013. Quantifying community assembly processes and identifying features that impose them. The ISME Journal **7**:2069-2079.

Stegen, J. C., X. Lin, A. E. Konopka, and J. K. Fredrickson. 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. The ISME Journal **6**:1653-1664.

Sun, S., S. Li, B. N. Avera, B. D. Strahm, and B. D. Badgley. 2017. Soil Bacterial and Fungal Communities Show Distinct Recovery Patterns during Forest Ecosystem Restoration. Applied and environmental microbiology **83**:e00966-00917.

Tang, M., J. Liu, W. Hou, R. M. Stubbendieck, H. Xiong, J. Jin, J. Gong, C. Cheng, X. Tang, Y. Liu, Z. Li, J. Wang, and Y. Yi. 2021. Structural variability in the bulk soil, rhizosphere, and root endophyte fungal communities of Themeda japonica plants under different grades of karst rocky desertification. Plant and Soil.

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

Watson, C. D., M. G. Gardner, R. J. Hodgson, C. Liddicoat, S. D. Peddle, and M. F. Breed. 2022. Global meta-analysis shows progress towards recovery of soil microbiota following revegetation. Biological Conservation **272**:109592.

Yates, M. C., A. M. Derry, and M. E. Cristescu. 2021. Environmental RNA: A Revolution in Ecological Resolution? Trends in Ecology & Evolution **36**:601-609.

Zhong, Y., P. O. Sorensen, G. Zhu, X. Jia, J. Liu, Z. Shangguan, R. Wang, and W. Yan. 2022. Differential microbial assembly processes and co-occurrence networks in the soil-root continuum along an environmental gradient. iMeta **n/a**:e18.

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Supplementary tables

Table S2.1. Our *Themeda triandra* sampling sites in southern Australia. Aridity data sourced from the Atlas of Living Australia (Belbin 2011, ALA 2014).

Site name	Latitude, longitude	Mean annual aridity index	Sampling date
Alligator Gorge	-32.71487, 138.10172	0.4450	15 Dec 2021
Mount Maria	-32.65862, 138.08985	0.3179	16 Dec 2021
Barlunga Gap	-33.82, 138.17392	0.3469	14 Dec 2021
Maitland	-34.37366, 137.71203	0.4532	21 Dec 2021
Neagles Rock Reserve	-33.85031, 138.60674	0.6507	14 Dec 2021
Scott Creek	-35.0872, 138.67266	0.9031	19 Dec 2021
Sturt Gorge	-35.03311, 138.57324	0.6345	13 Dec 2021
Frahn's Farm	-35.07231, 139.09781	0.4539	19 Dec 2021

Table S2.2. Explanatory variables included in canonical correspondence analysis (CCA) across bulk soil and plant rhizospheres.

Model variables	**Description**
Latitude	Latitude coordinates for each sampling site
Longitude	Longitude coordinates for each sampling site
Functional vegetation,	Relative abundance at each site for graminoids, herbs, shrubs, trees/canopy cover, litter, and bare soil.
Aridity	Mean annual aridity index for each sample site, Atlas of Living Australia (Belbin 2011, ALA 2014)
Aboveground biomass	Aboveground biomass from the host plant that each sample is centred around
T. triandra site-density	Values pertaining to the density of *T. triandra* individuals for each sampling site
Trace elements and macronutrients (for rhizosphere analyses only)	Boron, calcium, copper, iron, magnesium, manganese, Phosphorus, potassium, sodium, sulphur, total nitrogen, and zinc contained in the leaf tissue in sampled host plants
Physicochemical measurements (for bulk soil analyses only)	Ammonium nitrogen, nitrate, phosphorus, potassium, sulphur, organic carbon, electrical conductivity and pH ($CaCl_2$) contained in the soil sampled at either 2 m or 30 cm from host plants

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Table S2.3. Correlations for each bacterial phyla with mean annual aridity gradient. *Belowground zone* reflects samples obtained from bulk soil at 2 m, or 30 cm from *T. triandra* plants, or their rhizospheres. *Test* indicates if Pearson's correlation or Spearman's correlation was used. Estimate, between -1 to 1, shows *r* for Pearson's correlation, and *Rho* for Spearman's rank order correlation). Model *significance* is indicated by '*' at the level of 0.05. Only the top 11 bacterial phyla are shown. A decrease in site aridity is associated with an increase in aridity index (annual precipitation/annual potential evaporation), therefore an increase in relative abundance), accords with less dryness.

Belowground zone	**Phylum**	**Test**	**Estimate**	**Df**	**Test statistic**	**P-value**	**Significance**
Soil 2 m~	Acidobacteriota	Spearman	-0.4141	NA	26054.20	0.0034	*
	Actinobacteriota	Spearman	-0.0302	NA	18980.24	0.8386	NS
	Armatimonadota	Spearman	0.0604	NA	17311.51	0.6835	NS
	Bacteroidota	Spearman	0.2048	NA	14651.12	0.1627	NS
	Chloroflexi	Pearson	-0.2539	46	-1.78	0.0816	NS
	Firmicutes	Spearman	0.1398	NA	15848.35	0.3433	NS
	Gemmatimonadota	Pearson	-0.4321	46	-3.25	0.0022	*
	Myxococcota	Spearman	0.1149	NA	16307.86	0.4369	NS
	Patescibacteria	Spearman	0.4892	NA	9410.39	0.0004	*
	Proteobacteria	Pearson	0.3995	46	2.96	0.0049	*
	Verrucomicrobiota	Spearman	0.4561	NA	10019.89	0.0011	*
Soil 30 cm ~	Acidobacteriota	Spearman	-0.4397	NA	24901.49	0.0020	*
	Actinobacteriota	Pearson	-0.1935	45	-1.32	0.1926	NS
	Armatimonadota	Spearman	0.0935	NA	15678.63	0.5318	NS
	Bacteroidota	Spearman	0.2540	NA	12903.22	0.0849	NS
	Chloroflexi	Pearson	-0.1780	45	-1.21	0.2312	NS
	Firmicutes	Spearman	0.0157	NA	17024.92	0.9167	NS
	Gemmatimonadota	Pearson	-0.5182	45	-4.06	0.0002	*
	Myxococcota	Spearman	0.2694	NA	12636.16	0.0671	NS
	Patescibacteria	Spearman	0.4594	NA	9349.98	0.0012	*
	Proteobacteria	Pearson	0.4541	45	3.42	0.0013	*
	Verrucomicrobiota	Spearman	0.4260	NA	9928.34	0.0028	*
Rhizosphere ~	Acidobacteriota	Pearson	0.3409	43	2.38	0.0219	*
	Actinobacteriota	Pearson	-0.4294	43	-3.12	0.0032	*

Armatimonadota	Spearman	0.3301	NA	10168.40	0.0268	*
Bacteroidota	Spearman	-0.0021	NA	15212.25	0.9889	NS
Chloroflexi	Pearson	-0.2980	43	-2.05	0.0467	*
Firmicutes	Spearman	0.1031	NA	13615.64	0.5005	NS
Gemmatimonadota	Spearman	-0.3689	NA	20780.52	0.0126	*
Myxococcota	Spearman	0.1363	NA	13110.92	0.3720	NS
Patescibacteria	Spearman	0.0679	NA	14148.99	0.6575	NS
Proteobacteria	Spearman	0.3157	NA	10387.76	0.0346	*

Supplementary figures

Figure S2.1. Photographs of sampling populations across (i) Alligator Gorge, (ii) Burunga Gap, (iii) Frahn's Farm, (iv) Neagles Rock Reserve, (v) Maitland, (vi) Mount Maria, Scott Creek, and (viii) Sturt Gorge.

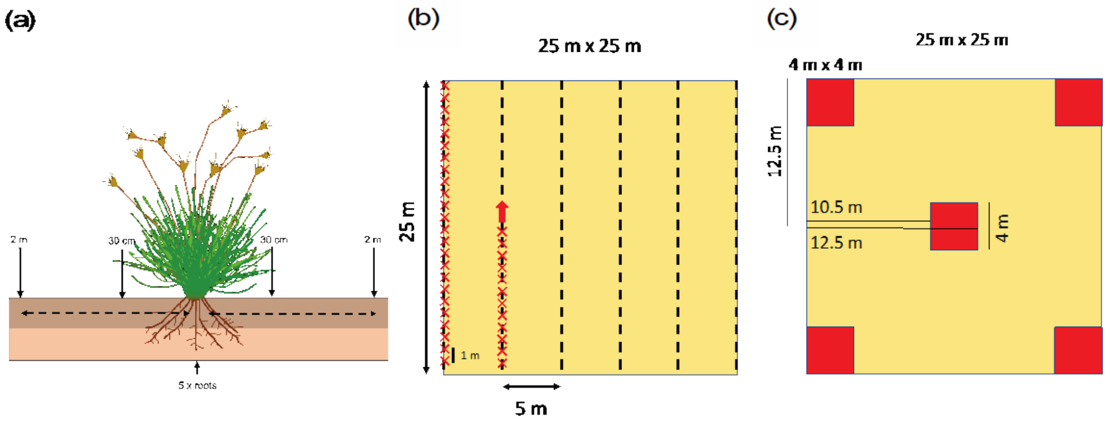

Figure S2.2. (a) Design for each of the six 25 m point-intercept transects spaced 5 m apart in a North-South direction (black dashed lines). At each 1 m interval along the transect, vegetation type was recorded (red crosses). (b) In each sampling site, five 4 x 4 m quadrats were used to measure *T. triandra* density (red squares). (c) Soil and rhizosphere sample collection for each targetted *T. triandra* plant within our sampling sites. Soil physicochemical analysis was performed in each soil sample, and bacterial profiling using amplicon sequencing occurred in the soils and rhizosphere samples.

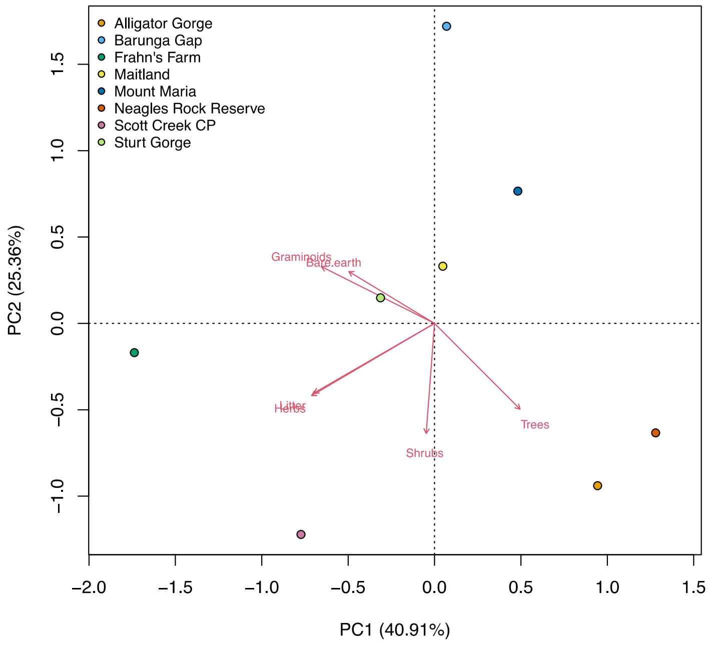

Figure S2.3. Principal components analysis of each site based on centroid point for relative abundance of functional vegetation.

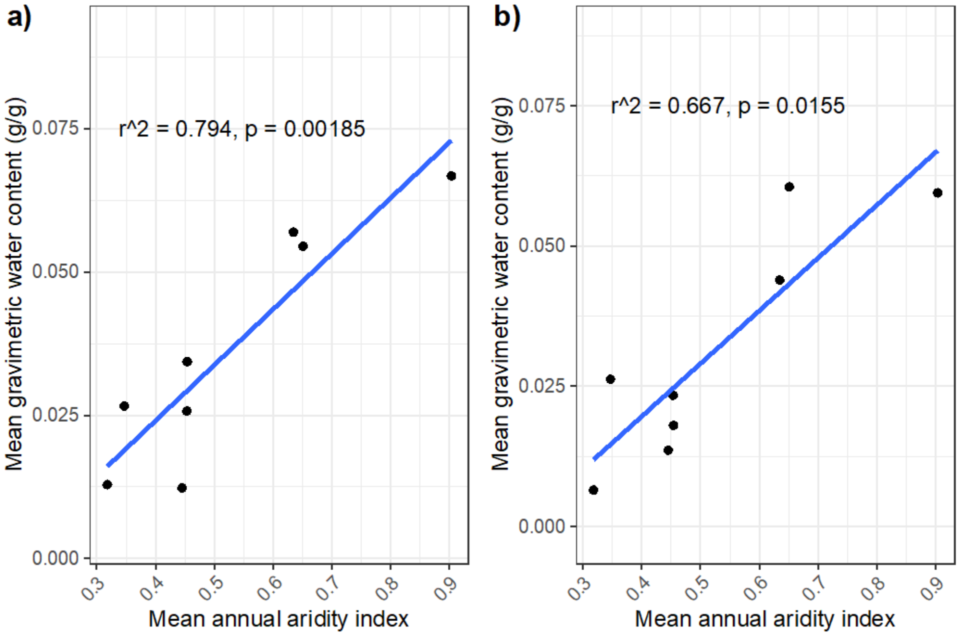

Figure S2.4. Mean gravimetric water content from soil samples according to each sampling site taken at (a) 2 m and (b) 30cm from *T. triandra* host plants, against mean annual aridity index values.

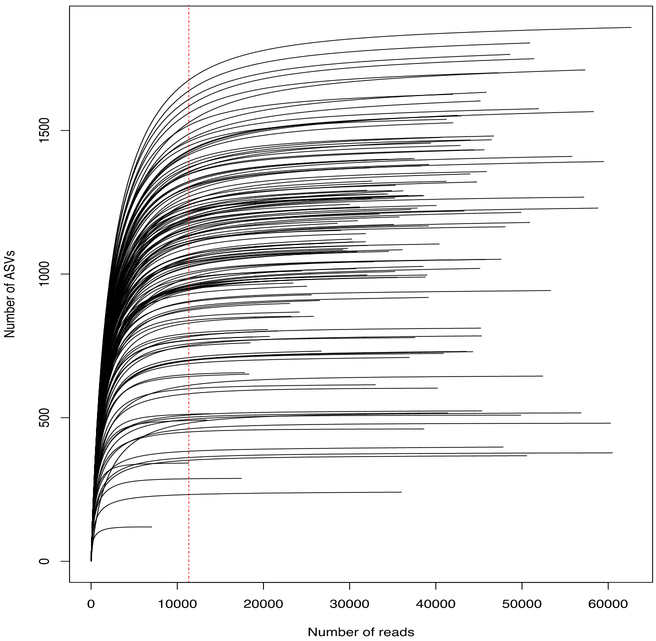

Figure S2.5. Rarefaction plot showing number of ASVs by number of reads per sample. Samples were rarified to 11,336 reads (red dashed line). Samples that did not meet the minimum threshold of reads were removed from analysis.

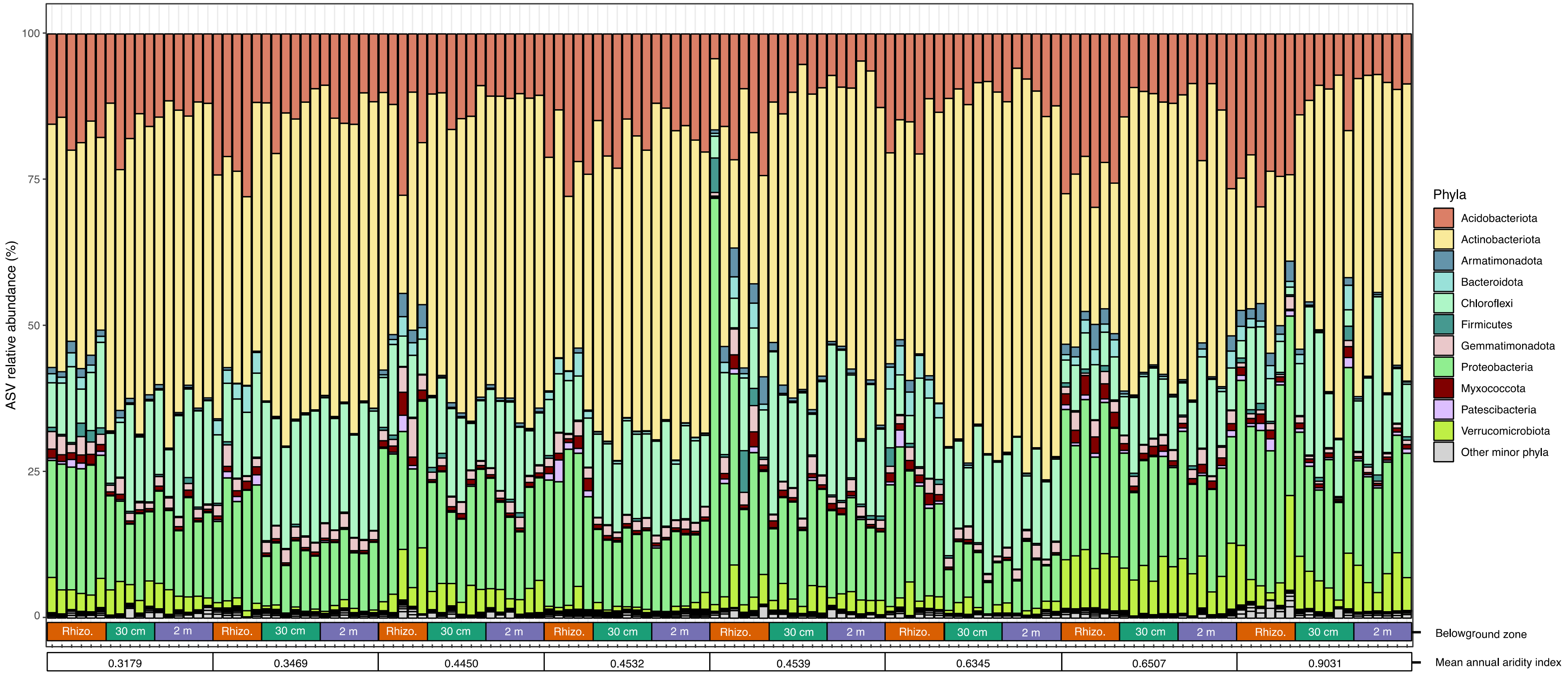

Figure S2.6. ASVs relative estimated abundance of bacterial phyla in samples across: soils at 2 m from host plant, soils at 30 cm from host plant, and rhizospheres, with mean annual aridity index. Samples represented 98.8% of reads for taxa at greater than 2% estimated abundances.

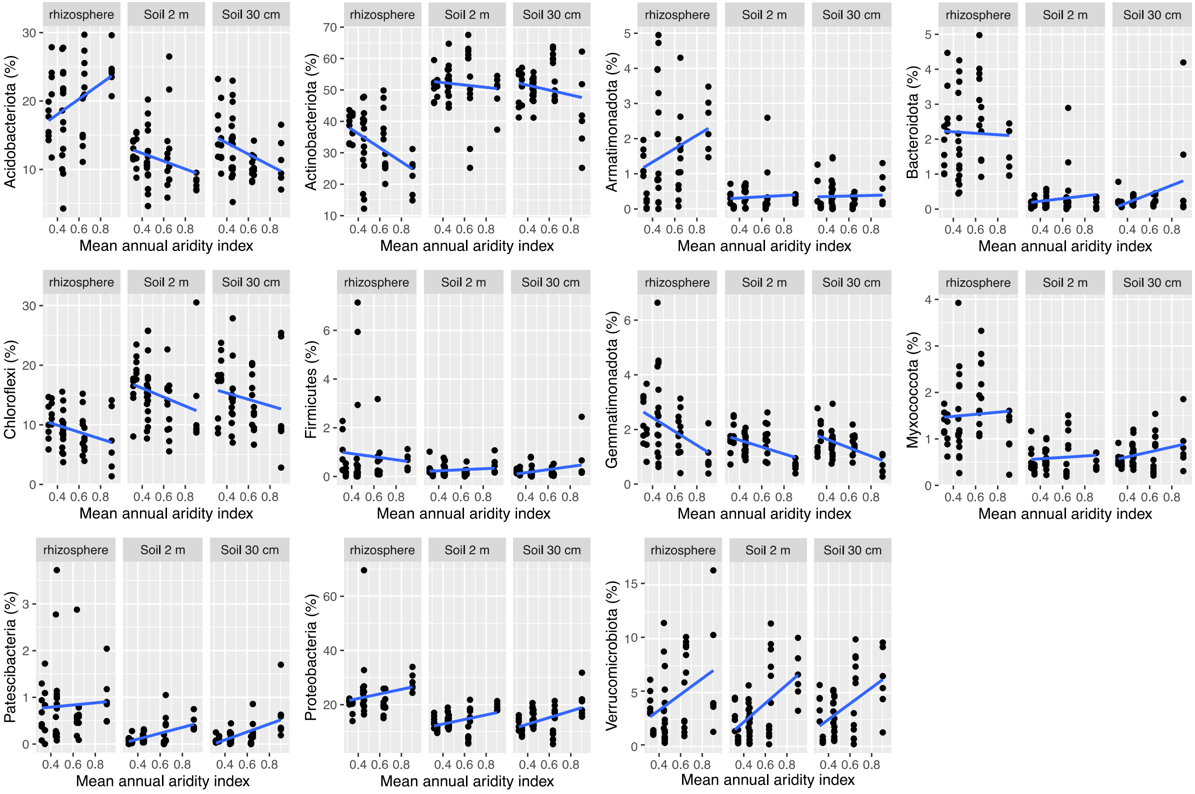

Figure S2.7. Change in relative abundance of major bacterial phyla in: rhizospheres, soils at 30 cm from host plants, and soils at 2 m from host plants. Taxa included represented 98.8% of reads at greater than 2% estimated abundances.

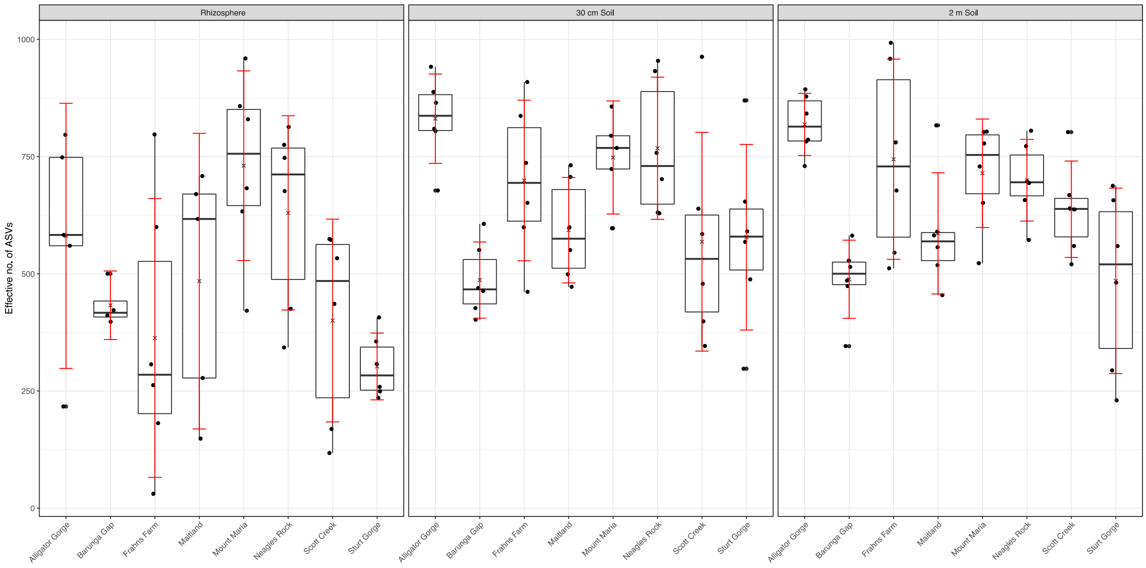

Figure S2.8. Effective number of ASVs across different sampling sites across: rhizospheres, bulk soil at 30 cm from host plant, and bulk soil at 2 m from host plant; crosses denote mean values and red error bars show 95% confidence intervals.

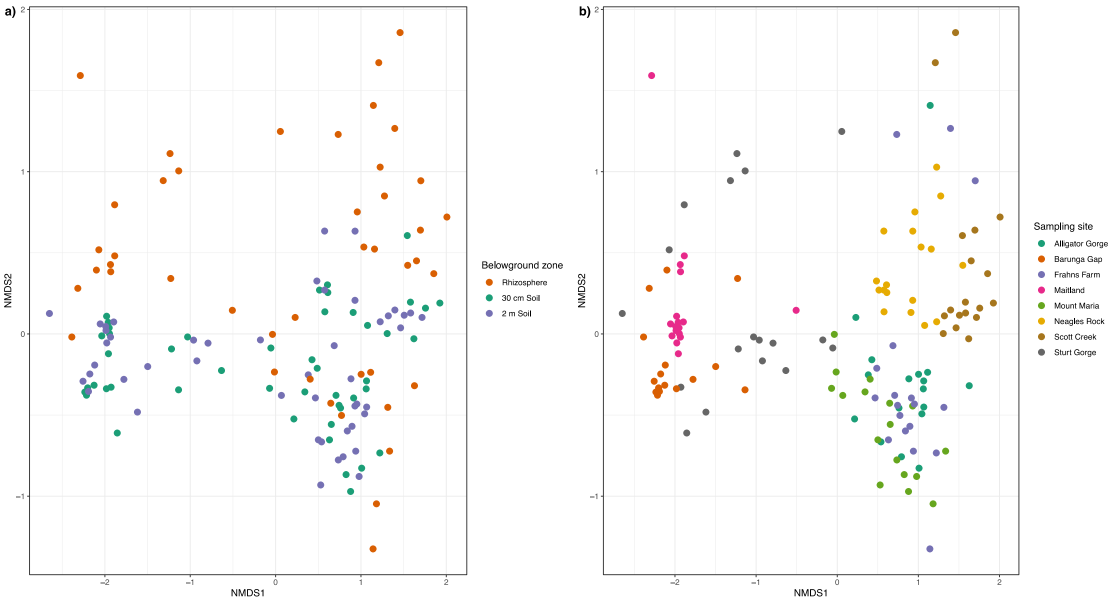

Figure S2.9. Non-metric multi-dimensional scaling (NMDS) plot of differences between bacterial community composition using Bray-Curtis distances (stress: 0.1097). Points represent samples with colour denoting (a) belowground zone, or (b) sampling sites.

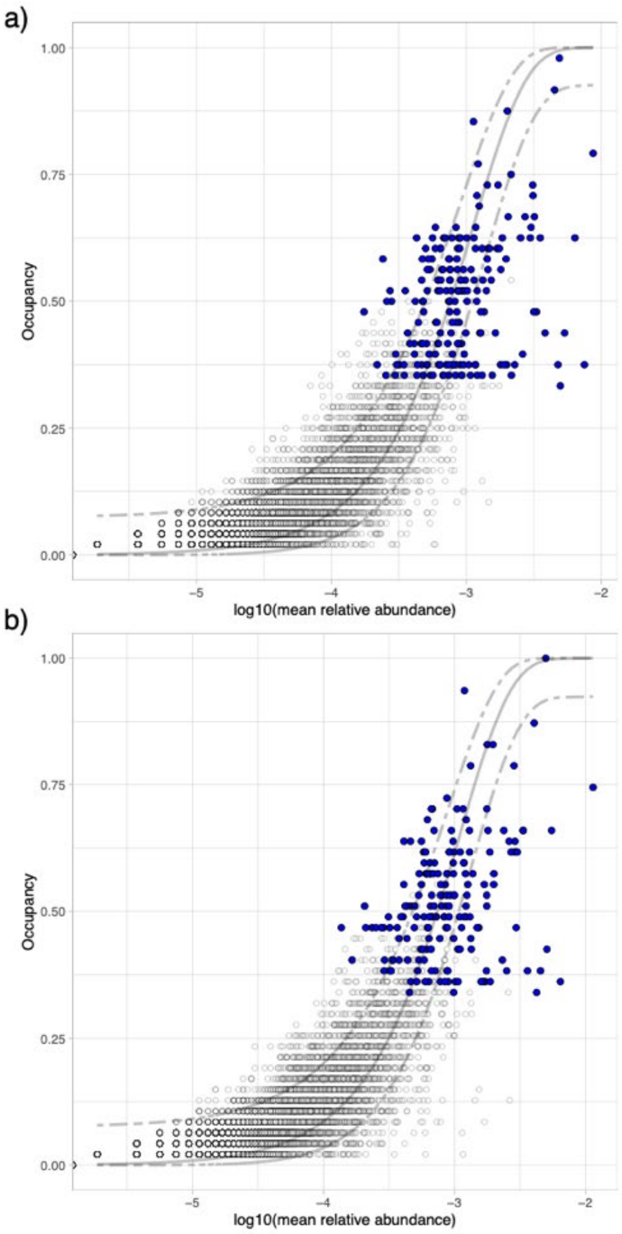

Figure S2.10. Abundance occupancy curves to prioritise candidate bacterial for core microbiome membership across (a) soils at 2 m from host plant (193 ASVs), and (b) soils at 30 cm from host plant (177 ASVs) Blue points represent amplicon sequence variants (ASVs) selected as prioritised candidates for core rhizosphere microbiome membership, white points represent non-prioritised candidates for core microbiome membership. The solid grey line represents a neutral model, with dashed lines showing 95% confidence intervals above and below the neutral taxa.

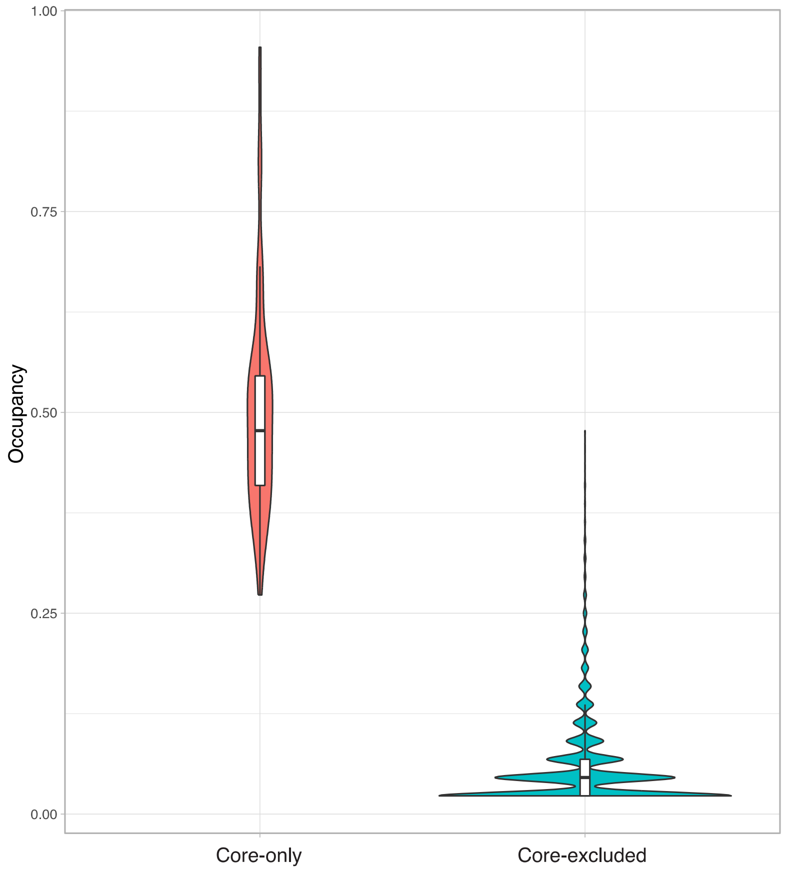

Figure S2.11. Occupany of core microbial taxa compared to core-excluded taxa determined using sloan neutral abundance occupancy curves.

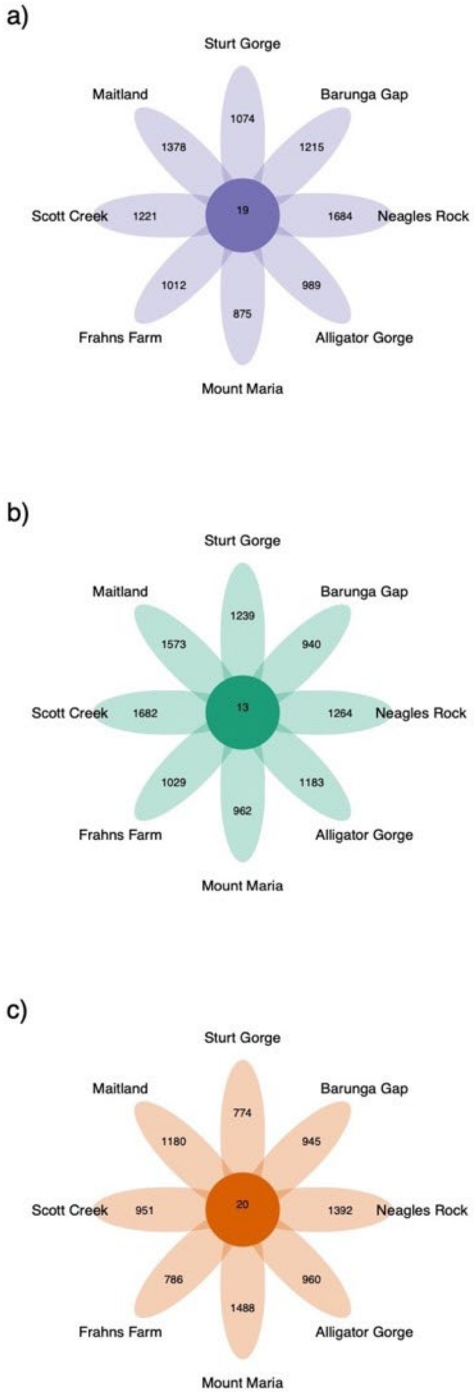

Figure S2.12. Venn diagram showing the number of unique taxa (ASVs) according to each sampling site for *T. triandra* plants across the (a) bulk soil at 2 m from host plants, 30 cm from host plants, and (c) rhizospheres. Taxa present in at least two, but fewer than all sites not represented.

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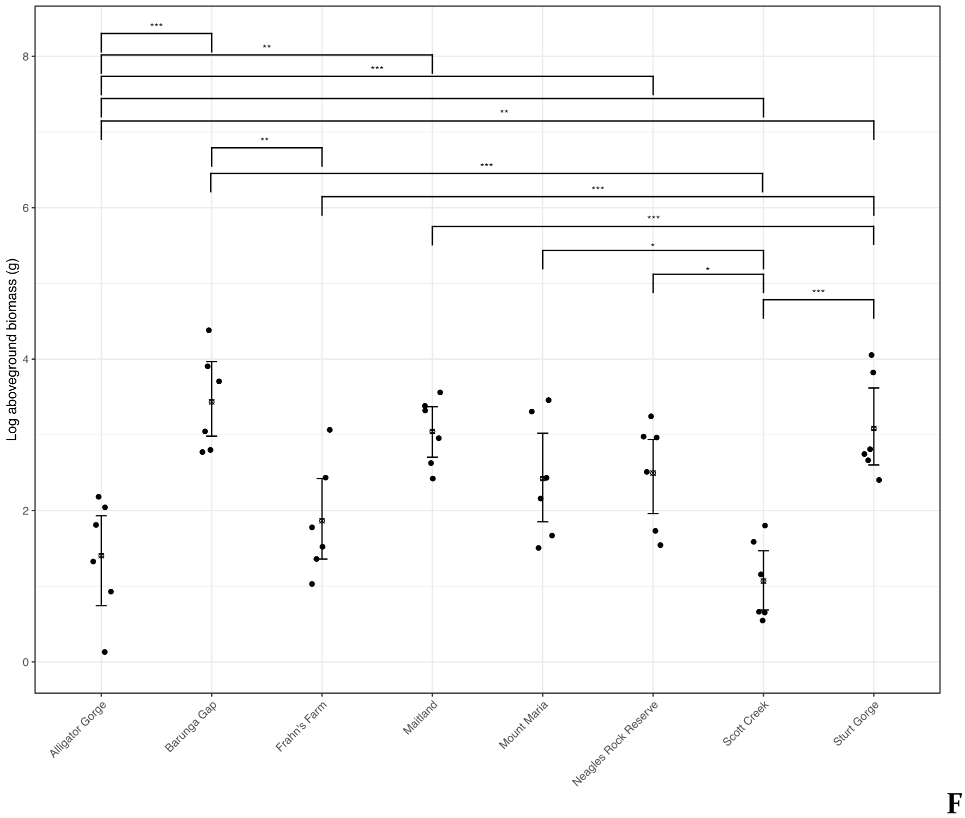

Figure S2.13. Above ground biomass for *T. triandra* across sampling sites

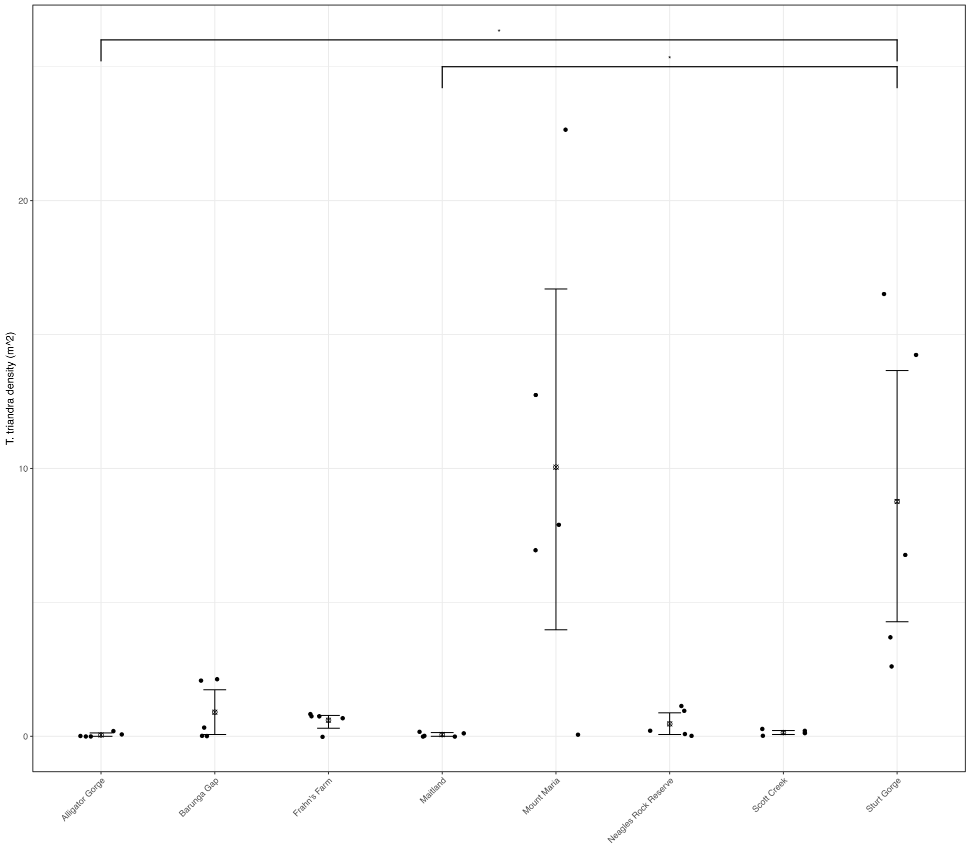

Figure S2.14. *T. triandra* density across sampling sites

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Figure S2.15. Canonical correspondence analysis (CCA) of the effect of the environmental variables on bacterial 16S community structure for soil at 2 m from host plant using: (a) CCA1 and CCA2, (b) CCA1 and CCA3, (c) CCA2 and CCA3. Bulk soil analyses considered soil physicochemical conditions potential environmental drivers for community structure (Table S2.2). Each point represents a sample from a given site based on colour.

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Figure S2.16. Canonical correspondence analysis (CCA) of the effect of the environmental variables on bacterial 16S community structure for soil at 30 cm from host plant using: (a) CCA1 and CCA2, (b) CCA1 and CCA3, (c) CCA2 and CCA3. Bulk soil analyses considered soil physicochemical conditions potential environmental drivers for community structure (Table S2.2). Each point represents a sample from a given site based on colour.

Figure S2.17. Canonical correspondence analysis (CCA) of the effect of the environmental variables on bacterial 16S community structure *T. triandra* rhizospheres: (a) CCA1 and CCA2, (b) CCA1 and CCA3, (c) CCA2 and CCA3. Rhizosphere analyses considered plant nutrient conditions as potential drivers for community structure (Table 2.2). Each point represents a rhizosphere sample from a given site based on colour.

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Figure S2.18. Canonical correspondence analysis (CCA) of the effect of the climatic, edaphic geographic, and host related variables on candidate core bacterial 16S community structure for soil at 2 m from host plant using: (a) CCA1 and CCA2, (b) CCA1 and CCA3, (c) CCA2 and CCA3. Bulk soil analyses considered soil physicochemical conditions potential environmental drivers for community structure (Table S2.2). Each point represents a sample from a given site based on colour.

Figure S2.19. Canonical correspondence analysis (CCA) of the effect of the climatic, edaphic geographic, and host related variables on candidate core bacterial 16S community structure for soil at 30 cm from host plant using: (a) CCA1 and CCA2, (b) CCA1 and CCA3, (c) CCA2 and CCA3. Bulk soil analyses considered soil physicochemical conditions potential environmental drivers for community structure (Table S2.2). Each point represents a sample from a given site based on colour.

Figure S2.20. Canonical correspondence analysis (CCA) of the effect of the climatic, edaphic geographic, and host related variables on candidate core bacterial 16S community structure for T. triandra rhizospheres using: (a) CCA1 and CCA2, (b) CCA1 and CCA3, (c) CCA2 and CCA3. Bulk soil analyses considered soil physicochemical conditions potential environmental drivers for community structure (Table S2.2). Each point represents a sample from a given site based on colour.

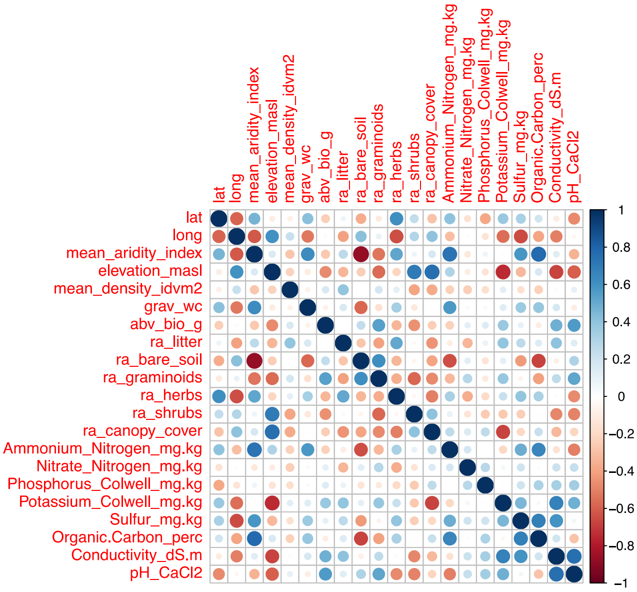

Figure S2.21. Correlation plot of environmental variables in soil at 2 m from host plants. Prior to canonical correspondance analysis. Where variable pairs contained correlations of greater than 0.75, one was removed.

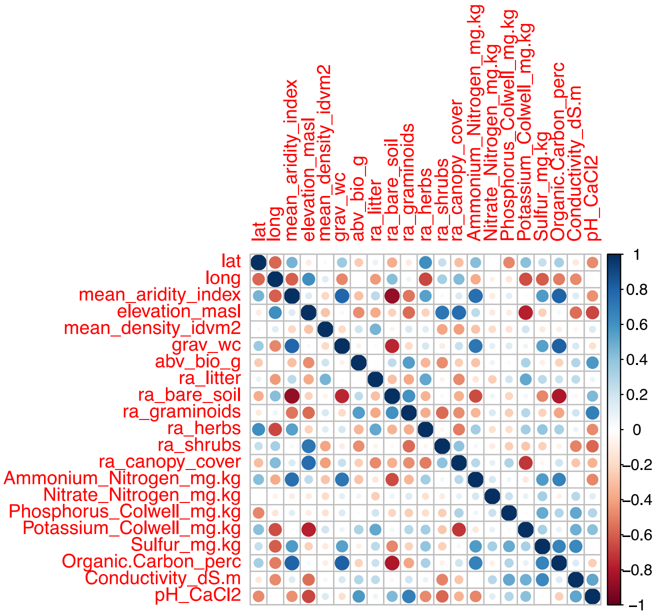

Figure S2.22. Correlation plot of environmental variables in soil at 30 cm from host plants at the sampling site. Prior to canonical correspondance analysis. Where variable pairs contained correlations of greater than 0.75, one was removed.

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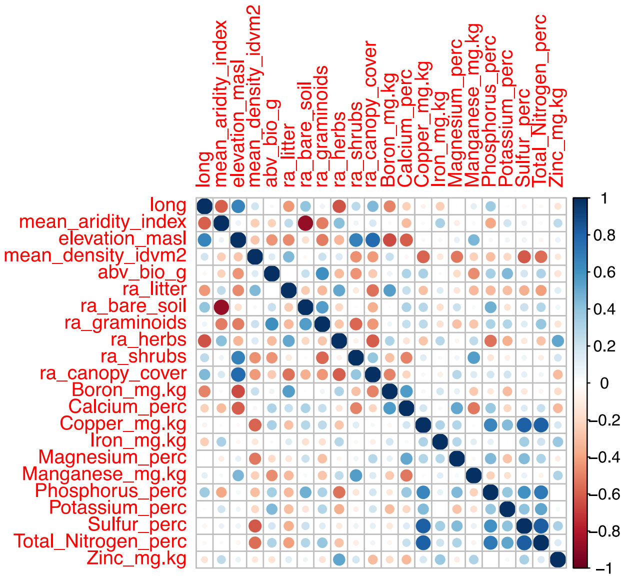

Figure S2.23. Correlation plot of environmental variables in host rhizospheres of the sampling site. Prior to canonical correspondance analysis. Where variable pairs contained correlations of greater than 0.75, one was removed.

Supplementary references

ALA. (2014). *Mean annual aridity index* Atlas of Living Australia. https://spatial.ala.org.au/layers, https://researchdata.edu.au/mean-annual-aridity-index/340830. Data layer, Accessed 21/12/2021

Belbin, L. 2011. The Atlas of Livings Australia's Spatial Portal. In, Proceedings of the Environmental Information Management Conference 2011 (EIM 2011).

Supplementary information: Chapter three

Supplementary methods

Pilot experiment

In March 2022, endospheres from *T. triandra* plants were obtained from 15 individuals at Flinders University (35° 1' 42.95", 138° 34' 37.38") to test the efficacy of different root cleaning methods to isolate endosphere microbiota (n=3). Endosphere microbe isolation is a common procedure undertaken across a variety of different plants, often studies employ sonication of the plant roots to remove surface microbiota, or chemical sterilisation with solutions such as NaOCl, for instance (Barra et al. 2016, Richter-Heitmann et al. 2016). These methods have been published for a host of model species (Bulgarelli et al. 2013, Urbina et al. 2018), outcomes and effectiveness, however, could differ depending on the species of plant and root types. For *T. triandra* plants obtained under field conditions, we tested four root washing treatments: sterilisation of root surfaces by submerging in either 2% or 4% NaOCl for a period of 3 minutes; sonication by probe in 0.02% Silwet L-77 amended PBS buffer for either 3 minutes or 5 minutes, each in 30 second burst and rest periods; and a wash only control treatment in the amended PBS buffer. Following these cleaning processes, plant roots were subsequently washed three times in 0.02% Silwet L-77 amended PBS buffer solution. During the final wash step a 100 uL samples was taken for each sample and plated on LB (Luria-Bertani Agar), and placed in an incubator at 28°C. Root samples were subsequently prepared for DNA extraction, in anticipation for amplicon sequencing of the 16S rRNA gene to identify bacteria present.

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Supplementary results

Pilot study

Following bioinformatic processing, we show that diversity was reduced in the bleached root samples, significantly below that of sonication and the control group (Figure S3.1a). Interestingly, the community composition did not appear to change much with sonication and control group, though there was a significant shift with the bleached treatments at 2% and 4% (Figure S3.1b-c).

Microbial colonies present on Luria Bertani agar showed different results following each the root cleaning processes. Results found that 2-4% bleach was the most effective process for removing contaminant taxa from root surfaces, followed 5 min sonication treatment compared to the control groups (Figure S3.2a). Log transformed concentrations of DNA extracted from root tissue across different cleaning methods (Figure S3.2b). Both bleach treatments appeared to have too great an effect on removing DNA from root samples, additionally removing DNA from the internal root structures, whereas in sonication treatments concentrations remained high, in similar quantities to the control group. Sonication of the roots for 5 minutes (30 second burst and rest periods) was identified as the most appropriate methods for use on *T. triandra* sampled roots based on our sampling procedure. To fine-tune sonication methods before applying this approach to samples from the field experiment, we explored the effect of increasing the number of different 0.02% Silwet L-77 amended PBS buffer wash steps before extraction. Additional wash steps improved the cleaning of the roots, as shown by the number of colonies identified on LB agar plates with the fewest colonies shown after 5 washes (Figure S3.2b).

Differential abundance analysis of ASVs and Phyla with neutral model fits

Supplementary information: Chapter three

When we performed the differential abundance analyses separately for each site, and compared site outcomes together, we found an average of 8.5 (±0.98 SE) differentially abundant phyla were present across rhizospheres and endospheres (Figure S3.7b). Specifically looking at directional trends, we found that 4.5 (±0.5 SE) phyla were more abundant in the endospheres (negative log fold changes) and 4 (±0.8 SE) phyla were more abundant in rhizospheres (positive log fold changes; Figure S3.11; Figure S3.12).

The ASVs that were differentially abundant between the *T. triandra* rhizospheres and endospheres were also tested with neutral assembly models, and revealed to be differently impacted by microbial community assembly selection dynamics (Figure S3.16). Differentially abundant rhizosphere-favoured ASVs (those with a significant positive log fold change, see Main document, Figure 4a) displayed a better fit to the neutral models in rhizospheres (R^2 = 0.286; Figure S3.16a), compared to endosphere abundant taxa (negative log fold change, see Main document, Figure 3.4a) (R^2 = 0.014; Figure S3.16e). ASVs that were not differentially abundant between the rhizospheres and endospheres still collectively deviated from the neutral models, suggesting that deterministic processes were influencing many taxa in these compartments. This pattern was consistent across compartments, with a similar deviation from neutral models measure in the non-differentially abundant ASVs in rhizospheres (R^2 =0.039, Figures S3.16c) as those in the endospheres (R^2 =0.028, Figure S3.16f).

When looking at how the neutral models fitted the endosphere-favoured ASVs (negative log fold change) within the rhizosphere samples (i.e., low abundance rhizosphere taxa), we observed strong neutral influences (R^2 = 0.695, Figure S3.16b); and similarly, the rhizosphere-favoured ASVs (positive log fold change) when found in endosphere samples (i.e., low abundance endosphere taxa) saw comparatively strong neutral influences (R^2 = 0.454, Figure S3.16d).

Supplementary tables

Table S3.1: Differentially abundant bacterial phyla with positive or negative magnitude of change across in the rhizospheres relative to root endosphere taxa. Data includes all differentially abundant phyla with a positive or negative direction of change, and log fold change.

Phylum	Direction	Log fold change
RCP2-54	Increasing	1.367
Gemmatimonadota	Increasing	1.172
Acidobacteriota	Increasing	1.1
Planctomycetota	Increasing	1.056
Nitrospirota	Increasing	0.911
Verrucomicrobiota	Increasing	0.853
WPS-2	Increasing	0.762
Chloroflexi	Increasing	0.545
Armatimonadota	Increasing	0.509
Patescibacteria	Decreasing	-0.531
Actinobacteriota	Decreasing	-0.75
Proteobacteria	Decreasing	-0.772
Myxococcota	Decreasing	-0.783

Table S3.2: Differentially abundant bacterial taxa with positive or negative magnitude of change across in the rhizospheres relative to root endosphere taxa. Data includes top 30 bacterial ASVs with a positive or negative direction of change, log fold change, and taxonomic rank.

ASV_ID	Direction	Log fold change	Phylum	Class	Order	Family	Genus	Species
ASV_2497	Increasing	2.161	Acidobacteriota	Holophagae	Subgroup_7	Subgroup_7	Subgroup_7	Unclassified
ASV_1181	Increasing	1.713	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2	Subgroup_2	uncultured_Acidobacteria
ASV_3182	Increasing	1.706	Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus_Solibacter	Unclassified
ASV_3081	Increasing	1.654	Myxococcota	bacteriap25	bacteriap25	bacteriap25	bacteriap25	Unclassified
ASV_1085	Increasing	1.628	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	Unclassified
ASV_8300	Increasing	1.619	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	uncultured	Unclassified
ASV_7131	Increasing	1.599	Actinobacteriota	Acidimicrobiia	Microtrichales	uncultured	uncultured	uncultured_bacterium
ASV_3741	Increasing	1.592	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Magnetospiraceae	uncultured	metagenome
ASV_2695	Increasing	1.501	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	uncultured_bacterium
ASV_1814	Increasing	1.466	Chloroflexi	TK10	TK10	TK10	TK10	uncultured_bacterium

ASV_6991	Increasing	1.462	Chloroflexi	Chloroflexia	Kallotenuales	AKIW781	AKIW781	uncultured_soil
ASV_2961	Increasing	1.443	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter	uncultured_Spartobacteria
ASV_317	Increasing	1.433	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	Unclassified
ASV_1480	Increasing	1.39	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	uncultured_bacterium
ASV_1102	Increasing	1.372	Acidobacteriota	Blastocatellia	Blastocatellales	Blastocatellaceae	uncultured	uncultured_Acidobacteria
ASV_464	Increasing	1.371	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	uncultured_Alphaproteobacteria
ASV_1670	Increasing	1.348	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	MND1	Unclassified
ASV_2122	Increasing	1.322	Chloroflexi	KD4-96	KD4-96	KD4-96	KD4-96	Unclassified
ASV_1754	Increasing	1.315	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured	uncultured_Gemmatimonadales
ASV_8348	Increasing	1.307	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	uncultured	Unclassified
ASV_331	Increasing	1.285	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter	uncultured_Spartobacteria
ASV_1661	Increasing	1.282	Acidobacteriota	Blastocatellia	Blastocatellales	Blastocatellaceae	JGI_0001001-H03	Unclassified
ASV_2171	Increasing	1.266	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	uncultured	uncultured	Unclassified
ASV_353	Increasing	1.241	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	Unclassified

ASV_1695	Increasing	1.219	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	uncultured	uncultured	Unclassified
ASV_579	Increasing	1.173	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured	uncultured	Unclassified
ASV_2518	Increasing	1.168	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter	Unclassified
ASV_1244	Increasing	1.158	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Xiphinematobacteraceae	Candidatus_Xiphinematobacter	Unclassified
ASV_959	Increasing	1.139	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter	Unclassified
ASV_745	Increasing	1.13	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured	uncultured	Unclassified
ASV_117	Decreasing	-1.897	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	uncultured_bacterium
ASV_54	Decreasing	-1.955	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Unclassified
ASV_167	Decreasing	-2	Actinobacteriota	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Actinocorallia	metagenome
ASV_28	Decreasing	-2.026	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	Unclassified
ASV_42	Decreasing	-2.028	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	Unclassified
ASV_73	Decreasing	-2.051	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	uncultured_bacterium

ASV_183	Decreasing	-2.074	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	Thermosporothrix	uncultured_bacterium
ASV_144	Decreasing	-2.102	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	uncultured_bacterium
ASV_264	Decreasing	-2.107	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	Leptothrix_sp.
ASV_100	Decreasing	-2.127	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	Unclassified
ASV_12	Decreasing	-2.171	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	Unclassified
ASV_49	Decreasing	-2.185	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	Unclassified
ASV_17	Decreasing	-2.226	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	uncultured_bacterium
ASV_45	Decreasing	-2.246	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Longimycelium	uncultured_bacterium
ASV_35	Decreasing	-2.279	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	Unclassified
ASV_225	Decreasing	-2.281	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	uncultured_bacterium
ASV_173	Decreasing	-2.334	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	Unclassified

ASV_26	Decreasing	-2.372	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	Actinophytocola_sp.
ASV_88	Decreasing	-2.465	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	uncultured	uncultured_actinomycete
ASV_129	Decreasing	-2.475	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Virgisporangium	Unclassified
ASV_13	Decreasing	-2.556	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	Unclassified
ASV_210	Decreasing	-2.635	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	Unclassified
ASV_53	Decreasing	-2.648	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Lechevalieria	Unclassified
ASV_37	Decreasing	-2.854	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	Unclassified
ASV_762	Decreasing	-2.97	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	uncultured_bacterium
ASV_81	Decreasing	-3.179	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	Unclassified
ASV_80	Decreasing	-3.775	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	Unclassified

ASV_14	Decreasing	-4.071	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	uncultured_bacterium
ASV_3	Decreasing	-4.672	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	Triticum_aestivum
ASV_1	Decreasing	-5.196	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	Triticum_aestivum

Table S3.3: List of endosphere HUB taxa from bacterial ASV network analysis showing taxonomic for all taxa and node degree with negative edges only.

ASV_ID	Phylum	Class	Order	Family	Genus	Node degrees (negative edges)
ASV_23	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	26
ASV_7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Unclassified	22
ASV_29	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	11
ASV_47	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	10
ASV_20	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Kibdelosporangium	9
ASV_36	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	8
ASV_12	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	7
ASV_13	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	6
ASV_35	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	6
ASV_77	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Saccharothrix	5
ASV_10	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	4

ASV_107	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	4
ASV_119	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	4
ASV_19	Actinobacteriota	Actinobacteria	Micrococcales	Promicromonosporaceae	Promicromonospora	4
ASV_28	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	4
ASV_43	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	4
ASV_117	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	3
ASV_18	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Kutzneria	3
ASV_197	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	3
ASV_207	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacter	3
ASV_26	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	3
ASV_152	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	uncultured	2
ASV_154	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2	Subgroup_2	2
ASV_239	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacter	2
ASV_24	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	2

ASV_306	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	2
ASV_313	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	2
ASV_359	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	2
ASV_90	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	2
ASV_1	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1
ASV_109	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	1
ASV_137	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	1
ASV_150	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	1
ASV_178	Proteobacteria	Alphaproteobacteria	Dongiales	Dongiaceae	Dongia	1
ASV_191	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia	1
ASV_3	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1
ASV_304	Actinobacteriota	Thermoleophilia	Solirubrobacterales	67-14	67-14	1
ASV_325	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas	1
ASV_336	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	uncultured	1

ASV_518	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	1
ASV_57	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	1
ASV_721	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	uncultured	1

Table S3.4: List of endosphere HUB taxa from bacterial ASV network analysis showing taxonomic for all taxa and node degree with positive edges only.

ASV_ID	Phylum	Class	Order	Family	Genus	Node degrees (positive edges)
ASV_13	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	29
ASV_12	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	25
ASV_28	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	25
ASV_35	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	25
ASV_19	Actinobacteriota	Actinobacteria	Micrococcales	Promicromonosporaceae	Promicromonospora	24
ASV_77	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Saccharothrix	22
ASV_26	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	21
ASV_117	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	19
ASV_107	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	16
ASV_152	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	uncultured	16

ASV_197	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	16
ASV_119	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	14
ASV_20	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Kibdelosporangium	14
ASV_207	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacter	14
ASV_313	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	14
ASV_10	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	13
ASV_239	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacter	13
ASV_29	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	13
ASV_23	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	9
ASV_359	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	9
ASV_155	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	8
ASV_74	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	8
ASV_178	Proteobacteria	Alphaproteobacteria	Dongiales	Dongiaceae	Dongia	7

ASV_90	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	7
ASV_325	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas	6
ASV_336	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	uncultured	6
ASV_357	Myxococcota	Polyangia	Polyangiales	BIrii41	BIrii41	6
ASV_36	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	6
ASV_7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Unclassified	6
ASV_129	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Virgisporangium	5
ASV_154	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2	Subgroup_2	4
ASV_47	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	4
ASV_502	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Unclassified	4
ASV_54	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	4
ASV_57	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	4
ASV_84	Actinobacteriota	Actinobacteria	Propionibacteriales	Nocardioidaceae	Kribbella	4
ASV_18	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Kutzneria	3

ASV_43	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	3
ASV_518	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	3
ASV_520	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	Unclassified	3
ASV_53	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Lechevalieria	3
ASV_88	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	uncultured	3
ASV_137	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	2
ASV_150	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	2
ASV_160	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Unclassified	2
ASV_180	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Phyllobacterium	2
ASV_191	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia	2
ASV_285	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	Steroidobacter	2
ASV_304	Actinobacteriota	Thermoleophilia	Solirubrobacterales	67-14	67-14	2
ASV_462	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	uncultured	2
ASV_67	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Lechevalieria	2

ASV_721	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	uncultured	2
ASV_1	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1
ASV_103	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Unclassified	1
ASV_109	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	1
ASV_112	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	1
ASV_115	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1
ASV_122	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	1
ASV_130	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	1
ASV_138	Acidobacteriota	Acidobacteriae	Bryobacterales	Bryobacteraceae	Bryobacter	1
ASV_15	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	1
ASV_198	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	1
ASV_218	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter	1
ASV_240	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	1
ASV_242	Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus_Solibacter	1

ASV_281	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces	1
ASV_3	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1
ASV_306	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	1
ASV_33	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria	1
ASV_34	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	1
ASV_37	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	1
ASV_380	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Occallatibacter	1
ASV_388	Actinobacteriota	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	1
ASV_395	Proteobacteria	Alphaproteobacteria	Dongiales	Dongiaceae	Dongia	1
ASV_42	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	1
ASV_49	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	1
ASV_62	Actinobacteriota	Actinobacteria	Frankiales	Geodermatophilaceae	Geodermatophilus	1
ASV_79	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	1
ASV_8	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Amycolatopsis	1

ASV_80	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Unclassified	1

Table S3.5: List of rhizosphere HUB taxa from bacterial ASV network analysis showing taxonomic for all taxa and node degree with negative edges only.

ASV_ID	Phylum	Class	Order	Family	Genus	Node degrees (negative edges)
ASV_7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Unclassified	20
ASV_23	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	11
ASV_109	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	4
ASV_36	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	4
ASV_240	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	3
ASV_10	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	2
ASV_12245	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacteraceae	2
ASV_185	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	2
ASV_317	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	2
ASV_490	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	2
ASV_497	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	2
ASV_683	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	2
ASV_79	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	2

ASV_950	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	2
ASV_107	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	1
ASV_119	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	1
ASV_12	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	1
ASV_152	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	uncultured	1
ASV_1670	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	MND1	1
ASV_2034	Actinobacteriota	Acidimicrobiia	Microtrichales	Iamiaceae	Iamia	1
ASV_239	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacter	1
ASV_259	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	1
ASV_262	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	1
ASV_2718	Proteobacteria	Gammaproteobacteria	PLTA13	PLTA13	PLTA13	1
ASV_313	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	1
ASV_402	Actinobacteriota	Thermoleophilia	Gaiellales	Unclassified	Unclassified	1
ASV_407	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	1
ASV_488	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	1

ASV_6	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	1
ASV_655	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	1
ASV_674	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Microlunatus	1
ASV_704	Actinobacteriota	Thermoleophilia	Solirubrobacterales	67-14	67-14	1
ASV_816	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	uncultured	1
ASV_987	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	1

Table S3.6: List of rhizosphere HUB taxa from bacterial ASV network analysis showing taxonomic for all taxa and node degree with positive edges only.

ASV_ID	Phylum	Class	Order	Family	Genus	Node degrees (positive edges)
ASV_109	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	18
ASV_497	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	12
ASV_185	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	11
ASV_240	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	9
ASV_683	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	9
ASV_10	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	8
ASV_262	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	8
ASV_987	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	8
ASV_674	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Microlunatus	6
ASV_152	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	uncultured	5
ASV_313	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	5
ASV_389	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	5

ASV_407	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	5
ASV_488	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	5
ASV_655	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	5
ASV_1197	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	4
ASV_359	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	4
ASV_402	Actinobacteriota	Thermoleophilia	Gaiellales	Unclassified	Unclassified	4
ASV_490	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	4
ASV_107	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	3
ASV_1102	Acidobacteriota	Blastocatellia	Blastocatellales	Blastocatellaceae	uncultured	3
ASV_12	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	3
ASV_331	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter	3
ASV_36	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	3
ASV_1670	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	MND1	2
ASV_202	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	2

ASV_23	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	2
ASV_464	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	2
ASV_7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Unclassified	2
ASV_950	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	2
ASV_104	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	1
ASV_1139	Actinobacteriota	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella	1
ASV_1715	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacteraceae	1
ASV_242	Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus_Solibacter	1
ASV_259	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	1
ASV_268	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces	1
ASV_292	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Unclassified	1
ASV_317	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	1
ASV_34	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	1
ASV_348	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Granulicella	1
ASV_353	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	1
ASV_377	Acidobacteriota	Acidobacteriae	Bryobacterales	Bryobacteraceae	Bryobacter	1

ASV_380	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Occallatibacter	1
ASV_579	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured	uncultured	1
ASV_587	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	uncultured	1
ASV_601	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Unclassified	1
ASV_638	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured	uncultured	1
ASV_704	Actinobacteriota	Thermoleophilia	Solirubrobacterales	67-14	67-14	1
ASV_742	Acidobacteriota	Acidobacteriae	Bryobacterales	Bryobacteraceae	Bryobacter	1
ASV_79	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	1
ASV_816	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	uncultured	1
ASV_879	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured	uncultured	1
ASV_8814	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Vicinamibacteraceae	1
ASV_898	Chloroflexi	KD4-96	KD4-96	KD4-96	KD4-96	1

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Supplementary figures

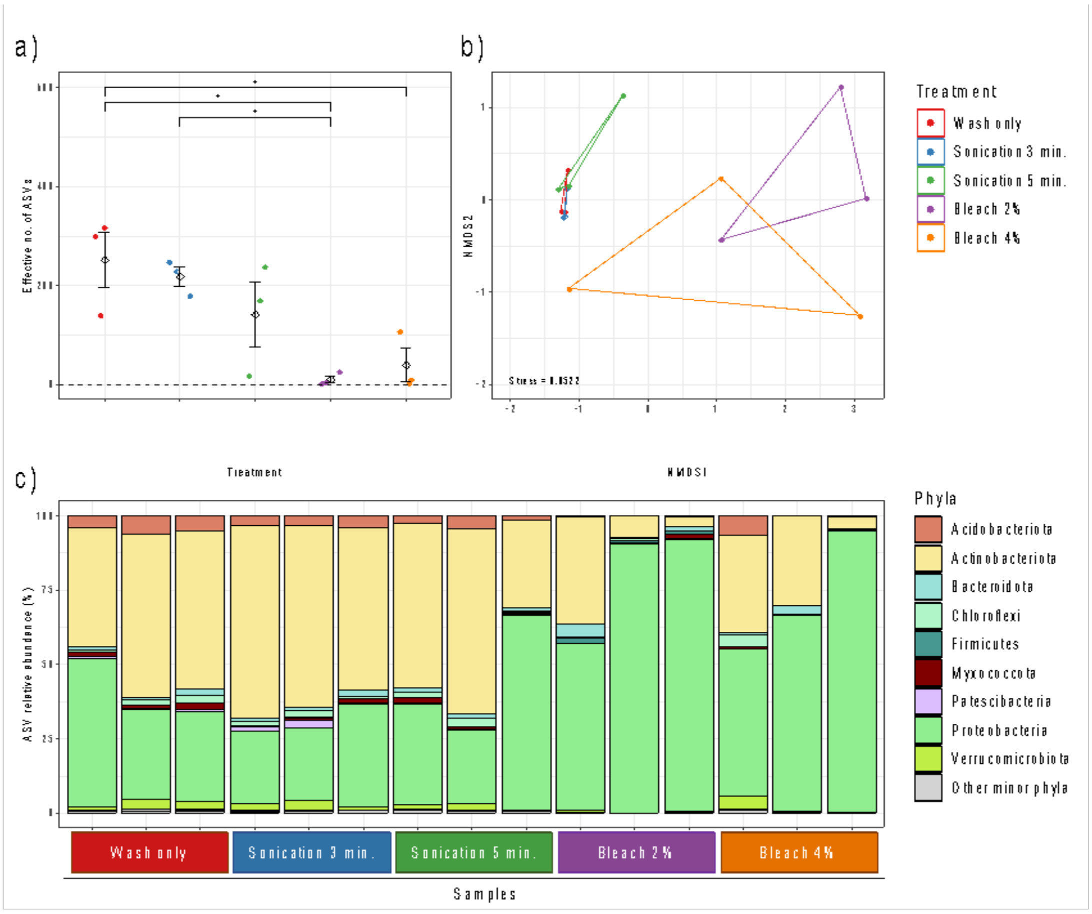

Figure S3.1. Root cleaning methods to isolate endosphere microbiota in *T. triandra* individuals. (a) bacterial diversity as effective number of ASVs, against chemical and mechanical cleaning methods. (b) NMDS ordination showing the effect of diferent cleaning methods on bacterial community composition. Polygons are coloured by treatment group. (c) Relative abundance of major bacterial phyla across samples and cleaning treatments.

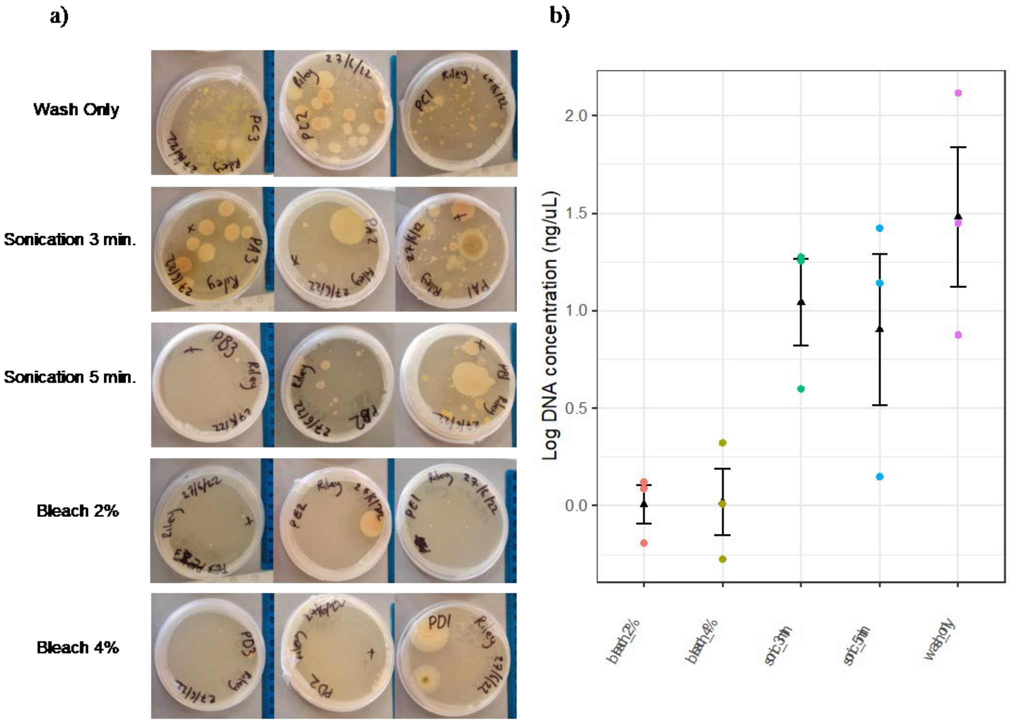

Figure S3.2. (a) Microbial colonies present on Luria Bertani agar following different root cleaning processes. Results found that 2-4% bleach was the most effective process for sterilising root surfaces, followed 5 min sonication, compared to the control groups. (b) Log transformed concentrations of DNA extracted from root tissue across different cleaning methods. Bleach appeared to have too great an effect on removing DNA from root samples, whereas sonication concentrations remained high, in similar quantities to the control group.

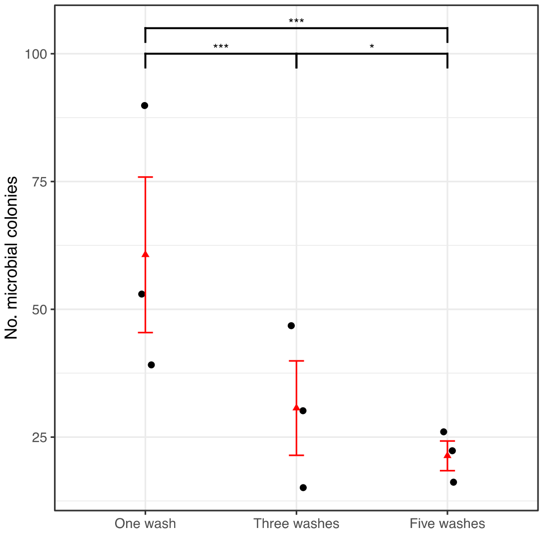

Figure S3.3. After establishing sonication as the most effective root cleaning method to isolate endosphere microbiota, with most sterilised wash mediums and best DNA yields. We compared the number of wash steps in 0.02% Silwet L-77 amended PBS, following primary cleaning of the roots to limit the formation of bacterial colonies.

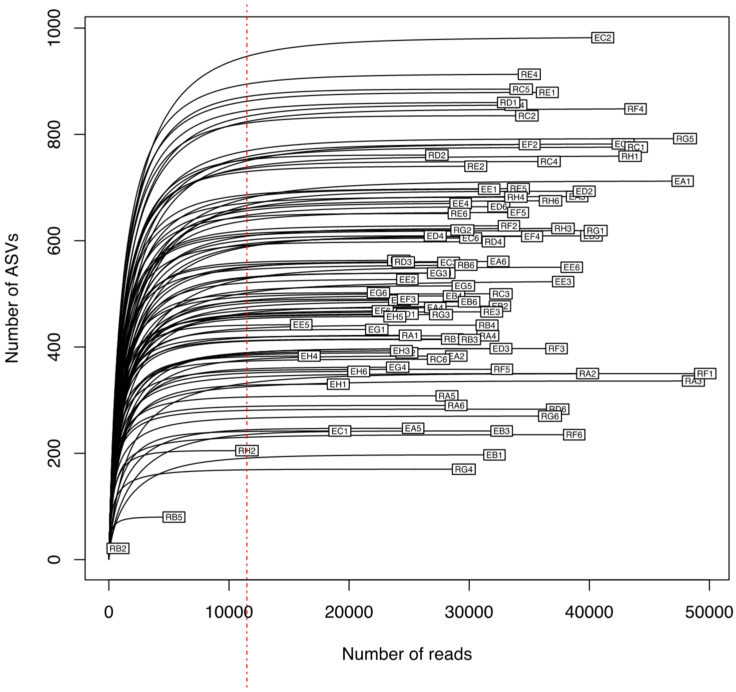

Figure S3.4. Rarefaction plot showing number of ASVs by number of reads per sample. Samples were rarified to 11,491 reads (red dashed line). Samples that did not meet the minimum threshold of reads were removed from analysis.

Figure S3.5. Faith's phylogenetic diversity for rhizospheres and endospheres communities.

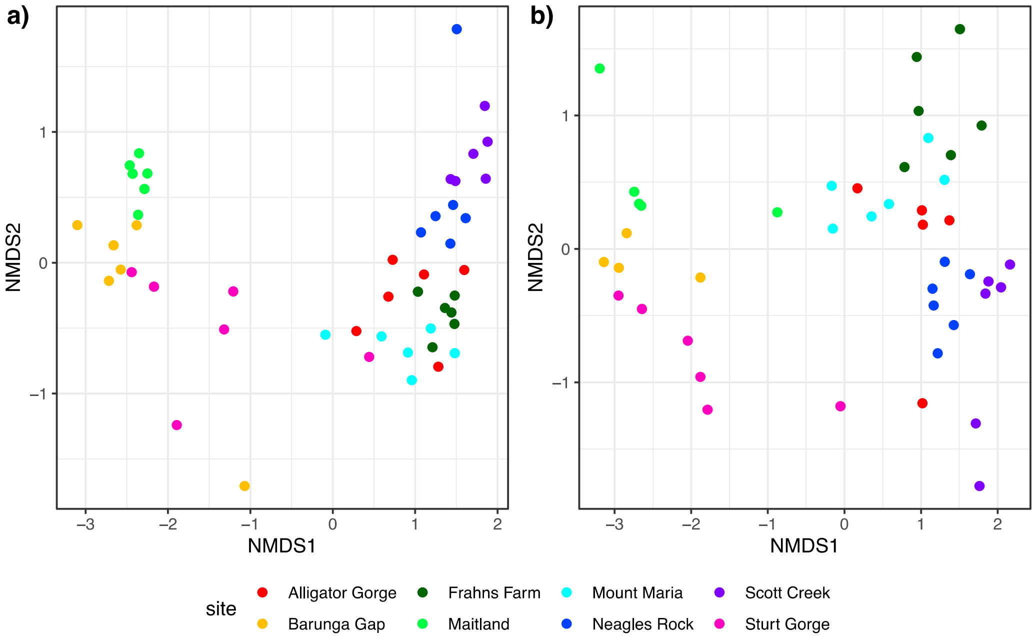

Figure S3.6. Non-metric multi-dimensional scaling (NMDS) ordinations with Bray-Curtis distances showing the difference between sampling sites across (a) endosphere samples (stress = 0.0922) and (b) rhizosphere samples (stress = 0.1200). Site are represented by colour.

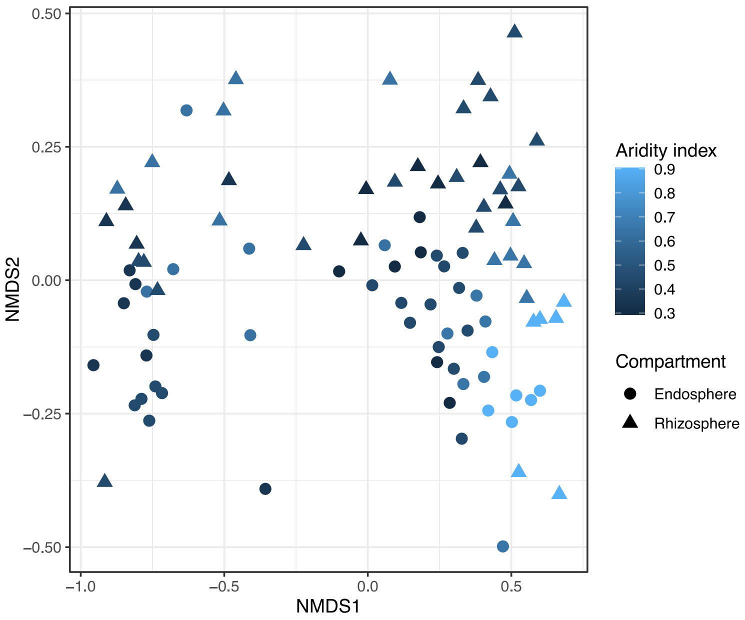

Figure S3.7. Non-metric dimensional scaling plot with Bray-Curtis distances showing the difference between plant compartments. Endosphere samples represented by circles, and rhizospheres by triangles. Mean annual aridity index of sampling sites is indicated by colour gradient with a lower aridity index values corresponding to higher site aridity estimates.

Figure S3.8. (a) Non-metric dimensional scaling plot with beta mean nearest taxon distances (bMNTD) showing the differences between bacterial community composition across plant rhizospheres (blue) and endospheres (red). (b) NMDS plot with beta mean nearest taxon distances (bMNTD) showing the differences between bacterial community composition. Endosphere samples represented by triangles, and rhizospheres by circles. Mean annual aridity index of sampling sites is indicated by colour gradient with a lower aridity index values corresponding to higher site aridity estimates. (c) Distance to centroid of samples comparing rhizosphere (blue) and endosphere (red) samples, calculated from bMNTD.

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Figure S3.9. (a) Non-metric dimensional scaling plot with weighted unifrac distances showing the differences between bacterial community composition across plant rhizospheres (blue) and endospheres (red). (b) NMDS plot with weighted unifrac distances showing the differences between bacterial community composition. Endosphere samples represented by triangles, and rhizospheres by circles. Mean annual aridity index of sampling sites is indicated by colour gradient with a lower aridity index values corresponding to higher site aridity estimates. (c) Distance to centroid of samples comparing rhizosphere (blue) and endosphere (red) samples, calculated from weighted unifrac distances.

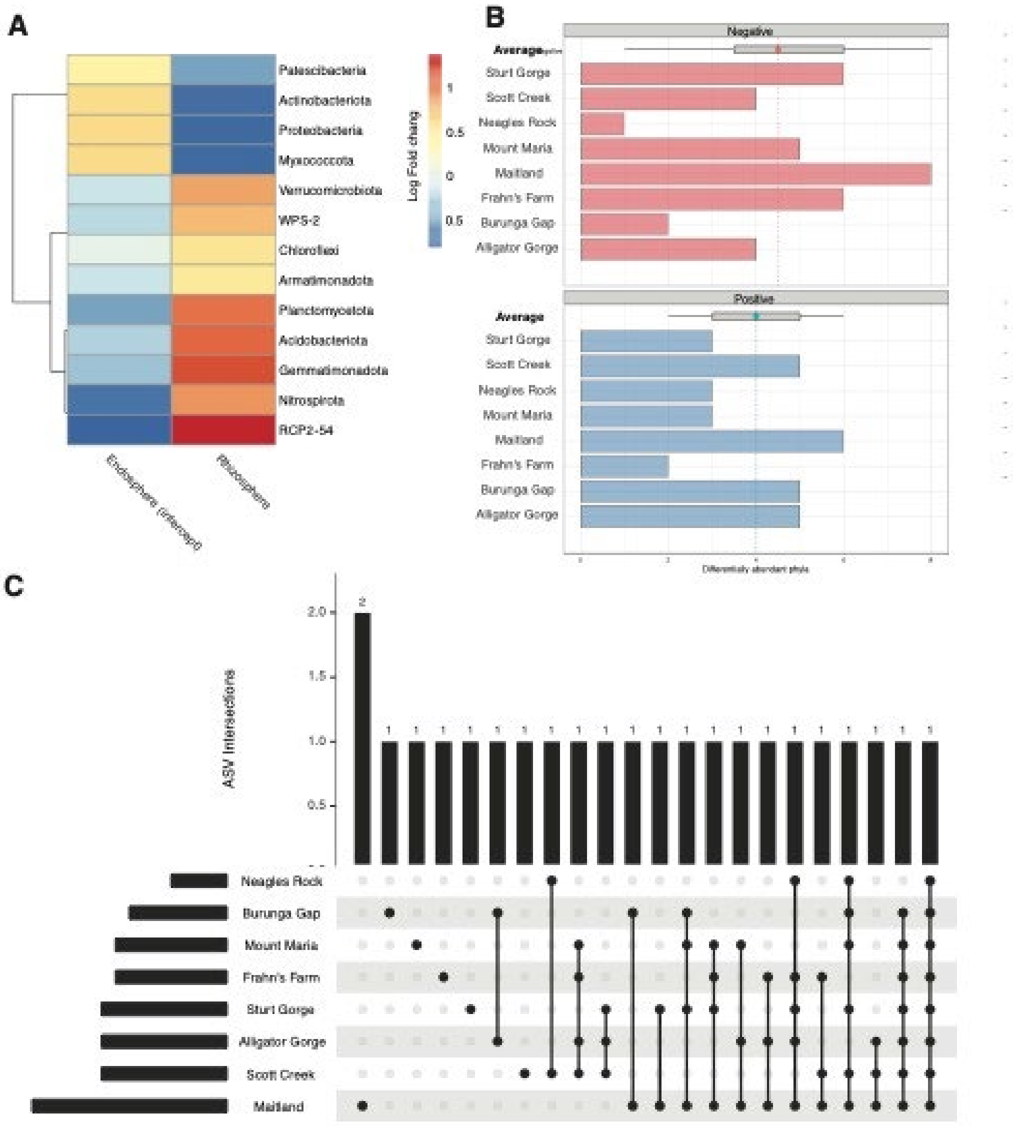

Figure S3.10. (a) Heatmap showing 13 differentially abundant bacterial phyla across *T. triandra* rhizospheres and endosphere samples and (b) the number of differentially abundant phyla calculated within each sampling site. The negative grouping includes those phyla favoured in the endosphere (negative log fold change), whereas the positive grouping includes phyla favoured in the rhizosphere (positive log fold change). (c) Upset plot showing the number of shared and unique bacterial phyla across each site that are differentially abundant. This plot shows only the first 22 most populated ASV intersections between sites (see Figure 10 for full figure).

Figure S3.11. Upset plot showing the number of shared and unique bacterial phyla across each sampling site that are differentially abundant.

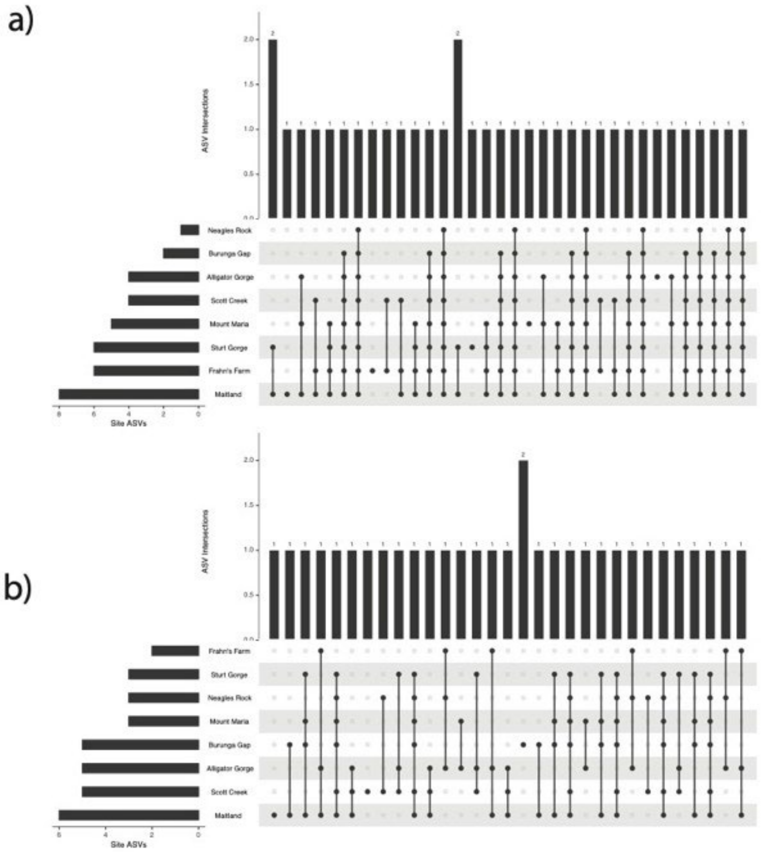

Figure S3.12. Upset plot showing the number of shared and unique bacterial phyla across each sampling site that are differentially abundant with either a (a) negative and (b) positive log fold change in the rhizosphere relative to the endosphere

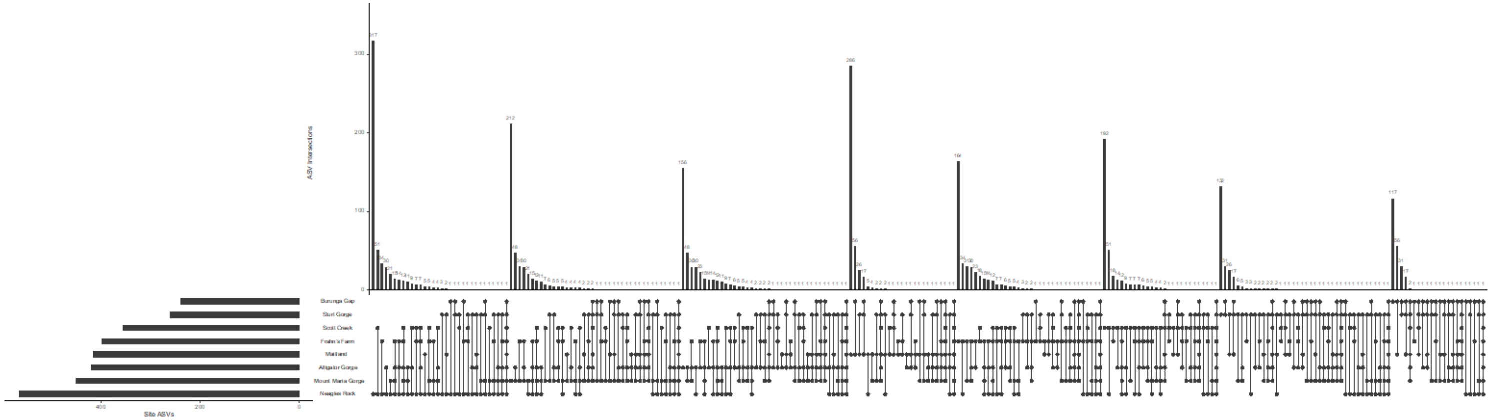

Figure S3.13: Upset plot showing the number of shared and unique bacterial ASVs across each sampling site that are differentially abundant. The order of the bars representing site intersections (overlapping ASVs between sites) are ordered first by the sites with the highest to lowest total number of ASVs (left panel bar), and then within sites by the groupings from highest to lowest intersection counts with other sites.

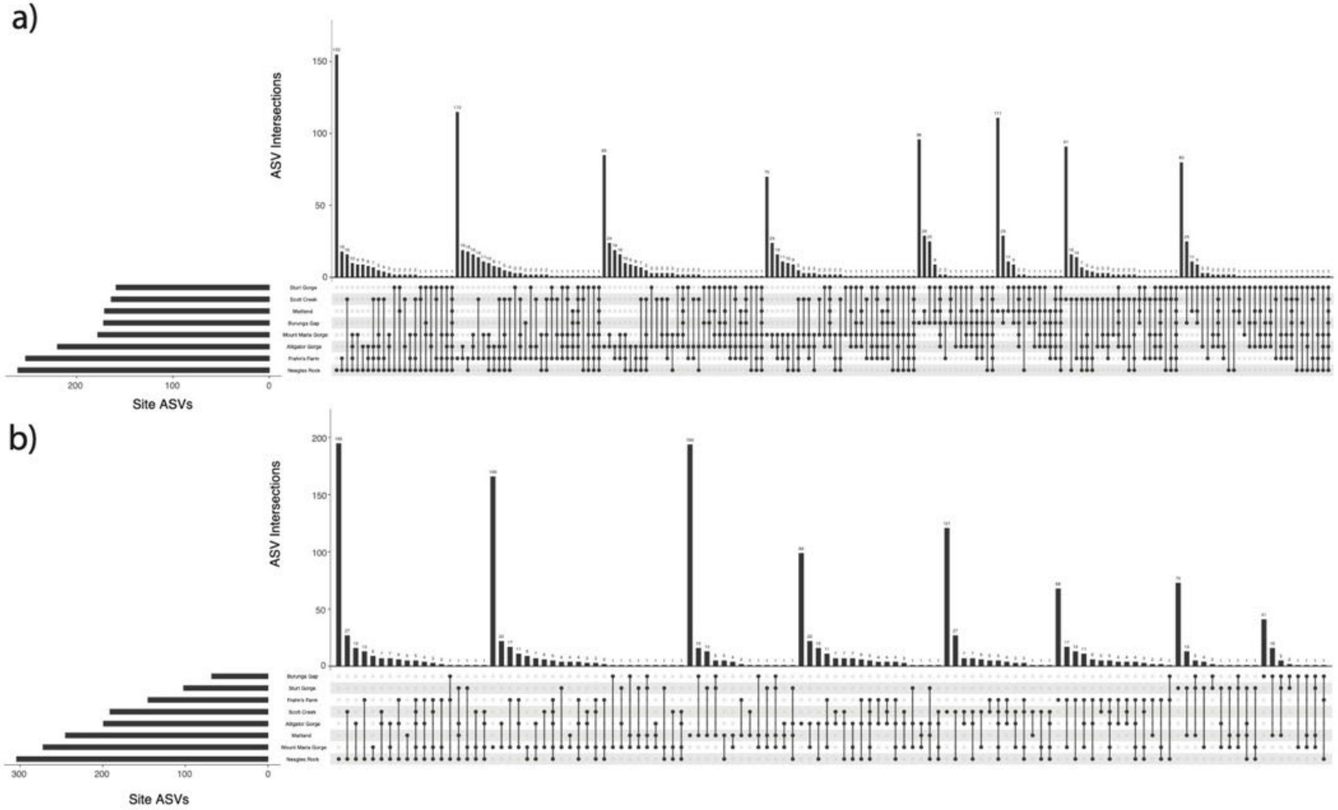

Figure S3.14: Upset plot showing the number of shared and unique bacterial ASVs across each sampling site that are differentially abundant with either a (a) negative and (b) positive log fold change in the rhizosphere relative to the endosphere. The order of the bars representing site intersections (overlapping ASVs between sites) are ordered first by the sites with the highest to lowest total number of ASVs (left panel bar), and then within sites by the groupings from highest to lowest intersection counts with other sites.

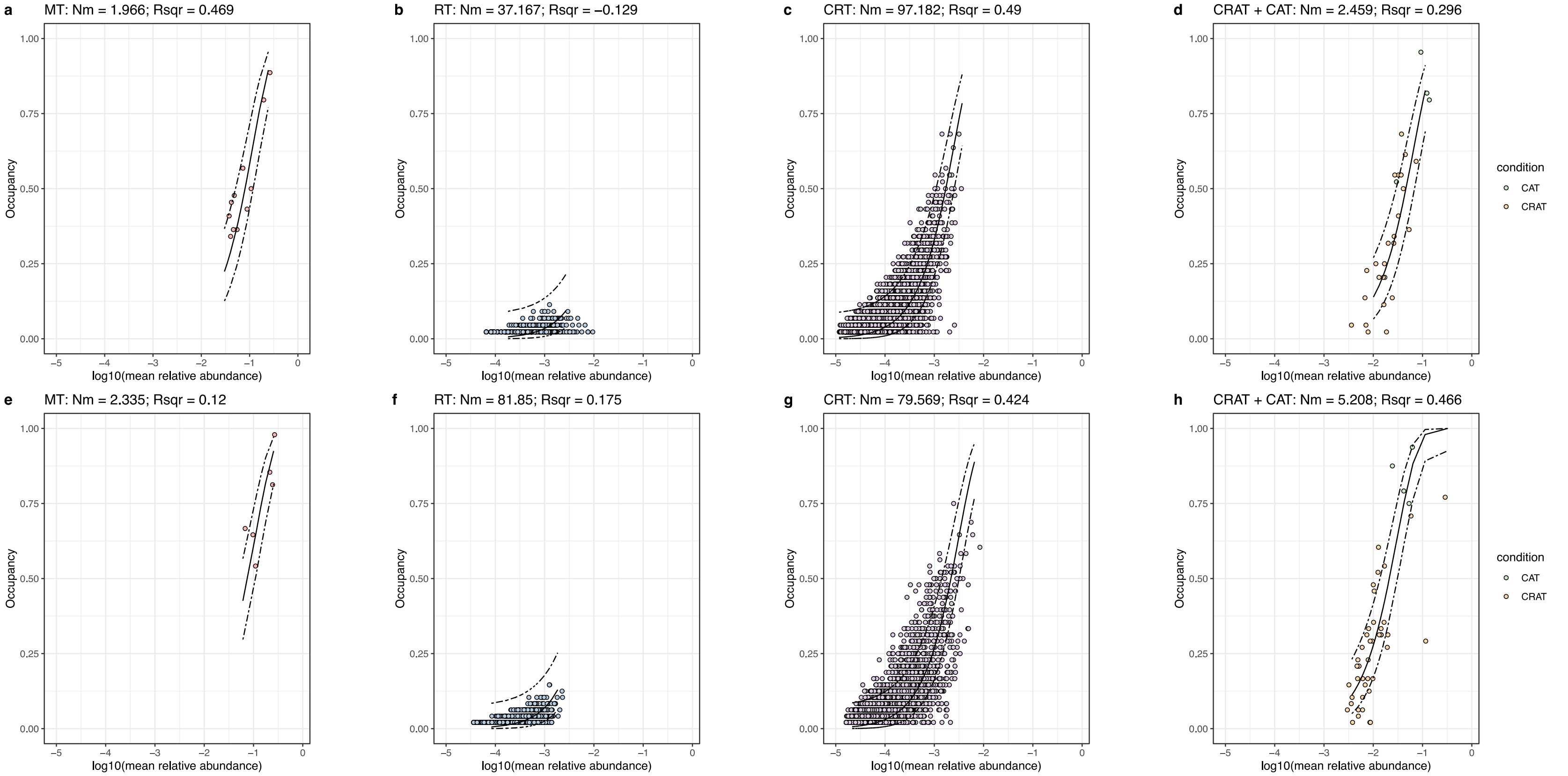

Figure S3.15. Abundance-occupancy curves fitted with Sloan neutral model in *T. triandra* (a-d) rhizospheres and (e-h) endospheres. Each point represents a bacterial ASV that was categorised as moderate taxa (MT, orange; panels a and e), rare taxa (RT, blue; panels b and f), conditionally rare taxa (CRT, pink; panels c and f), and the conditionally rare and abundant taxa plus the conditionally abundant taxa (CRAT+CAT, yellow and green, respectively; panels d and h), against a neutral model (black line) with 95% confidence intervals (dashed lines). The negative coefficient of determination for rare taxa (panel b) indicates failure to fit a model.

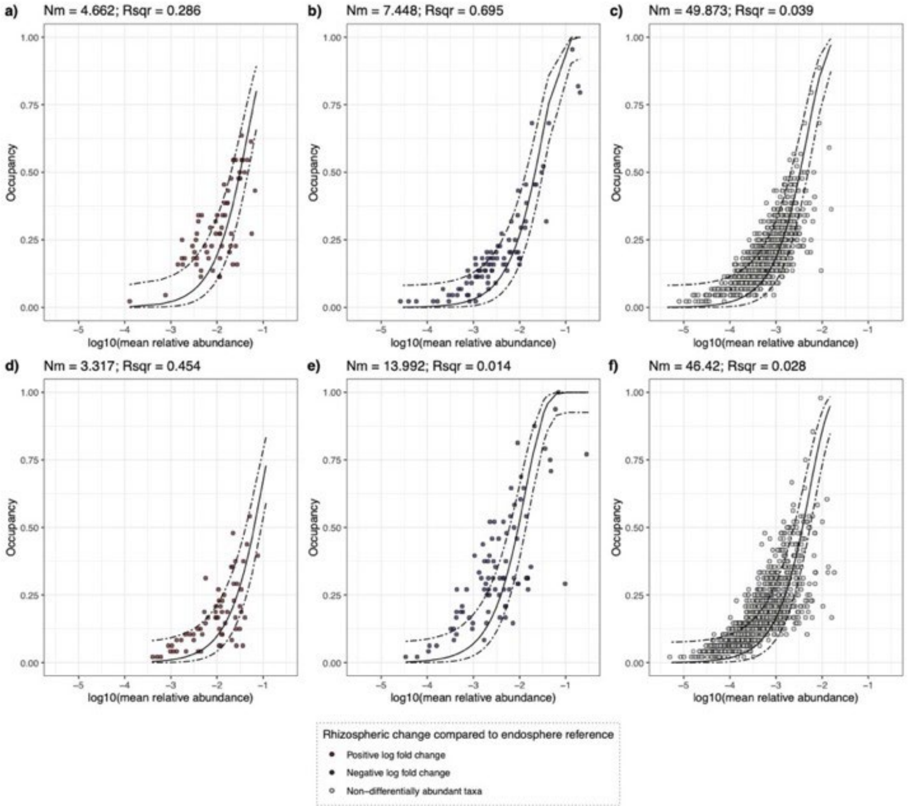

Figure S3.16. Abundance-occupancy curves fitted with Sloan neutral model in *T. triandra* (a-c) rhizosphere only samples and (d-f) endosphere only samples. Each point represents a bacterial ASV that was either differentially abundant with positive log fold change in the rhizosphere (rhizosphere-favoured, red; a and d), those with a negative log fold change compared to the rhizosphere (endosphere-favoured, blue; b and e), or those that were not differentially abundant (white; c and f). Each was plotted against a neutral model (black line) with 95% confidence intervals (dashed lines).

Figure S3.17. βNTI values for rhizospheres and endosphere within each site (See main document Figure 5c), but pooled for simpler interpretation. Aridity index values indicate the aridity level for each of the sampling sites (0.318-0.907), where low aridity index indicates drier conditions, and high aridity index indicates wetter conditions. Heterogeneous and homogeneous selection is attributed βNTI values of > +2 or < -2, respectively. Communities without significant βNTI values (|βNTI | <2) indicate the influences of stochastic processes on microbial community assembly.

Supplementary references

Barra, P. J., N. G. Inostroza, J. J. Acuña, M. L. Mora, D. E. Crowley, and M. A. Jorquera. 2016. Formulation of bacterial consortia from avocado (Persea americana Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Applied Soil Ecology **102**:80-91.

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. v. Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology **64**:807-838.

Richter-Heitmann, T., T. Eickhorst, S. Knauth, M. W. Friedrich, and H. Schmidt. 2016. Evaluation of Strategies to Separate Root-Associated Microbial Communities: A Crucial Choice in Rhizobiome Research. Frontiers in Microbiology **7**.

Urbina, H., M. F. Breed, W. Zhao, K. Lakshmi Gurrala, S. G. E. Andersson, J. Ågren, S. Baldauf, and A. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biology **122**:231-240.

Supplementary information: Chapter four

Supplementary methods

Vegetation surveys

At each study population (Figure S4.1a, e), *T. triandra* density estimates and vegetation assessments were taken alongside plant and soil samples within 25 m x 25 m quadrats. *T. triandra* density was measured from five 4 m x 4 m quadrats within the target area. To characterise vegetation at each population, we ran six point-intercept transects within our 25 x 25 m quadrats (Bonham 2013). Each transect was spaced 5 m apart in a North-South direction and involved observations of the occurrence of plant species found every meter. Functional categories for the vegetation were as follows: graminoids, herbs (forbs), shrubs, trees/canopy cover, litter, and bare earth (exposed dirt or rock). Where more than one functional unit occurred at a given point, all were recorded (Figure S4.1f).

Analysis of plant and soil physicochemical conditions

We collected *T. triandra* leaf samples and analysed them for nutrient concentrations, as were the bulk soil samples that were collected at 30 cm from the base of these plants, along with other physicochemical conditions. Soil physicochemical conditions at 30 cm from host included: phosphorus and potassium (Colwell 1965), sulphur (KCl 40 method) (Blair et al. 1991), organic carbon (Walkley and Armstrong 1934), nitrate, ammonium, electrical conductivity and pH ($CaCl_2$) at CSBP Laboratories (Bibra Lake, Australia), in addition to mean gravimetric water content (McPherson et al. 2018), which correlated with mean aridity index values across sampling sites (Figure S4.4). Nutrient analysis in *T. triandra* root and leaf samples were also conducted at CSBP Laboratories (Bibra Lake, Australia) using inductively coupled plasma (ICP) spectroscopy to measure trace elements and macronutrients within the plant, including: nitrogen, phosphorus, potassium, sulphur, copper, zinc, manganese, calcium, magnesium, sodium, iron, and boron.

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Supplementary tables

Table S4.1. *T. triandra* sampling sites across southern Australia

Site name	Latitude, longitude	Aridity index	Sampling date
Alligator Gorge	-32.71487, 138.10172	0.4450	15 Dec 2021
Barlunga Gap	-33.82, 138.17392	0.3469	14 Dec 2021
Frahn's Farm	-35.07231, 139.09781	0.4539	19 Dec 2021
Maitland	-34.37366, 137.71203	0.4532	21 Dec 2021
Mount Maria	-32.65862, 138.08985	0.3179	16 Dec 2021
Neagles Rock	-33.85031, 138.60674	0.6507	14 Dec 2021
Scott Creek	-35.0872, 138.67266	0.9031	19 Dec 2021
Sturt Gorge	-35.03311, 138.57324	0.6345	13 Dec 2021

Table S4.2. Upper and lower 95% confidence intervals for linear mixed-effect model estimates of the slope coefficient denoting a trend relationship between functional subsystems (level 1) of *Themeda triandra* soil/root microbiomes (i.e., see response variables listed) and mean annual aridity index, and the coefficient of determination (R^2), following bootstrapping at 2000 permutations. Subsystem denotes the functional process at subsystem level 1: motility = motility and chemotaxis, stress = stress response, nitrogen = nitrogen metabolism, phosphorus = phosphorus metabolism, signal = regulation and cell signalling, and secondary metabolism. Compartment refers to whether these associations were tested with functions in *T. triandra* endospheres, rhizosphere, or bulk soils. Trend denotes the significant directional relationship in response to increasing aridity index (where high aridity index corresponds to increasingly wetter conditions, and low aridity index reflects drier conditions; i.e., a positive trend means the response variable increases with wetter conditions).

Response	**Subsystem**	**Compartment**	**R^2: Lower confidence interval**	**R^2: Upper confidence interval**	**Estimate: Lower confidence interval**	**Estimate: Upper confidence interval**	**Trend**
Beta diversity ~ (Bray-Curtis distances)	motility	endosphere	0.0000	0.0268	-0.1137	0.035	None
	motility	rhizosphere	0.0019	0.5947	0.0048	0.1568	Positive
	motility	soil	0.0004	0.3187	-0.1825	0.0221	None
	stress	endosphere	0.0000	0.1435	-0.1044	0.0107	None
	stress	rhizosphere	0.0886	0.7645	0.0541	0.2494	Positive
	stress	soil	0.0001	0.2397	-0.1106	0.0366	None
	nitrogen	endosphere	0.000	0.074	-0.0676	0.0304	None

	nitrogen	rhizosphere	0.0226	0.6326	0.0357	0.1975	Positive
	nitrogen	soil	0.0000	0.1274	-0.1044	0.0702	None
	phosphorus	endosphere	0.0000	0.0754	-0.0203	0.0454	None
	phosphorus	rhizosphere	0.0616	0.6841	0.0394	0.1543	Positive
	phosphorus	soil	0.0016	0.3217	-0.1486	-0.0013	Negative
	signal	endosphere	0.0000	0.0906	-0.1262	0.0244	None
	signal	rhizosphere	0.0375	0.7237	0.0435	0.2314	Positive
	signal	soil	0.0002	0.2976	-0.1403	0.0111	
	secondary metabolism	endosphere	0.0000	0.0812	-0.1247	0.0849	None
	secondary metabolism	rhizosphere	0.0857	0.7320	0.0641	0.2989	Positive
	secondary metabolism	soil	0.0015	0.3437	-0.1611	-0.0039	Negative
Alpha diversity ~ (Richness of functions)			0.0004	0.2936			None
	motility	endosphere			-7.55	76.04	
	motility	rhizosphere	0.0000	0.1980	-34.07	73.07	None
	motility	soil	0.0000	0.0171	-89.77	54.368	None
	stress	endosphere	0.0002	0.2939	-48	266.6	None
	stress	rhizosphere	0.0003	0.3262	-36.9	365.3	None
	stress	soil	0.0000	0.0590	-355.3	193.46	None
	nitrogen	endosphere	0.0001	0.2187	-23.1	74.96	None
	nitrogen	rhizosphere	0.0001	0.2683	-26.32	96.88	None

	nitrogen	soil	0.0000	0.0635	-97.58	54.87	None
	phosphorus	endosphere	0.0010	0.3479	-1.47	60.46	None
	phosphorus	rhizosphere	0.0001	0.2478	-15.29	65.22	None
	phosphorus	soil	0.0000	0.0285	-59.403	38.683	None
	signal	endosphere	0.0002	0.2858	-33.25	184.55	None
	signal	rhizosphere	0.0008	0.3168	-8.4	262.3	None
	signal	soil	0.0000	0.0402	-14.39	26.77	None
	secondary metabolism	endosphere	0.0000	0.0000	-23.9613	17.9999	None
	secondary metabolism	rhizosphere	0.0002	0.3272	-2.79	52.21	None
	secondary metabolism	soil	0.0001	0.1970	-61.51	18.56	None
Alpha diversity ~ (Effective no. functions)			0.0000	0. 3322			
	motility	endosphere			-13.187	6.722	None
	motility	rhizosphere	0.0024	0.5325	-30.82	-1.36	Negative
	motility	soil	0.0000	0.0912	-7.532	9.931	None
	stress	endosphere	0.0000	0.0022	-18.333	25.395	None
	stress	rhizosphere	0.0036	0.5750	-88.04	-1.14	Negative
	stress	soil	0.0258	0.4823	14.28	63.76	Positive
	nitrogen	endosphere	0.0052	0.2738	1.646	12.909	Positive
	nitrogen	rhizosphere	0.0101	0.6302	-25.79	-2.81	Negative
	nitrogen	soil	0.0000	0.0635	-6.916	9.843	None

	phosphorus	endosphere	0.0401	0.4046	3.225	9.818	Positive
	phosphorus	rhizosphere	0.0018	0.5681	-15.259	0.534	None
	phosphorus	soil	0.0745	0.4982	3.661	11.959	Positive
	signal	endosphere	0.0002	0.3477	-22	0.08	None
	signal	rhizosphere	0.0008	0.4691	-41.71	3.78	None
	signal	soil	0.0000	0.3084	-211.14	130.3	None
	secondary metabolism	endosphere	0.0000	0.0388	-12.167	9.179	None
	secondary metabolism	rhizosphere	0.0015	0.4358	-0.282	6.03	None
	secondary metabolism	soil	0.0438	0.6264	1.236	4.968	Positive
Functional gene relative abundance (%) ~			0.0122	0.3092			
	motility	endosphere			0.1525	0.7833	Positive
	motility	rhizosphere	0.0004	0.2945	-0.7116	0.0611	None
	motility	soil	0.0105	0.4205	0.1665	1.1414	Positive
	stress	endosphere	0.0005	0.2292	-0.2662	-0.0076	Negative
	stress	rhizosphere	0.0000	0.0006	-0.3769	0.6685	None
	stress	soil	0.0716	0.4520	0.665	1.97	Positive
	nitrogen	endosphere	0.0000	0.1055	-0.1795	0.0757	None
	nitrogen	rhizosphere	0.0012	0.5083	-0.4657	0.0436	None
	nitrogen	soil	0.0001	0.3014	-0.124	0.3465	None
	phosphorus	endosphere	0.0001	0.2348	-0.1369	0.0296	None

phosphorus	rhizosphere	0.0000	0.0279	-0.2017	0.1573	None
phosphorus	soil	0.0000	0.0062	-0.1209	0.1447	None
signal	endosphere	0.0000	0.0005	-0.1899	0.1518	None
signal	rhizosphere	0.000	0.111	-0.1227	0.3295	None
signal	soil	0.0392	0.4837	0.154	0.6785	Positive
secondary metabolism	endosphere	0.0000	0.0278	-0.0564	0.1011	None
secondary metabolism	rhizosphere	0.0041	0.6476	-0.1138	-0.0102	Negative
secondary metabolism	soil	0.0017	0.3213	-0.1121	-0.0061	Negative

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Table S4.3. Upper and lower 95% confidence intervals for linear mixed effect model estimates of the slope coefficient denoting a trend between *Themeda triandra* soil/root bacterial taxonomic beta diversity (Bray-Curtis distances), R^2, and mean annual aridity index following bootstrapping at 2000 permutations. Compartment refers to whether these associations were tested in *T. triandra* endospheres, rhizospheres, or bulk soils. Trend denotes the directional relationship in response to increasing aridity index (where high aridity index corresponds to increasingly wetter conditions, and low aridity index reflects drier conditions, i.e., a positive trend means the response variable increases with wetter conditions).

Response	**Compartment**	**R2: Lower confidence interval**	**R2: Upper confidence interval**	**Estimate: Lower confidence interval**	**Estimate: Upper confidence interval**	**Trend**
Beta diversity ~ (Bray-Curtis distances)	Endosphere	0.0454	0.3003	-0.1570	-0.0375	Negative
	Rhizosphere	0.0044	0.6779	0.0472	0.3420	Positive
	Soil	0.0000	0.0714	-0.1229	0.1252	None

Table S4.4. Explanatory variables included in canonical correspondence analysis (CCA) across *T. triandra* bulk soils, rhizospheres and endospheres.

Model variables	**Description**
Aboveground biomass	Aboveground biomass of the host plant that each sample attributed to (bulk soil, rhizosphere or endosphere)
Aridity	Mean annual aridity index for each sample site, Atlas of Living Australia (Belbin 2011, ALA 2014)
Functional vegetation,	Relative abundance at each site for graminoids, herbs, shrubs, trees/canopy cover, litter, and bare soil.
Latitude	Latitude coordinates for each sampling site
Longitude	Longitude coordinates for each sampling site
Physicochemical measurements (for bulk soil analyses only)	Ammonium nitrogen, nitrate, phosphorus, potassium, sulphur, organic carbon, electrical conductivity and pH ($CaCl_2$) contained in the soil sampled at either 2 m or 30 cm from host plants
Themeda triandra site-density	Values pertaining to the density of *T. triandra* individuals for each sampling site
Trace elements and macronutrients (for rhizosphere analyses only)	Boron, calcium, copper, iron, magnesium, manganese, Phosphorus, potassium, sodium, sulphur, total nitrogen, and zinc contained in the leaf tissue in sampled host plants

Table S4.5. Sample sequencing information for taxonomic and functional annotation. Reads shows taxonomic and functional library sizes. The number of unique species and functions are also represented. All values reported are after data processing (i.e., removal of non-representative taxa, and quality control filtering).

Sample ID	Compartment	Site	Plant ID	Aridity index	Reads (taxonomy)	Unique Species (bacteria)	Reads (functions)	Unique functions
EA2	endosphere	Sturt Gorge	A2	0.6344784	148,881	7,546	168,228	11,712
EA3	endosphere	Sturt Gorge	A3	0.6344784	197,097	7,800	228,762	12,622
EA4	endosphere	Sturt Gorge	A4	0.6344784	176,143	7,958	205,948	12,399
EA5	endosphere	Sturt Gorge	A5	0.6344784	1,295,071	9,157	1,339,565	18,345
EA6	endosphere	Sturt Gorge	A6	0.6344784	1,544,200	9,061	1,603,085	18,441
EB1	endosphere	Barunga Gap	B1	0.3468773	70,931	5,721	56,953	7,899
EB2	endosphere	Barunga Gap	B2	0.3468773	264,316	7,959	274,509	12,865
EB3	endosphere	Barunga Gap	B3	0.3468773	71,402	7,111	65,065	8,658
EB5	endosphere	Barunga Gap	B5	0.3468773	384,699	8,086	401,163	14,189
EB6	endosphere	Barunga Gap	B6	0.3468773	231,724	7,951	260,666	13,059
EC1	endosphere	Neagles Rock	C1	0.6507292	22,793	5,744	26,975	6,580
EC2	endosphere	Neagles Rock	C2	0.6507292	409,149	8,641	550,736	15,406
EC3	endosphere	Neagles Rock	C3	0.6507292	98,305	7,325	117,101	10,836
EC4	endosphere	Neagles Rock	C4	0.6507292	365,726	9,084	583,958	14,859

ED2	endosphere	Alligator Gorge	D2	0.4450030	141,325	7,319	160,209	11,887
ED3	endosphere	Alligator Gorge	D3	0.4450030	137,210	7,252	158,492	11,229
ED4	endosphere	Alligator Gorge	D4	0.4450030	187,026	7,520	203,397	12,400
ED5	endosphere	Alligator Gorge	D5	0.4450030	229,766	7,801	245,451	12,923
ED6	endosphere	Alligator Gorge	D6	0.4450030	432,808	7,878	488,464	14,716
EE1	endosphere	Mount Maria	E1	0.2927616	820,531	8,740	888,789	16,588
EE2	endosphere	Mount Maria	E2	0.2927616	327,595	8,250	353,716	14,114
EE3	endosphere	Mount Maria	E3	0.2927616	269,652	7,878	294,926	13,110
EE4	endosphere	Mount Maria	E4	0.2927616	324,325	7,891	372,015	14,052
EE5	endosphere	Mount Maria	E5	0.2927616	238,982	7,555	271,555	12,827
EE6	endosphere	Mount Maria	E6	0.2927616	129,403	7,697	141,205	11,447
EF1	endosphere	Frahns Farm	F1	0.4539200	234,588	7,727	277,724	13,685
EF2	endosphere	Frahns Farm	F2	0.4539200	227,154	7,779	264,451	13,021
EF3	endosphere	Frahns Farm	F3	0.4539200	353,919	8,040	408,296	14,239
EF4	endosphere	Frahns Farm	F4	0.4539200	240,658	7,738	263,158	13,115
EF5	endosphere	Frahns Farm	F5	0.4539200	227,168	7,773	263,060	13,215
EF6	endosphere	Frahns Farm	F6	0.4539200	148,847	7,436	164,995	11,526
EG1	endosphere	Scott Creek	G1	0.9030759	564,358	8,430	737,550	15,364

EG2	endosphere	Scott Creek	G2	0.9030759	566,530	8,708	772,271	16,035
EG3	endosphere	Scott Creek	G3	0.9030759	358,633	8,387	464,984	14,137
EG4	endosphere	Scott Creek	G4	0.9030759	125,461	6,958	156,709	11,062
EG5	endosphere	Scott Creek	G5	0.9030759	174,958	8,618	218,455	12,880
EG6	endosphere	Scott Creek	G6	0.9030759	767,711	8,789	1,009,624	16,987
EH1	endosphere	Maitland	H1	0.4532474	1,171,644	9,050	1,302,793	17,825
EH2	endosphere	Maitland	H2	0.4532474	1,496,065	8,884	1,552,796	17,979
EH3	endosphere	Maitland	H3	0.4532474	505,697	8,154	541,837	14,978
EH4	endosphere	Maitland	H4	0.4532474	370,951	7,981	408,061	14,540
EH5	endosphere	Maitland	H5	0.4532474	216,793	6,791	215,328	12,081
EH6	endosphere	Maitland	H6	0.4532474	432,286	8,127	464,166	14,225
RA1	rhizosphere	Sturt Gorge	A1	0.6344784	979,435	8,865	124,7696	17,327
RA2	rhizosphere	Sturt Gorge	A2	0.6344784	196,556	8,039	273,390	12,459
RA4	rhizosphere	Sturt Gorge	A4	0.6344784	169,331	8,467	328,812	12,010
RA5	rhizosphere	Sturt Gorge	A5	0.6344784	195,745	8,610	365,039	12,368
RA6	rhizosphere	Sturt Gorge	A6	0.6344784	186,873	8,595	336,609	11,891
RB1	rhizosphere	Barunga Gap	B1	0.3468773	112,966	8,035	212,035	10,655
RB2	rhizosphere	Barunga Gap	B2	0.3468773	183,869	8,551	349,185	12,025
RD1	rhizosphere	Alligator Gorge	D1	0.4450030	228,563	8,647	443,737	13,329
RD6	rhizosphere	Alligator Gorge	D6	0.4450030	145,898	8,539	305,006	11,544
RE1	rhizosphere	Mount Maria	E1	0.3178943	172,850	8,563	356,732	12,279

RE3	rhizosphere	Mount Maria	E3	0.3178943	110,858	8,410	237,807	10,897
RE4	rhizosphere	Mount Maria	E4	0.3178943	398,165	8,626	623,623	15,223
RF3	rhizosphere	Frahns Farm	F3	0.4539200	298,688	8,796	673,559	13,349
RF4	rhizosphere	Frahns Farm	F4	0.4539200	198,734	8,595	382,951	13,157
RF5	rhizosphere	Frahns Farm	F5	0.4539200	116,606	8,342	251,405	11,611
RG3	rhizosphere	Scott Creek	G3	0.9030759	497,08	7,466	118,077	8,851
RG4	rhizosphere	Scott Creek	G4	0.9030759	7,115	3,128	11,911	3,843
RG5	rhizosphere	Scott Creek	G5	0.9030759	12,214	4,362	23,651	5,246
RG6	rhizosphere	Scott Creek	G6	0.9030759	NA	NA	13,609	4,181
RH1	rhizosphere	Maitland	H1	0.4532474	19,271	4,986	44,488	6,050
RH3	rhizosphere	Maitland	H3	0.4532474	128,490	8,354	257,185	11,120
RH4	rhizosphere	Maitland	H4	0.4532474	179,149	8,240	398,595	10,286
RH6	rhizosphere	Maitland	H6	0.4532474	158,816	8,540	384,701	11,222
SA1	soil	Sturt Gorge	A1	0.6344784	119,443	8,500	264,693	10,268
SA2	soil	Sturt Gorge	A2	0.6344784	72,704	7,675	171,912	8,648
SA3	soil	Sturt Gorge	A3	0.6344784	87,519	8,126	190,795	9,474
SA4	soil	Sturt Gorge	A4	0.6344784	68,022	8,006	155,098	9,204
SA5	soil	Sturt Gorge	A5	0.6344784	137,506	8,640	292,297	10,950
SA6	soil	Sturt Gorge	A6	0.6344784	108,078	8,273	246,037	9,986
SB1	soil	Barunga Gap	B1	0.3468773	90,468	7,741	277,375	8,903

SB2	soil	Barunga Gap	B2	0.3468773	84,868	7,609	234,708	8,546
SB3	soil	Barunga Gap	B3	0.3468773	78,110	7,670	186,826	8,710
SB4	soil	Barunga Gap	B4	0.3468773	106,651	8,349	275,450	10,070
SB5	soil	Barunga Gap	B5	0.3468773	95,049	8,100	255,235	9,552
SB6	soil	Barunga Gap	B6	0.3468773	19,462	5,592	56,302	6,131
SC1	soil	Neagles Rock	C1	0.6507292	74,654	8,043	171,753	9,334
SC2	soil	Neagles Rock	C2	0.6507292	105,413	8,408	238,124	10,622
SC3	soil	Neagles Rock	C3	0.6507292	133,725	7,414	279,538	9,167
SC4	soil	Neagles Rock	C4	0.6507292	72,670	7,952	169,533	9,444
SC5	soil	Neagles Rock	C5	0.6507292	117,833	8,450	275,391	10,572
SC6	soil	Neagles Rock	C6	0.6507292	137,826	8657	315,805	11,432
SD1	soil	Alligator Gorge	D1	0.4450030	118,696	8,533	291,863	11,174
SD2	soil	Alligator Gorge	D2	0.4450030	146,304	8,709	380,494	11,880
SD3	soil	Alligator Gorge	D3	0.4450030	113,813	8,570	288,153	10,961
SD4	soil	Alligator Gorge	D4	0.4450030	166,267	8,671	354,156	12,263
SE3	soil	Mount Maria	E3	0.3178943	60,442	6,726	151,270	8,240
SF2	soil	Frahns Farm	F2	0.4539200	53,889	7,506	121,066	8,303
SF3	soil	Frahns Farm	F3	0.4539200	125,891	8,696	297,857	11,428

SF4	soil	Frahns Farm	F4	0.4539200	87,930	8,301	216,252	10,307
SF5	soil	Frahns Farm	F5	0.4539200	87,996	8,325	196,589	10,059
SG1	soil	Scott Creek	G1	0.9030759	61,711	6,388	111,736	7,574
SG2	soil	Scott Creek	G2	0.9030759	95,039	8,465	268,302	10,368
SG3	soil	Scott Creek	G3	0.9030759	110,860	8,474	308,153	11,551
SG4	soil	Scott Creek	G4	0.9030759	641,625	9,342	1,530,255	15,849
SG5	soil	Scott Creek	G5	0.9030759	629,842	9,368	1,417,307	15,966
SG6	soil	Scott Creek	G6	0.9030759	963,770	9,289	1,976,684	16,507
SH1	soil	Maitland	H1	0.4532474	175,366	8,800	421,075	12,147
SH2	soil	Maitland	H2	0.4532474	115,035	8,513	271,665	11,007
SH3	soil	Maitland	H3	0.4532474	107,227	7,534	264,682	9,185
SH4	soil	Maitland	H4	0.4532474	129,221	8,148	343,810	10,307
SH5	soil	Maitland	H5	0.4532474	109,460	8,397	275,408	10,378
SH6	soil	Maitland	H6	0.4532474	156,345	8,731	389,924	11,421

Table S4.6. Mean relative abundance of reads, and standard deviations (SD) attributed to six isolated functional categories at SEED subsystem level 1 across the bulk soils, rhizospheres, and endospheres of *T. triandra* plants.

Subsystem 1	Compartment	Mean relative abundance (%)	SD relative abundance (%)
Motility and Chemotaxis	endosphere	1.438	0.224
Motility and Chemotaxis	rhizosphere	1.375	0.263
Motility and Chemotaxis	soil	1.052	0.314
Nitrogen Metabolism	endosphere	1.235	0.084
Nitrogen Metabolism	rhizosphere	0.9	0.137
Nitrogen Metabolism	soil	0.677	0.122
Phosphorus Metabolism	endosphere	1.221	0.05
Phosphorus Metabolism	rhizosphere	1.302	0.095
Phosphorus Metabolism	soil	1.304	0.081
Regulation and Cell signalling	endosphere	1.99	0.081
Regulation and Cell signalling	rhizosphere	1.821	0.121
Regulation and Cell signalling	soil	1.655	0.155
Secondary Metabolism	endosphere	0.228	0.051
Secondary Metabolism	rhizosphere	0.207	0.029
Secondary Metabolism	soil	0.209	0.035
Stress Response	endosphere	4.188	0.092
Stress Response	rhizosphere	4.086	0.291
Stress Response	soil	3.67	0.469

Table S4.7. Full statistical output for taxonomic alpha diversity linear mixed effects models and subsequent pairwise comparisons.

Type	Test	Full model	Response variable	predictor	Random Effects	df	Test statistic type	Test statistic value	P-value	Significance
taxonomy alpha diversity	LMEM	Bacterial Richness ~ site + compartment + (1|plant_id)	Bacterial Richness	site	Plant_ID	7	χ2	11.3733	0.1231	NS
				compartment	Plant_ID	2	χ2	2.5627	0.2777	NS
taxonomy alpha diversity	LMEM	Bacterial richness ~ compartment + (1|plant_id)	Bacterial Richness	compartment	Plant_ID	2	χ2	2.9945	0.2237	NS
taxonomy alpha diversity	LMEM	Shannon diversity ~ site + compartment + (1|plant_id)	Shannon's diveristy	site	Plant_ID	7	χ2	19.999	0.005572	**
	Tukey	site pairwise comparisons		Barunga Gap - Alligator Gorge		-	z value	-2.993	0.0549	NS
				Frahns Farm - Alligator Gorge		-		0.133	1	NS
				Maitland - Alligator Gorge		-		-2.077	0.4273	NS
				Mount Maria - Alligator Gorge		-		-0.57	0.9992	NS

	Neagles Rock - Alligator Gorge	-	0.103	1	NS
	Scott Creek - Alligator Gorge	-	-1.681	0.6978	NS
	Sturt Gorge - Alligator Gorge	-	-1.878	0.5641	NS
	Frahns Farm - Barunga Gap	-	3.258	0.0247	*
	Maitland - Barunga Gap	-	1.104	0.9556	NS
	Mount Maria - Barunga Gap	-	2.285	0.3	NS
	Neagles Rock - Barunga Gap	-	3.013	0.0525	NS
	Scott Creek - Barunga Gap	-	1.477	0.8184	NS
	Sturt Gorge - Barunga Gap	-	1.303	0.8971	NS
	Maitland - Frahns Farm	-	-2.325	0.278	NS
	Mount Maria - Frahns Farm	-	-0.723	0.9963	NS
	Neagles Rock - Frahns Farm	-	-0.022	1	NS
	Scott Creek - Frahns Farm	-	-1.903	0.5461	NS

		Sturt Gorge - Frahns Farm		-		-2.117	0.4013	NS
		Mount Maria - Maitland		-		1.383	0.8642	NS
		Neagles Rock - Maitland		-		2.104	0.4095	NS
		Scott Creek - Maitland		-		0.408	0.9999	NS
		Sturt Gorge - Maitland		-		0.214	1	NS
		Neagles Rock - Mount Maria		-		0.644	0.9982	NS
		Scott Creek - Mount Maria		-		-1.008	0.9731	NS
		Sturt Gorge - Mount Maria		-		-1.195	0.9331	NS
		Scott Creek - Neagles Rock		-		-1.731	0.665	NS
		Sturt Gorge - Neagles Rock		-		-1.908	0.5431	NS
		Sturt Gorge - Scott Creek		-		-0.197	1	NS
LMEM		compartment	Plant_ID	2		92.283	2.20E-16	***
Tukey	compartment pairwise comparisons	rhizosphere - soil		-	z value	-2.529	0.0304	*
		endosphere - soil		-		-9.41	<0.001	***

				rhizosphere - endosphere		-		-5.379	<0.001	***
taxonomy alpha diversity	LMEM	Shannon ~ compartment + (1|plant_id)	Shannon's diveristy	compartment	Plant_ID	2	χ2	80.453	<0.001	***
	Tukey	compartment pairwise comparisons		rhizosphere - soil		-	z value	-2.551	0.0286	*
				endosphere - soil		-		-8.854	<0.001	***
				endosphere - rhizosphere		-		-4.858	<0.001	***
taxonomy alpha diversity	LMEM	effective no species ~ site + compartment + (1|plant_id)	Effective no. species	site	Plant_ID	7	χ2	22.448	0.002126	**
	Tukey	site pairwise comparisons		Barunga Gap - Alligator Gorge		-	z value	-3.2	0.0294	*
				Frahns Farm - Alligator Gorge		-		-0.027	1	NS
				Maitland - Alligator Gorge		-		-2.436	0.2215	NS
				Mount Maria - Alligator Gorge		-		-0.978	0.9773	NS
				Neagles Rock - Alligator Gorge		-		-0.319	1	NS

Scott Creek - Alligator Gorge	-	-1.865	0.5731	NS
Sturt Gorge - Alligator Gorge	-	-2.62	0.1468	NS
Frahns Farm - Barunga Gap	-	3.307	0.0211	*
Maitland - Barunga Gap	-	0.955	0.9803	NS
Mount Maria - Barunga Gap	-	2.063	0.4371	NS
Neagles Rock - Barunga Gap	-	2.773	0.1009	NS
Scott Creek - Barunga Gap	-	1.508	0.802	NS
Sturt Gorge - Barunga Gap	-	0.751	0.9953	NS
Maitland - Frahns Farm	-	-2.526	0.1833	NS
Mount Maria - Frahns Farm	-	-0.993	0.9753	NS
Neagles Rock - Frahns Farm	-	-0.304	1	NS
Scott Creek - Frahns Farm	-	-1.923	0.5319	NS
Sturt Gorge - Frahns Farm	-	-2.721	0.1151	NS

			Mount Maria - Maitland		-		1.287	0.903	NS
			Neagles Rock - Maitland		-		1.994	0.4843	NS
			Scott Creek - Maitland		-		0.597	0.9989	NS
			Sturt Gorge - Maitland		-		-0.213	1	NS
			Neagles Rock - Mount Maria		-		0.63	0.9985	NS
			Scott Creek - Mount Maria		-		-0.75	0.9953	NS
			Sturt Gorge - Mount Maria		-		-1.469	0.8226	NS
			Scott Creek - Neagles Rock		-		-1.457	0.8292	NS
			Sturt Gorge - Neagles Rock		-		-2.166	0.3701	NS
			Sturt Gorge - Scott Creek		-		-0.804	0.9929	NS
LMEM		Effective no. species	compartment	Plant_ID	2	χ2	109.074	<0.001	***
Tukey	compartment pairwise comparisons		rhizosphere - endosphere		-	z value	5.49	<0.001	***
			soil - endosphere		-		10.308	<0.001	***

				soil - rhizosphere	-			3.157	0.00455	**
taxonomy alpha diversity	LMEM	effective no species ~ compartment + (1|plant_id)	Effective no. species	compartment	Plant_ID	2	χ2	95.27	<0.001	***
	Tukey	compartment pairwise comparisons		rhizosphere - endosphere	-		z value	4.884	<0.001	***
				soil - endosphere	-			9.698	<0.001	***
				soil - rhizosphere	-			3.206	0.00368	**
taxonomy alpha diversity	LMEM	Pielou's evenness ~ site + compartment + (1|plant_id)	Pielou's evenness index	site	Plant_ID	7	χ2	12.744	0.0786	NS
				compartment	Plant_ID	2	χ2	73.731	<0.001	***
	Tukey	compartment pairwise comparisons		rhizosphere - endosphere	-		z value	5.056	<0.001	***
				soil - endosphere	-			8.345	<0.001	***
				soil - rhizosphere	-			1.966	0.12	
taxonomy alpha diversity	LMEM	Pielou's evenness ~ site + compartment + (1|plant_id)	Pielou's evenness index	compartment	Plant_ID	2	χ2	68.538	<0.001	***
	Tukey	compartment pairwise comparisons		rhizosphere - endosphere	-		z value	4.784	<0.001	***
				soil - endosphere	-			8.108	<0.001	***

				soil - rhizosphere		-		2.022	0.106	NS
taxonomy alpha diversity	LMEM	Bacterial richness ~ Aridity index + (1|plant_id)	Bacterial Richness	Aridity index	Plant_ID	1	χ2	1.667	0.1967	NS
taxonomy alpha diversity	LMEM	Shannons diversity ~ Aridity index + (1|plant_id)	Shannon's diveristy	Aridity index	Plant_ID	1	χ2	0.2774	0.5984	NS
taxonomy alpha diversity	LMEM	Pielou's evenness~ Aridity index + (1|plant_id)	Pielou's evenness	Aridity index	Plant_ID	1	χ2	0.2448	0.6207	NS

Table S4.8. Beta diversity analysis output showing the effects of plant compartment (bulk soil, rhizosphere and endosphere) on bacterial community compositions using PERMANOVAS via the *adonis2* function in the R package Vegan.

Response variable	Statistical test	Predictor variable	Degrees of freedom	F-statistic	R squared value	P value
Bacterial taxonomy	PERMANOVA	~Compartment	2 and 101	55.96	0.53	<0.001***
Bacterial taxonomy	PERMANOVA	~Aridity index	1 and 102	3.53	0.03	0.027*
Bacterial taxonomy	PERMANOVA	~Sampling site	7 and 96	2.73	0.17	0.002**
Microbial functions	PERMANOVA	~Compartment	2 and 102	45.73	0.47	<0.001***
Microbial functions	PERMANOVA	~Aridity index	2 and 102	4.61	0.04	0.008**

Table S4.9. Full statistical output for distance to centroid estimates for taxonomic and functional beta diversity

Response variable	Statistical test	Permutations	Predictor variable	Degrees of freedom	F-statistic	P value
Distance to centroid - taxonomic beta diversity~	Permutation test for homogeneity of multivariate dispersions	999	Compartment	2 and 102	4.7072	0.015
	Pairwise compartment test		Rhizosphere-Endosphere			0.0090047
	Pairwise compartment test		Soil-Endosphere			0.7666314
	Pairwise compartment test		Soil-Rhizosphere			0.0488275
Distance to centroid - functional beta diversity~	Permutation test for homogeneity of multivariate dispersions	999	Compartment	2 and 102	14.647	<0.001
	Pairwise compartment test		Rhizosphere-Endosphere			0.0004887
	Pairwise compartment test		Soil-Endosphere			0.0000068
	Pairwise compartment test		Soil-Rhizosphere			0.9255204

Table S4.10. Full statistical output for functional alpha diversity linear mixed effects models and subsequent pairwise comparisons

Type	Test	Full model	Response variable	predictor	Random Effects	df	Test statistic type	Test statistic value	P-value	Significance
functional alpha diversity	LMEM	Functional richness ~ site + compartment + (1|plant_id)	Functional richness	site	Plant_ID	7	χ2	2.6844	0.912583	NS
			Functional richness	compartment	Plant_ID	2	χ2	10.0471	0.006581	**
	Tukey	compartment pairwise comparisons		rhizosphere - endosphere		-	z value	2.887	0.0107	*
				soil - endosphere		-		2.328	0.0515	NS
				soil - rhizosphere		-		-0.865	0.661	NS
functional alpha diversity	LMEM	Functional richness ~ compartment + (1|plant_id)	Functional richness	compartment	Plant_ID	2	χ2	10.3	0.005799	**
	Tukey	compartment pairwise comparisons		rhizosphere - endosphere		-	z value	2.84	0.0124	*
				soil - endosphere		-		2.519	0.0315	*
				soil - rhizosphere		-		-0.676	0.7765	NS
functional alpha diversity	LMEM	Shannon's diversity ~ site + compartment + (1|plant_id)	Shannon's diversity	site	Plant_ID	7	χ2	30.992	<0.001	***

Tukey	site pairwise comparisons	Barunga Gap - Alligator Gorge	-	z value	-4.372	<0.001	***
		Frahns Farm - Alligator Gorge	-		-0.264	1	NS
		Maitland - Alligator Gorge	-		-2.465	0.2095	NS
		Mount Maria - Alligator Gorge	-		-0.927	0.9833	NS
		Neagles Rock - Alligator Gorge	-		-1.583	0.7591	NS
		Scott Creek - Alligator Gorge	-		-2.028	0.4605	NS
		Sturt Gorge - Alligator Gorge	-		-0.498	0.9997	NS
		Frahns Farm - Barunga Gap	-		4.281	<0.001	***
		Maitland - Barunga Gap	-		2.209	0.3439	NS

Mount Maria - Barunga Gap	-	3.242	0.0255	*
Neagles Rock - Barunga Gap	-	2.595	0.1565	NS
Scott Creek - Barunga Gap	-	2.667	0.1315	NS
Sturt Gorge - Barunga Gap	-	4.256	<0.001	***
Maitland - Frahns Farm	-	-2.296	0.2938	NS
Mount Maria - Frahns Farm	-	-0.709	0.9967	NS
Neagles Rock - Frahns Farm	-	-1.381	0.865	NS
Scott Creek - Frahns Farm	-	-1.837	0.592	NS
Sturt Gorge - Frahns Farm	-	-0.233	1	NS
Mount Maria - Maitland	-	1.371	0.8695	NS
Neagles Rock - Maitland	-	0.66	0.9979	NS
Scott Creek - Maitland	-	0.485	0.9997	NS

				Sturt Gorge - Maitland		-		2.178	0.3625	NS
				Neagles Rock - Mount Maria		-		-0.627	0.9985	NS
				Scott Creek - Mount Maria		-		-0.95	0.9808	NS
				Sturt Gorge - Mount Maria		-		0.521	0.9996	NS
				Scott Creek - Neagles Rock		-		-0.24	1	NS
				Sturt Gorge - Neagles Rock		-		1.221	0.9251	NS
				Sturt Gorge - Scott Creek		-		1.694	0.6894	NS
	LMEM		Shannon's diversity	compartment	Plant_ID	2	χ2	182.969	<0.001	***
	Tukey	compartment pairwise comparisons		rhizosphere - endosphere		-	z value	-8.463	<0.001	***
				soil - endosphere		-		-13.013	<0.001	***
				soil - rhizosphere		-		-2.663	0.0207	*
functional alpha diversity	LMEM	Shannon's diversity ~ compartment + (1|plant_id)	Shannon's diversity	compartment	Plant_ID	2	χ2	159.95	<0.001	***

	Tukey			rhizosphere - endosphere		-	z value	-7.576	<0.001	***
				soil - endosphere		-		-12.334	<0.001	***
				soil - rhizosphere		-		-2.93	0.00959	**
functional alpha diversity	LMEM	Effective no. functions ~ site + compartment + (1|plant_id)	Effective no. functions	site	Plant_ID	7	χ2	31.449	<0.001	***
	TUkey	site pairwise comparisons		Barunga Gap - Alligator Gorge		-	z value	-4.376	<0.001	***
				Frahns Farm - Alligator Gorge		-		-0.282	1	NS
				Maitland - Alligator Gorge		-		-2.548	0.1741	NS
				Mount Maria - Alligator Gorge		-		-0.845	0.9903	NS
				Neagles Rock - Alligator Gorge		-		-1.772	0.6368	NS
				Scott Creek - Alligator Gorge		-		-1.976	0.4959	NS

Sturt Gorge - Alligator Gorge	-	-0.502	0.9997	NS
Frahns Farm - Barunga Gap	-	4.267	<0.001	***
Maitland - Barunga Gap	-	2.126	0.3953	NS
Mount Maria - Barunga Gap	-	3.329	0.0196	*
Neagles Rock - Barunga Gap	-	2.401	0.2389	NS
Scott Creek - Barunga Gap	-	2.726	0.1136	NS
Sturt Gorge - Barunga Gap	-	4.257	<0.001	***
Maitland - Frahns Farm	-	-2.364	0.258	NS
Mount Maria - Frahns Farm	-	-0.607	0.9988	NS
Neagles Rock - Frahns Farm	-	-1.559	0.7729	NS
Scott Creek - Frahns Farm	-	-1.764	0.6427	NS

	Sturt Gorge - Frahns Farm		-		-0.218	1	NS
	Mount Maria - Maitland		-		1.539	0.7845	NS
	Neagles Rock - Maitland		-		0.536	0.9995	NS
	Scott Creek - Maitland		-		0.635	0.9984	NS
	Sturt Gorge - Maitland		-		2.266	0.3105	NS
	Neagles Rock - Mount Maria		-		-0.887	0.9872	NS
	Scott Creek - Mount Maria		-		-0.989	0.9759	NS
	Sturt Gorge - Mount Maria		-		0.429	0.9999	NS
	Scott Creek - Neagles Rock		-		0.014	1	NS
	Sturt Gorge - Neagles Rock		-		1.421	0.8467	NS
	Sturt Gorge - Scott Creek		-		1.632	0.7287	NS
Effective no. functions	compartment	Plant_ID	2	χ2	218.189	<0.001	***

		compartment pairwise comparisons		rhizosphere - endosphere		-	z value	-9.664	<0.001	***
				soil - endosphere		-		-14.64	<0.001	***
				soil - rhizosphere		-		-2.858	0.0118	*
functional alpha diversity	LMEM	Effective no. functions ~ compartment + (1|plant_id)	Effective no. functions	compartment	Plant_ID	2	χ2	202.35	<0.001	***
						-	z value	-8.632	<0.001	***
						-		-13.841	<0.001	***
						-		-3.16	0.00455	**
functional alpha diversity	LMEM	Pielou's evenness ~ site + compartment + (1|plant_id)	Pielou's evenness	site	Plant_ID	7	χ2	4.0765	0.7709	NS
				compartment	Plant_ID	2	χ2	40.3641	<0.001	***
		compartment pairwise comparisons		rhizosphere - endosphere		-	z value	-4.844	<0.001	***
				soil - endosphere		-		-5.672	<0.001	***
				soil - rhizosphere		-		-0.045	0.999	NS
functional alpha diversity	LMEM	Pielou's evenness ~ compartment + (1|plant_id)	Pielou's evenness	compartment	Plant_ID	2	χ2	159.95	<0.001	***
		compartment pairwise comparisons		rhizosphere - endosphere		-	z value	-7.576	<0.001	***

				soil - endosphere		-			12.334	<0.001	***
				soil - rhizosphere		-			-2.93	0.00928	**
functional alpha diversity	LMEM	Functional richness ~ Aridity index + (1|plant_id)	Functional Richness	Aridity index	Plant_ID		1	χ2	1.367	0.2423	NS
functional alpha diversity	LMEM	Shannons diversity ~ Aridity index + (1|plant_id)	Shannon's diveristy	Aridity index	Plant_ID		1	χ2	0.0817	0.775	NS
functional alpha diversity	LMEM	Effective no. functions ~ Aridity index + (1|plant_id)	Effective no. functions	Aridity index	Plant_ID		1	χ2	0.1165	0.7328	NS
functional alpha diversity	LMEM	Pielou's evenness~ Aridity index + (1|plant_id)	Pielou's evenness	Aridity index	Plant_ID		1	χ2	0.4785	0.4891	NS

Table S4.11. Statistical output for linear mixed effects model (LMEM) comparing bacterial alpha diversity by functional alpha diversity.

Test	Full model	Response variable	Predictor variable	Random Effects	df	Test statistic type	Test statistic value	P-value	Significance
LMEM	Effective no. functions ~ Effective no. species + compartment + (1|compartment)	Effective no. functions	Effective no. species	Compartment	1	χ2	29.0432	<0.001	***
			Compartment	Compartment	2	χ2	5.3444	0.0691	NS

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Supplementary information: Chapter four

Table S4.12. Log fold change (LFC) of differentially abundant bacterial phyla across bulk soils, rhizospheres and endospheres. Significance value is indicated by '*' for $p < 0.05$, '**' for $p < 0.01$, and '***' for $p < 0.001$. NS indicates non-significant taxa within a given comparison.

Bacterial Phylum	LFC Rhizosphere vs. Endosphere	LFC Soil vs. Endosphere	LFC Soil vs. Rhizosphere
Coprothermobacterota	0.479 NS	0.824***	0.345 NS
Chrysiogenota	0.453*	0.482**	0.029 NS
Caldisericota	0.869**	1.8***	0.931**
Nitrospinota	0.609**	0.203 NS	-0.407*
Atribacterota	1.151***	1.915***	0.764*
Elusimicrobiota	0.71**	1.496***	0.786**
Dictyoglomota	0.795*	1.783***	0.987**
Calditrichota	1.085***	1.292***	0.207 NS
Aquificota	0.694***	1.355***	0.661***
Deferribacterota	0.755**	1.481***	0.726**
Candidatus_Bipolaricaulota	0.082 NS	-0.906***	-0.988***
Candidatus_Fervidibacteria	0.854***	0.981***	0.127 NS
Candidatus_Absconditabacteria	0.756*	1.565***	0.809*
Candidatus_Saccharibacteria	1.04**	1.055***	0.015 NS
Bdellovibrionota	0.699**	1.134***	0.435*
Thermotogota	0.818***	1.515***	0.697**
Nitrospirota	0.834***	0.798***	-0.036 NS
Fusobacteriota	0.465*	1.387***	0.923***
Myxococcota	-1.02***	-1.984***	-0.964**
Spirochaetota	0.546**	0.991***	0.446*
Acidobacteriota	0.41 NS	-0.438 NS	-0.848*
Thermodesulfobacteriota	0.254 NS	0.125 NS	-0.129 NS
Campylobacterota	0.555*	1.282***	0.727**
Candidatus_Omnitrophota	0.965***	0.848***	-0.117 NS
Lentisphaerota	0.786***	1.376***	0.59*
Kiritimatiellota	0.438*	0.205 NS	-0.233 NS
Chlamydiota	1.192***	2.084***	0.893***
Verrucomicrobiota	0.321 NS	-0.123 NS	-0.444*
Planctomycetota	-0.055 NS	-0.661***	-0.606*
Pseudomonadota	-0.735***	-1.374***	-0.639**
Fibrobacterota	0.808***	0.925***	0.116 NS
Candidatus_Cloacimonadota	0.9**	1.692***	0.792**
Gemmatimonadota	0.047 NS	-1.201***	-1.247**

Balneolota	0.827***	0.926***	0.099 NS
Rhodothermota	0.054 NS	-0.627***	-0.681**
Ignavibacteriota	1.438***	2.033***	0.596*
Chlorobiota	0.675***	0.675***	-0.001 NS
Bacteroidota	0.929***	1.231***	0.302 NS
Thermomicrobiota	0.024 NS	-0.714***	-0.738*
Armatimonadota	0.562*	-0.092 NS	-0.654*
Deinococcota	-0.328 NS	-0.972***	-0.644*
Chloroflexota	0.346*	0.618***	0.272 NS
Mycoplasmatota	0.455*	1.319***	0.864**
Cyanobacteriota	0.712***	1.234***	0.522**
Bacillota	0.623***	1.094***	0.47*
Actinomycetota	-1.794***	-2.691***	-0.897*

Table S4.13. Statistical output for Kruskal-Wallis test and Dunn's Test comparing edge average weights from the network analysis of functional genes from subsystem 1 with. These tests compared bulk soils, rhizospheres and endospheres networks low, medium and high aridity levels. Global significance denotes significance level at P<0.05 = '*', P<0.01 = '**', P<0.001 = '***', and P≥0.05 = 'NS' (non-significant result).

Subsystem 1	Zone	Test	Aridity level comparison	df	Test statistic	Statistic value	P unadj.	Adj. P value	Global Significance
Motility and chemotaxis	Soil	Kruskal-Wallis		2	χ2	4.696	0.096		NS
	Rhizosphere	Kruskal-Wallis		2	χ2	15.628	0.000		***
		Dunn's Test	High aridity - Low aridity		z	-3.773	0.000	0.000	
			High aridity - Medium aridity		z	-3.428	0.001	0.001	
			Low aridity - Medium aridity		z	1.230	0.219	0.219	
	Endosphere	Kruskal-Wallis		2	χ2	9.732	0.008		**
		Dunn's Test	High aridity - Low aridity		z	3.045	0.002	0.007	
			High aridity - Medium aridity		z	2.511	0.012	0.024	
			Low aridity - Medium aridity		z	-0.729	0.466	0.466	
Nitrogen	Soil	Kruskal-Wallis		2	χ2	4.164	0.125		NS

	Rhizosphere			2	χ2	0.523	0.770		NS
	Endosphere			2	χ2	7.750	0.021		*
		Dunn's Test	High aridity - Low aridity		z	-2.363	0.018	0.036	
			High aridity - Medium aridity		z	-2.698	0.007	0.021	
			Low aridity - Medium aridity		z	-0.422	0.673	0.673	
Phosphorus	Soil	Kruskal-Wallis		2	χ2	1.678	0.432		NS
	Rhizosphere	Kruskal-Wallis		2	χ2	36.122	1.432e-08		***
		Dunn's Test	High aridity - Low aridity		z	-5.762	8.305e-09	2.491e-08	
			High aridity - Medium aridity		z	-5.061	4.176e-07	8.353e-07	
			Low aridity - Medium aridity		z	1.495	0.135	0.135	
	Endosphere	Kruskal-Wallis		2	χ2	3.437	0.179		NS
Secondary metabolism	Soil	Kruskal-Wallis		2	χ2	3.822	0.148		NS
	Rhizosphere	Kruskal-Wallis		2	χ2	10.547	0.005		**
		Dunn's Test	High aridity - Low aridity		z	3.190	0.001	0.004	
			High aridity - Medium aridity		z	0.984	0.325	0.325	

			Low aridity - Medium aridity		z	-2.252	0.024	0.049	
	Endosphere	Kruskal-Wallis		2	χ2	1.031	0.597		NS
Regulation and cell signalling	Soil	Kruskal-Wallis		2	χ2	7.107	0.029		*
		Dunn's Test	High aridity - Low aridity		z	-2.098	0.036	0.108	
			High aridity - Medium aridity		z	-1.627	0.104	0.104	
			Low aridity - Medium aridity		z	1.843	0.065	0.131	
	Rhizosphere	Kruskal-Wallis		2	χ2	60.927	5.886e-14		***
		Dunn's Test	High aridity - Low aridity		z	-7.760	8.526e-15	2.558e-14	
			High aridity - Medium aridity		z	-6.831	8.445e-12	1.689e-11	
			Low aridity - Medium aridity		z	2.506	0.012	0.012	
	Endosphere	Kruskal-Wallis		2	χ2	6.949	0.031		*
		Dunn's Test	High aridity - Low aridity		z	-2.491	0.013	0.038	
			High aridity - Medium aridity		z	-2.169	0.030	0.060	
			Low aridity - Medium aridity		z	0.077	0.939	0.939	
Stress response	Soil	Kruskal-Wallis		2	χ2	4.232	0.120		NS

Rhizosphere	Kruskal-Wallis		2	χ2	51.016	8.355e-12		***
	Dunn Test	High aridity - Low aridity		Z	-6.891	5.526e-12	1.658e-11	
		High aridity - Medium aridity		Z	-6.515	7.290e-11	1.458e-10	
		Low aridity - Medium aridity		Z	1.748	0.080	0.080	
Endosphere	Kruskal-Wallis		2	χ2	7.785	0.020		*
	Dunn Test	High aridity - Low aridity		Z	-2.788	0.005	0.016	
		High aridity - Medium aridity		Z	-1.762	0.078	0.156	
		Low aridity - Medium aridity		Z	0.993	0.321	0.321	

Table S4.14. Hub functions for the stress response (subsystem 1) networks for each plant compartment (bulk soil, rhizosphere and endosphere) at three different aridity levels (low aridity, medium aridity, and high aridity). The top 20 bub functions in each network were selected based on highest node degree, then closeness centrality.

Compartment	**Aridity**	**Function**	**Subsystem 2**	**Subsystem 3**	**Degree**	**Closeness centrality**
Bulk soil~	Low aridity~	FUN28191	-	Dimethylarginine metabolism	108	0.0049
		FUN28255	-	Flavohaemoglobin	111	0.0053
		FUN28405	-	Universal stress protein family	114	0.005
		FUN28464	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	124	0.0051
		FUN28467	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	110	0.0052
		FUN28636	Heat shock	At5g63290	113	0.0049
		FUN28649	Heat shock	At5g63290	119	0.0052
		FUN28655	Heat shock	At5g63290	108	0.0051
		FUN28661	Heat shock	At5g63290	112	0.0054
		FUN28686	Heat shock	Heat shock dnaK gene cluster extended	106	0.0052
		FUN28697	Heat shock	Heat shock dnaK gene cluster extended	112	0.0052
		FUN28706	Heat shock	Heat shock dnaK gene cluster extended	117	0.0053
		FUN28707	Heat shock	Heat shock dnaK gene cluster extended	107	0.0049
		FUN28717	Heat shock	Heat shock dnaK gene cluster extended	110	0.0051
		FUN28718	Heat shock	Heat shock dnaK gene cluster extended	108	0.0048
		FUN28719	Heat shock	Heat shock dnaK gene cluster extended	110	0.0048
		FUN28725	Heat shock	Heat shock dnaK gene cluster extended	118	0.005
		FUN28735	Heat shock	Heat shock dnaK gene cluster extended	114	0.0052

	FUN28871	Osmotic stress	Osmoregulation	108	0.0052
	FUN28893	Osmotic stress	Synthesis of osmoregulated periplasmic glucans	107	0.0053
	FUN28901	Oxidative stress	Glutaredoxins	114	0.0054
	FUN29012	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	112	0.0049
	FUN29123	Oxidative stress	Oxidative stress	123	0.0053
	FUN29158	Oxidative stress	Oxidative stress	107	0.0052
	FUN29191	Oxidative stress	Oxidative stress	108	0.0051
	FUN29240	Oxidative stress	Oxidative stress	112	0.0051
	FUN29356	Oxidative stress	Rubrerythrin	117	0.0049
	FUN29365	Oxidative stress	Rubrerythrin	126	0.0053
	FUN29376	Periplasmic Stress	Periplasmic Stress Response	129	0.0052
	FUN29382	Periplasmic Stress	Periplasmic Stress Response	110	0.0049
Medium aridity~	FUN281911	-	Dimethylarginine metabolism	111	0.0066
	FUN282721	-	Flavohaemoglobin	110	0.0067
	FUN283001	-	Flavohaemoglobin	111	0.007
	FUN283311	-	Hfl operon	115	0.0066
	FUN284641	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	115	0.0065

FUN284671	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	109	0.0065
FUN286031	Detoxification	Uptake of selenate and selenite	110	0.0063
FUN286301	Heat shock	At5g63290	115	0.0066
FUN286361	Heat shock	At5g63290	117	0.0064
FUN286411	Heat shock	At5g63290	116	0.007
FUN286491	Heat shock	At5g63290	104	0.0068
FUN286531	Heat shock	At5g63290	112	0.0065
FUN286581	Heat shock	At5g63290	106	0.0065
FUN286861	Heat shock	Heat shock dnaK gene cluster extended	118	0.0069
FUN286971	Heat shock	Heat shock dnaK gene cluster extended	114	0.0069
FUN287061	Heat shock	Heat shock dnaK gene cluster extended	109	0.007
FUN287131	Heat shock	Heat shock dnaK gene cluster extended	106	0.0066
FUN287171	Heat shock	Heat shock dnaK gene cluster extended	113	0.0068
FUN287251	Heat shock	Heat shock dnaK gene cluster extended	116	0.0067
FUN287711	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	112	0.0065
FUN288571	Osmotic stress	Gycosylglycerates	104	0.0066
FUN288881	Osmotic stress	Synthesis of osmoregulated periplasmic glucans	108	0.0062
FUN289541	Oxidative stress	Glutaredoxins	117	0.0066
FUN290981	Oxidative stress	Glutathione: Redox cycle	104	0.0066
FUN291581	Oxidative stress	Oxidative stress	110	0.0065
FUN291951	Oxidative stress	Oxidative stress	111	0.0066

	FUN292911	Oxidative stress	Protection from Reactive Oxygen Species	114	0.0065
	FUN293251	Oxidative stress	Redox-dependent regulation of nucleus processes	112	0.0068
	FUN293561	Oxidative stress	Rubrerythrin	107	0.0066
	FUN293681	Oxidative stress	Rubrerythrin	104	0.0063
High aridity~	FUN281542	-	Bacterial hemoglobins	22	0.0085
	FUN281912	-	Dimethylarginine metabolism	14	0.0079
	FUN283312	-	Hfl operon	18	0.0085
	FUN283732	-	SigmaB stress responce regulation	12	0.0083
	FUN283802	-	SigmaB stress responce regulation	14	0.0085
	FUN284052	-	Universal stress protein family	23	0.0085
	FUN284192	Cold shock	Cold shock, CspA family of proteins	20	0.0085
	FUN284232	Cold shock	Cold shock, CspA family of proteins	17	0.0092
	FUN284902	Detoxification	Nucleoside triphosphate pyrophosphohydrolase MazG	9	0.0081
	FUN285002	Detoxification	Nudix KE	25	0.0093
	FUN286362	Heat shock	At5g63290	15	0.0093
	FUN286532	Heat shock	At5g63290	12	0.0083
	FUN286712	Heat shock	At5g63290	9	0.0083
	FUN286772	Heat shock	At5g63290	16	0.0085
	FUN287072	Heat shock	Heat shock dnaK gene cluster extended	18	0.0086
	FUN287112	Heat shock	Heat shock dnaK gene cluster extended	17	0.0087
	FUN287352	Heat shock	Heat shock dnaK gene cluster extended	14	0.0081

		FUN287732	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	25	0.0096
		FUN287962	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	16	0.0084
		FUN289342	Oxidative stress	Glutaredoxins	10	0.0086
		FUN289972	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	21	0.0095
		FUN290302	Oxidative stress	Glutathione: Non-redox reactions	18	0.0085
		FUN290982	Oxidative stress	Glutathione: Redox cycle	19	0.0092
		FUN291232	Oxidative stress	Oxidative stress	21	0.0084
		FUN291752	Oxidative stress	Oxidative stress	9	0.0064
		FUN292872	Oxidative stress	Protection from Reactive Oxygen Species	9	0.0065
		FUN292932	Oxidative stress	Protection from Reactive Oxygen Species	9	0.0065
		FUN293562	Oxidative stress	Rubrerythrin	18	0.0083
		FUN293822	Periplasmic Stress	Periplasmic Stress Response	17	0.0088
		FUN293902	Periplasmic Stress	Periplasmic Stress Response	19	0.0084
Rhizosphere	Low aridity~	FUN281702	-	Carbon Starvation	63	0.0054
		FUN281772	-	Carbon Starvation	55	0.0054

FUN281913	-	Dimethylarginine metabolism	58	0.0059
FUN282452	-	Flavohaemoglobin	47	0.0055
FUN283372	-	Hfl operon	67	0.0057
FUN283722	-	SigmaB stress responce regulation	52	0.0057
FUN284053	-	Universal stress protein family	55	0.0051
FUN284193	Cold shock	Cold shock, CspA family of proteins	45	0.0053
FUN284803	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	44	0.0056
FUN286202	Detoxification	Uptake of selenate and selenite	47	0.0054
FUN286323	Heat shock	At5g63290	50	0.0053
FUN286552	Heat shock	At5g63290	53	0.0058
FUN287233	Heat shock	Heat shock dnaK gene cluster extended	53	0.0057
FUN287362	Heat shock	Heat shock dnaK gene cluster extended	62	0.0056
FUN287733	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	49	0.0053
FUN287862	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	47	0.0052
FUN287963	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	43	0.0055
FUN287992	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	47	0.0052
FUN288181	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	51	0.0059
FUN288552	Osmotic stress	Gycosylglycerates	46	0.005
FUN288612	Osmotic stress	Gycosylglycerates	57	0.0055
FUN289222	Oxidative stress	Glutaredoxins	59	0.0054

	FUN289343	Oxidative stress	Glutaredoxins	44	0.0053
	FUN290303	Oxidative stress	Glutathione: Non-redox reactions	67	0.0059
	FUN290422	Oxidative stress	Glutathione: Non-redox reactions	51	0.0057
	FUN290613	Oxidative stress	Glutathione: Non-redox reactions	48	0.0056
	FUN291682	Oxidative stress	Oxidative stress	67	0.0055
	FUN291912	Oxidative stress	Oxidative stress	53	0.0057
	FUN292263	Oxidative stress	Oxidative stress	67	0.0057
	FUN293222	Oxidative stress	Redox-dependent regulation of nucleus processes	48	0.0057
Medium aridity~	FUN281603	-	Bacterial hemoglobins	119	0.0052
	FUN281703	-	Carbon Starvation	116	0.0054
	FUN281773	-	Carbon Starvation	117	0.0054
	FUN281914	-	Dimethylarginine metabolism	117	0.0052
	FUN282723	-	Flavohaemoglobin	115	0.0053
	FUN284644	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	124	0.0053
	FUN284673	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	121	0.0054
	FUN285572	Detoxification	Nudix proteins (nucleoside triphosphate hydrolases)	115	0.0051
	FUN286304	Heat shock	At5g63290	120	0.0053

FUN286363	Heat shock	At5g63290	120	0.0051
FUN286864	Heat shock	Heat shock dnaK gene cluster extended	118	0.0051
FUN287024	Heat shock	Heat shock dnaK gene cluster extended	115	0.0054
FUN287074	Heat shock	Heat shock dnaK gene cluster extended	115	0.0053
FUN287613	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	115	0.0055
FUN288884	Osmotic stress	Synthesis of osmoregulated periplasmic glucans	124	0.0054
FUN289974	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	118	0.0054
FUN289992	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	115	0.0052
FUN290124	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	116	0.005
FUN290271	Oxidative stress	Glutathione: Non-redox reactions	117	0.0056
FUN290984	Oxidative stress	Glutathione: Redox cycle	120	0.0052
FUN291343	Oxidative stress	Oxidative stress	117	0.0054
FUN291834	Oxidative stress	Oxidative stress	122	0.0051
FUN291953	Oxidative stress	Oxidative stress	115	0.0053
FUN292044	Oxidative stress	Oxidative stress	118	0.0053
FUN292264	Oxidative stress	Oxidative stress	117	0.0052
FUN292773	Oxidative stress	Protection from Reactive Oxygen Species	116	0.0053

	FUN292914	Oxidative stress	Protection from Reactive Oxygen Species	116	0.0055
	FUN293074	Oxidative stress	Protection from Reactive Oxygen Species	116	0.0051
	FUN293653	Oxidative stress	Rubrerythrin	115	0.0052
	FUN293823	Periplasmic Stress	Periplasmic Stress Response	116	0.0053
High aridity~	FUN281915	-	Dimethylarginine metabolism	23	0.0027
	FUN283344	-	Hfl operon	21	0.0027
	FUN283514	-	SigmaB stress responce regulation	20	0.0027
	FUN283735	-	SigmaB stress responce regulation	20	0.0027
	FUN285495	Detoxification	Nudix proteins (nucleoside triphosphate hydrolases)	23	0.0027
	FUN286134	Detoxification	Uptake of selenate and selenite	22	0.0026
	FUN286204	Detoxification	Uptake of selenate and selenite	20	0.0027
	FUN286614	Heat shock	At5g63290	20	0.0029
	FUN286694	Heat shock	At5g63290	21	0.0027
	FUN287143	Heat shock	Heat shock dnaK gene cluster extended	25	0.0027
	FUN287255	Heat shock	Heat shock dnaK gene cluster extended	27	0.003
	FUN287295	Heat shock	Heat shock dnaK gene cluster extended	24	0.0027
	FUN287715	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	20	0.0027
	FUN287735	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	21	0.0029
	FUN287903	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	20	0.0026

FUN287965	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	22	0.0025
FUN288613	Osmotic stress	Gycosylglycerates	22	0.0026
FUN289913	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	22	0.0027
FUN289975	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	22	0.0026
FUN289993	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	25	0.0027
FUN290272	Oxidative stress	Glutathione: Non-redox reactions	25	0.0027
FUN290565	Oxidative stress	Glutathione: Non-redox reactions	24	0.0028
FUN290615	Oxidative stress	Glutathione: Non-redox reactions	20	0.0026
FUN291004	Oxidative stress	Glutathione: Redox cycle	23	0.0026
FUN291324	Oxidative stress	Oxidative stress	21	0.0026
FUN291425	Oxidative stress	Oxidative stress	22	0.0028
FUN291914	Oxidative stress	Oxidative stress	20	0.0025
FUN292275	Oxidative stress	Oxidative stress	20	0.0028
FUN293805	Periplasmic Stress	Periplasmic Stress Response	23	0.0025
FUN293905	Periplasmic Stress	Periplasmic Stress Response	21	0.0025

Endosphere	Low aridity~	FUN281705	-	Carbon Starvation	63	0.0054
		FUN281774	-	Carbon Starvation	55	0.0054
		FUN281916	-	Dimethylarginine metabolism	58	0.0059
		FUN282454	-	Flavohaemoglobin	47	0.0055
		FUN283375	-	Hfl operon	67	0.0057
		FUN283723	-	SigmaB stress responce regulation	52	0.0057
		FUN284056	-	Universal stress protein family	55	0.0051
		FUN284196	Cold shock	Cold shock, CspA family of proteins	45	0.0053
		FUN284806	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	44	0.0056
		FUN286205	Detoxification	Uptake of selenate and selenite	47	0.0054
		FUN286326	Heat shock	At5g63290	50	0.0053
		FUN286555	Heat shock	At5g63290	53	0.0058
		FUN287236	Heat shock	Heat shock dnaK gene cluster extended	53	0.0057
		FUN287365	Heat shock	Heat shock dnaK gene cluster extended	62	0.0056
		FUN287736	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	49	0.0053
		FUN287864	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	47	0.0052
		FUN287966	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	43	0.0055
		FUN287995	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	47	0.0052
		FUN288184	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	51	0.0059
		FUN288554	Osmotic stress	Gycosylglycerates	46	0.005
		FUN288614	Osmotic stress	Gycosylglycerates	57	0.0055

	FUN289225	Oxidative stress	Glutaredoxins	59	0.0054
	FUN289346	Oxidative stress	Glutaredoxins	44	0.0053
	FUN290306	Oxidative stress	Glutathione: Non-redox reactions	67	0.0059
	FUN290425	Oxidative stress	Glutathione: Non-redox reactions	51	0.0057
	FUN290616	Oxidative stress	Glutathione: Non-redox reactions	48	0.0056
	FUN291685	Oxidative stress	Oxidative stress	67	0.0055
	FUN291915	Oxidative stress	Oxidative stress	53	0.0057
	FUN292266	Oxidative stress	Oxidative stress	67	0.0057
	FUN293225	Oxidative stress	Redox-dependent regulation of nucleus processes	48	0.0057
Medium aridity~	FUN281605	-	Bacterial hemoglobins	119	0.0052
	FUN281706	-	Carbon Starvation	116	0.0054
	FUN281775	-	Carbon Starvation	117	0.0054
	FUN281917	-	Dimethylarginine metabolism	117	0.0052
	FUN282726	-	Flavohaemoglobin	115	0.0053
	FUN284647	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	124	0.0053
	FUN284676	Detoxification	Housecleaning nucleoside triphosphate pyrophosphatases	121	0.0054

FUN285573	Detoxification	Nudix proteins (nucleoside triphosphate hydrolases)	115	0.0051
FUN286307	Heat shock	At5g63290	120	0.0053
FUN286365	Heat shock	At5g63290	120	0.0051
FUN286867	Heat shock	Heat shock dnaK gene cluster extended	118	0.0051
FUN287027	Heat shock	Heat shock dnaK gene cluster extended	115	0.0054
FUN287077	Heat shock	Heat shock dnaK gene cluster extended	115	0.0053
FUN287615	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	115	0.0055
FUN288887	Osmotic stress	Synthesis of osmoregulated periplasmic glucans	124	0.0054
FUN289977	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	118	0.0054
FUN289995	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	115	0.0052
FUN290127	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	116	0.005
FUN290274	Oxidative stress	Glutathione: Non-redox reactions	117	0.0056
FUN290987	Oxidative stress	Glutathione: Redox cycle	120	0.0052
FUN291346	Oxidative stress	Oxidative stress	117	0.0054
FUN291837	Oxidative stress	Oxidative stress	122	0.0051
FUN291955	Oxidative stress	Oxidative stress	115	0.0053
FUN292047	Oxidative stress	Oxidative stress	118	0.0053

	FUN292267	Oxidative stress	Oxidative stress	117	0.0052
	FUN292776	Oxidative stress	Protection from Reactive Oxygen Species	116	0.0053
	FUN292917	Oxidative stress	Protection from Reactive Oxygen Species	116	0.0055
	FUN293077	Oxidative stress	Protection from Reactive Oxygen Species	116	0.0051
	FUN293655	Oxidative stress	Rubrerythrin	115	0.0052
	FUN293824	Periplasmic Stress	Periplasmic Stress Response	116	0.0053
High aridity~	FUN281918	-	Dimethylarginine metabolism	23	0.0027
	FUN283347	-	Hfl operon	21	0.0027
	FUN283517	-	SigmaB stress responce regulation	20	0.0027
	FUN283738	-	SigmaB stress responce regulation	20	0.0027
	FUN285498	Detoxification	Nudix proteins (nucleoside triphosphate hydrolases)	23	0.0027
	FUN286137	Detoxification	Uptake of selenate and selenite	22	0.0026
	FUN286207	Detoxification	Uptake of selenate and selenite	20	0.0027
	FUN286617	Heat shock	At5g63290	20	0.0029
	FUN286696	Heat shock	At5g63290	21	0.0027
	FUN287146	Heat shock	Heat shock dnaK gene cluster extended	25	0.0027
	FUN287258	Heat shock	Heat shock dnaK gene cluster extended	27	0.003
	FUN287298	Heat shock	Heat shock dnaK gene cluster extended	24	0.0027
	FUN287718	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	20	0.0027

FUN287738	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	21	0.0029
FUN287905	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	20	0.0026
FUN287968	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	22	0.0025
FUN288615	Osmotic stress	Gycosylglycerates	22	0.0026
FUN289916	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	22	0.0027
FUN289978	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	22	0.0026
FUN289996	Oxidative stress	Glutathione: Biosynthesis and gamma-glutamyl cycle	25	0.0027
FUN290275	Oxidative stress	Glutathione: Non-redox reactions	25	0.0027
FUN290568	Oxidative stress	Glutathione: Non-redox reactions	24	0.0028
FUN290618	Oxidative stress	Glutathione: Non-redox reactions	20	0.0026
FUN291007	Oxidative stress	Glutathione: Redox cycle	23	0.0026
FUN291327	Oxidative stress	Oxidative stress	21	0.0026
FUN291428	Oxidative stress	Oxidative stress	22	0.0028
FUN291917	Oxidative stress	Oxidative stress	20	0.0025
FUN292278	Oxidative stress	Oxidative stress	20	0.0028

	FUN293808	Periplasmic Stress	Periplasmic Stress Response	23	0.0025
	FUN293908	Periplasmic Stress	Periplasmic Stress Response	21	0.0025

Table S15. Statistical output for distance to centroid estimates for functional genes at subsystem level 1.

Response variable	Statistical test	Permutations	Predictor variable	Degrees of freedom	F-statistic	P value
Motility and chemotaxis~	Permutation test for homogeneity of multivariate dispersions	999	Compartment	2 and 102	8.9856	0.002
	Pairwise compartment test	Rhizosphere-Endosphere				0.001
	Pairwise compartment test	Soil-Endosphere				0.003
	Pairwise compartment test	Soil-Rhizosphere				0.716
Phosphorus metabolism~	Permutation test for homogeneity of multivariate dispersions	999	Compartment	2 and 102	8.8053	0.002
	Pairwise compartment test	Rhizosphere-Endosphere				0.0087402
	Pairwise compartment test	Soil-Endosphere				0.0005252
	Pairwise compartment test	Soil-Rhizosphere				0.9526302
Nitrogen metabolism~	Permutation test for homogeneity of multivariate dispersions	999	Compartment	2 and 102	0.001	0.002
	Pairwise compartment test	Rhizosphere-Endosphere				0.0000039
	Pairwise compartment test	Soil-Endosphere				0.0000002
	Pairwise compartment test	Soil-Rhizosphere				0.9874346

Regulation and cell signalling~	Permutation test for homogeneity of multivariate dispersions		999	Compartment	2 and 102	8.5343	0.001
	Pairwise compartment test	Rhizosphere-Endosphere					0.0008591
	Pairwise compartment test	Soil-Endosphere					0.00671
	Pairwise compartment test	Soil-Rhizosphere					0.5392789
Secondary metabolism~	Permutation test for homogeneity of multivariate dispersions		999	Compartment	2 and 102	7.3946	0.002
	Pairwise compartment test	Rhizosphere-Endosphere					0.1191559
	Pairwise compartment test	Soil-Endosphere					0.0006778
	Pairwise compartment test	Soil-Rhizosphere					0.4261641
Stress response~	Permutation test for homogeneity of multivariate dispersions		999	Compartment	2 and 102	11.477	<0.001
	Pairwise compartment test	Rhizosphere-Endosphere					0.0010103
	Pairwise compartment test	Soil-Endosphere					0.0027863
	Pairwise compartment test	Soil-Rhizosphere					0.7161791

Table S16. Statistical output for differential abundance analysis of each functional subsystem, across each plant compartment (soil, rhizosphere, and endosphere). Table shows statistical output for global models from ANCOMBC differential abundance analysis, and pairwise log fold change differences.

Subsystem 1	Subsystem 3	Test statistic (W)	P value	Adjusted P value	LFC Endosphere vs Rhizosphere	LFC Endosphere vs Soil	LFC Soil vs Rhizosphere
Motility and Chemotaxis	Archaeal Flagellum	23.874	4E-06	4E-06	0.521	0.876	0.355
Motility and Chemotaxis	Bacterial Chemotaxis	90.032	6E-23	4E-22	-0.449	-0.830	-0.381
Motility and Chemotaxis	Bacterial motility:Gliding	68.997	2E-19	7E-19	0.675	0.606	-0.070
Motility and Chemotaxis	Flagellar motility	25.574	2E-09	3E-09	-0.199	-0.458	-0.259
Motility and Chemotaxis	Flagellum	19.312	2E-07	2E-07	-0.224	-0.327	-0.102
Motility and Chemotaxis	Flagellum in Campylobacter	39.728	3E-13	7E-13	-0.469	-0.634	-0.165
Nitrogen metabolism	Allantoin Utilization	46.818	8E-15	2E-14	-0.260	-0.770	-0.510
Nitrogen metabolism	Amidase clustered with urea and nitrile hydratase functions	110.377	6E-26	4E-25	-0.767	-1.960	-1.193
Nitrogen metabolism	Ammonia assimilation	22.530	2E-08	3E-08	0.193	-0.458	-0.650
Nitrogen metabolism	Cyanate hydrolysis	6.088	6E-03	6E-03	0.093	-0.240	-0.333

Nitrogen metabolism	Denitrification	6.869	3E-03	3E-03	0.569	0.120	-0.449
Nitrogen metabolism	Dissimilatory nitrite reductase	25.879	2E-09	3E-09	-0.358	-0.653	-0.295
Nitrogen metabolism	Nitrate and nitrite ammonification	69.727	2E-19	7E-19	0.026	-0.833	-0.860
Nitrogen metabolism	Nitric oxide synthase	180.086	7E-34	8E-33	-0.914	-1.425	-0.510
Nitrogen metabolism	Nitrilase	9.940	2E-04	3E-04	0.452	-0.263	-0.715
Nitrogen metabolism	Nitrogen fixation	26.420	1E-09	3E-09	0.794	0.400	-0.394
Nitrogen metabolism	Nitrogen Metabolism in Aspergillus nidulans	8.452	8E-04	1E-03	-0.643	-0.522	0.121
Nitrogen metabolism	Nitrosative stress	16.237	2E-06	2E-06	0.681	-0.194	-0.876
Phosphorus metabolism	High affinity phosphate transporter and control of PHO regulon	22.747	1E-08	8E-08	0.144	0.423	0.279
Phosphorus metabolism	P uptake (cyanobacteria)	14.151	2E-05	3E-05	0.703	0.816	0.113
Phosphorus metabolism	Phosphate metabolism	15.917	2E-06	4E-06	0.155	0.320	0.165
Phosphorus metabolism	Phosphonate metabolism	21.438	4E-08	1E-07	-0.313	-0.660	-0.347
Secondary metabolism	2-isocapryloyl-3R-hydroxymethyl-gamma-butyrolactone and other bacterial morphogens	145.431	2E-21	9E-21	-1.782	-2.480	-0.699

Secondary metabolism	Alkaloid biosynthesis from L-lysine	72.838	4E-20	2E-19	0.873	1.434	0.561
Secondary metabolism	Apigenin derivatives	4.609	3E-02	4E-02	-0.205	0.348	0.554
Secondary metabolism	Auxin biosynthesis	91.308	2E-16	5E-16	-1.829	-2.190	-0.361
Secondary metabolism	Auxin degradation	8.747	6E-04	9E-04	-0.156	-0.527	-0.371
Secondary metabolism	Biflavanoid biosynthesis	21.011	5E-08	8E-08	-0.582	-0.829	-0.247
Secondary metabolism	Caffeic acid derivatives	110.847	7E-24	5E-23	-1.790	-1.888	-0.099
Secondary metabolism	Clavulanic acid biosynthesis	51.818	5E-15	1E-14	-1.311	-1.388	-0.076
Secondary metabolism	Flavanones and dihydroflavonols biosynthesis in plants	17.417	2E-05	2E-05	-0.258	-0.867	-0.609
Secondary metabolism	Homomethionine biosynthesis and methionine chain elongation pathway for glucosinolates in plants	54.358	1E-10	2E-10	-0.901	-1.600	-0.699
Secondary metabolism	Nonribosomal peptide synthetases (NRPS) in Frankia sp. Ccl3	204.046	4E-29	8E-28	-2.536	-2.981	-0.445
Secondary metabolism	Paerucumarin Biosynthesis	5.402	1E-02	2E-02	-0.569	-0.162	0.407
Secondary metabolism	Phenazine biosynthesis	35.076	5E-12	1E-11	-0.806	-1.151	-0.345

Secondary metabolism	Phenylpropanoids general biosynthesis in plants	157.371	1E-26	1E-25	-2.333	-2.364	-0.031
Secondary metabolism	Phenylpropionate Degradation	67.171	6E-17	2E-16	-1.341	-1.633	-0.292
Secondary metabolism	Salicylic acid biosynthesis1	16.722	2E-06	3E-06	-0.956	-0.842	0.114
Secondary metabolism	Sinapate ester biosynthesis in plants	110.145	5E-21	2E-20	-2.519	-2.001	0.517
Secondary metabolism	Tannin biosynthesis	21.011	5E-08	8E-08	-0.582	-0.829	-0.247
Regulation and cell signalling	A conserved operon linked to TyrR and possibly involved in virulence	44.364	4E-07	7E-07	0.412	-0.757	-1.170
Regulation and cell signalling	Acyl Homoserine Lactone (AHL) Autoinducer Quorum Sensing_	14.921	8E-06	1E-05	0.048	-0.583	-0.631
Regulation and cell signalling	Autoinducer 2 (AI-2) transport and processing (lsrACDBFGE operon)	39.295	7E-13	2E-12	0.908	1.595	0.687
Regulation and cell signalling	Bacterial Caspases	6.951	4E-03	5E-03	0.191	-0.324	-0.515
Regulation and cell signalling	Biofilm Adhesin Biosynthesis	9.597	5E-04	8E-04	-0.010	-0.484	-0.474
Regulation and cell signalling	Biofilm formation in Staphylococcus	318.381	3E-42	4E-41	-1.277	-2.957	-1.681
Regulation and cell signalling	cAMP signalling in bacteria	4.604	2E-02	3E-02	0.242	-0.145	-0.387

Regulation and cell signalling	Cell envelope-associated LytR-CpsA-Psr transcriptional attenuators	58.856	2E-17	6E-17	-0.749	-1.282	-0.533
Regulation and cell signalling	Coagulation cascade	6.302	9E-03	1E-02	0.541	0.028	-0.513
Regulation and cell signalling	CytR regulation	32.491	7E-10	2E-09	-0.067	-0.915	-0.848
Regulation and cell signalling	DNA-binding regulatory proteins, strays	103.573	6E-25	4E-24	-0.558	-1.538	-0.980
Regulation and cell signalling	G-protein-coupled receptor (GPCR) system in Actinobacteria	662.394	5E-51	2E-49	-2.840	-4.664	-1.824
Regulation and cell signalling	Global Two-component Regulator PrrBA in Proteobacteria	438.940	7E-46	1E-44	-1.138	-3.187	-2.049
Regulation and cell signalling	HPr catabolite repression system	9.896	2E-04	4E-04	0.524	0.251	-0.273
Regulation and cell signalling	Iojap	5.976	7E-03	9E-03	0.328	-0.159	-0.487
Regulation and cell signalling	MazEF toxin-antitoxing (programmed cell death) system	84.684	4E-22	2E-21	1.100	1.187	0.087
Regulation and cell signalling	Murein hydrolase regulation and cell death	81.755	2E-21	7E-21	-0.454	-1.095	-0.641
Regulation and cell signalling	Orphan regulatory proteins	40.181	3E-13	8E-13	-0.132	-0.771	-0.638
Regulation and cell signalling	Oxygen and light sensor PpaA-PpsR	25.022	3E-09	7E-09	0.712	0.427	-0.285

Regulation and cell signalling	P38 MAP kinase pathways	9.067	5E-04	7E-04	0.287	0.552	0.265
Regulation and cell signalling	Phd-Doc, YdcE-YdcD toxin-antitoxin (programmed cell death) systems	29.771	1E-10	4E-10	0.647	0.550	-0.098
Regulation and cell signalling	Phosphoinositides biosynthesis in plants	4.052	4E-02	5E-02	-0.521	0.008	0.529
Regulation and cell signalling	Plastidial (p)ppGpp-mediated response in plants	19.437	1E-07	3E-07	-0.023	-0.598	-0.575
Regulation and cell signalling	Quorum sensing in Yersinia	12.947	2E-05	4E-05	0.518	0.474	-0.045
Regulation and cell signalling	Rcs phosphorelay signal transduction pathway	10.875	1E-04	2E-04	0.228	-0.536	-0.764
Regulation and cell signalling	Sex pheromones in Enterococcus faecalis and other Firmicutes	157.903	1E-31	1E-30	-0.595	-1.423	-0.828
Regulation and cell signalling	Stringent Response, (p)ppGpp metabolism	9.938	2E-04	4E-04	0.474	0.118	-0.356
Regulation and cell signalling	Symbiotic colonization and sigma-dependent biofilm formation gene cluster	5.699	1E-02	1E-02	0.242	-0.235	-0.477
Regulation and cell signalling	The Chv regulatory system of Alphaproteobacteria	110.951	1E-25	9E-25	-0.631	-2.022	-1.391
Regulation and cell signalling	Toxin-antitoxin replicon stabilization systems	20.147	9E-07	2E-06	1.005	0.586	-0.419
Regulation and cell signalling	Toxin-antitoxin system in Mycobacterium	72.721	2E-18	8E-18	-0.381	-1.390	-1.009
Regulation and cell signalling	Toxin-antitoxin systems (other than RelBE and MazEF)	61.093	7E-18	2E-17	1.011	0.832	-0.179

Regulation and cell signalling	Trans-envelope signalling system VreARI in Pseudomonas	70.536	6E-18	2E-17	-0.511	-1.777	-1.266
Regulation and cell signalling	Two-component regulatory systems in Campylobacter	21.357	4E-08	8E-08	0.606	0.394	-0.212
Regulation and cell signalling	Two-component Response Regulator of Virulence ResDE	114.817	2E-23	1E-22	1.380	2.078	0.698
Regulation and cell signalling	WhiB and WhiB-type regulatory proteins_	5.741	9E-03	1E-02	0.286	0.221	-0.065
Regulation and cell signalling	Zinc regulated enzymes	4.717	2E-02	3E-02	0.009	-0.225	-0.234
Stress response	At5g63290	30.123	1E-10	2E-10	0.436	0.359	-0.077
Stress response	Bacterial hemoglobins	79.606	3E-21	1E-20	-0.567	-0.774	-0.206
Stress response	Carbon Starvation	24.907	3E-09	6E-09	0.027	-0.528	-0.555
Stress response	Choline and Betaine Uptake and Betaine Biosynthesis	150.352	8E-31	7E-30	-0.614	-1.014	-0.399
Stress response	Cold shock, CspA family of proteins	96.177	7E-24	4E-23	0.783	0.744	-0.039
Stress response	Commensurate regulon activation	4.878	3E-02	3E-02	0.386	0.375	-0.011
Stress response	D-tyrosyl-tRNA(Tyr) deacylase	74.126	4E-20	1E-19	0.786	0.806	0.020
Stress response	Dimethylarginine metabolism	5.332	1E-02	2E-02	-0.250	-0.194	0.056
Stress response	Ectoine biosynthesis and regulation	223.447	6E-36	7E-35	-1.522	-2.116	-0.594
Stress response	Flavohaemoglobin	55.767	9E-17	2E-16	-0.315	-0.671	-0.356
Stress response	FOL Commensurate regulon activation	9.338	4E-04	6E-04	-0.009	-0.496	-0.488

Stress response	Glutamate transporter involved in acid tolerance in Streptococcus	44.877	1E-07	2E-07	0.966	-0.080	-1.046
Stress response	Glutathione analogs: mycothiol	511.520	3E-49	1E-47	-2.443	-3.722	-1.279
Stress response	Glutathione-dependent pathway of formaldehyde detoxification	205.311	5E-36	7E-35	-0.757	-1.466	-0.709
Stress response	Glutathione: Biosynthesis and gamma-glutamyl cycle	86.491	2E-22	1E-21	-0.379	-0.732	-0.353
Stress response	Glutathione: Non-redox reactions	73.049	4E-20	2E-19	-0.454	-0.706	-0.251
Stress response	Glutathionylspermidine and Trypanothione	101.258	6E-24	4E-23	-0.457	-1.448	-0.991
Stress response	Gycosylglycerates	12.866	2E-05	4E-05	0.575	0.675	0.101
Stress response	Heat shock dnaK gene cluster extended	20.420	7E-08	1E-07	0.257	0.296	0.039
Stress response	Hfl operon	5.171	1E-02	2E-02	0.104	0.167	0.063
Stress response	Housecleaning nucleoside triphosphate pyrophosphatases	8.075	1E-03	1E-03	0.124	0.248	0.124
Stress response	Nucleoside triphosphate pyrophosphohydrolase MazG	12.449	3E-05	5E-05	0.163	0.364	0.200
Stress response	Nudix KE	11.854	5E-05	7E-05	-0.120	-0.285	-0.165
Stress response	Nudix proteins (nucleoside triphosphate hydrolases)	107.852	1E-25	1E-24	-0.473	-0.731	-0.259
Stress response	O-antigen capsule important for environmental persistence	5.752	2E-02	2E-02	-0.471	-0.448	0.024

Stress response	Osmoprotectant ABC transporter YehZYXW of Enterobacteriales	101.394	3E-19	1E-18	-0.228	-1.419	-1.191
Stress response	Osmoregulation	10.347	2E-04	2E-04	0.092	0.322	0.230
Stress response	Osmotic stress cluster	30.560	2E-10	4E-10	-0.446	-0.894	-0.447
Stress response	Oxidative stress	9.698	3E-04	4E-04	0.172	0.193	0.021
Stress response	Oxygen stress response / Human gut microbiome	30.906	8E-11	2E-10	0.483	0.779	0.295
Stress response	Periplasmic Acid Stress Response in Enterobacteria	23.817	2E-06	3E-06	-0.042	0.593	0.635
Stress response	Periplasmic Stress Response	52.341	5E-16	1E-15	0.392	0.574	0.182
Stress response	Phage shock protein (psp) operon	61.473	1E-17	4E-17	0.007	-1.018	-1.025
Stress response	Redox-dependent regulation of nucleus processes	7.726	2E-03	2E-03	-0.161	-0.189	-0.028
Stress response	Regulation of Oxidative Stress Response	9.146	4E-04	6E-04	-0.050	-0.273	-0.223
Stress response	Rubrerythrin	65.849	9E-19	3E-18	0.526	0.681	0.155
Stress response	SigmaB stress responce regulation	62.569	4E-18	1E-17	-0.364	-0.613	-0.249
Stress response	Synthesis of osmoregulated periplasmic glucans	15.679	2E-06	4E-06	0.028	-0.402	-0.430
Stress response	Tellurite resistance: Chromosomal determinants	12.926	3E-05	5E-05	0.001	0.847	0.846
Stress response	Uptake of selenate and selenite	223.777	1E-37	2E-36	-0.710	-1.261	-0.551

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Supplementary figures

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Figure S4.1. Sampling sites and aridity index with schematic of *Themeda triandra* plant compartments. (a) Pan-palaeotropical distribution of *T. triandra* based on observations (points) from the Global Biodiversity Information Facility between years 2000-2023 (GBIF.org 2023). Points are likely underrepresented geographically across its distribution due to different practises of obtaining reliable records of occurrence. The colour gradient represents mean annual aridity index from Version 3 of the Global Aridity Index and Potential Evapotranspiration Database (Zomer et al. 2022). (b) Map of sampling sites (points) with aridity index with sourced from the Global AI-PET database (version 3), (c) Mantel test showing no correlation between comparisons of site-site geographic distances and aridity distances across each of the sampling sites (Mantel: $p = 0.489$; $r = -0.021$). (d) Density plot of global *T. triandra* occurrences based on records from the Global Biodiversity Information Facility between the years 2000–2023 with upper and lower limits from the Atlas of Living Australia aridity index data which included 87% of *T. triandra* occurrences (blue bar), and the Global AI-PET database (version 3) which included 41% of *T. triandra* occurrences (red bar). (e) Diagram of *T. triandra* plant compartments showing bulk soil, rhizosphere, and endospheres microbiota. (f) Photographs of sampling populations across (i) Alligator Gorge, (ii) Burunga Gap, (iii) Frahn's Farm, (iv) Neagles Rock, (v) Maitland, (vi) Mount Maria, (vii) Scott Creek, and (viii) Sturt Gorge. (g) Bar plot of relative abundance of functional vegetation groups in each site. Functional categories for the vegetation were as follows: graminoids, forbs (herbs), shrubs, trees/canopy cover, litter, and bare earth (exposed dirt or rock).

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Figure S4.2. Non-metric multidimensional scaling ordination with Bray-Curtis distances showing bacterial taxonomic community differences across sampling sites. Plots represent: (a) bulk soils (triangles), (b) rhizospheres (squares), and (c) endospheres (circles) (see Table S4.4).

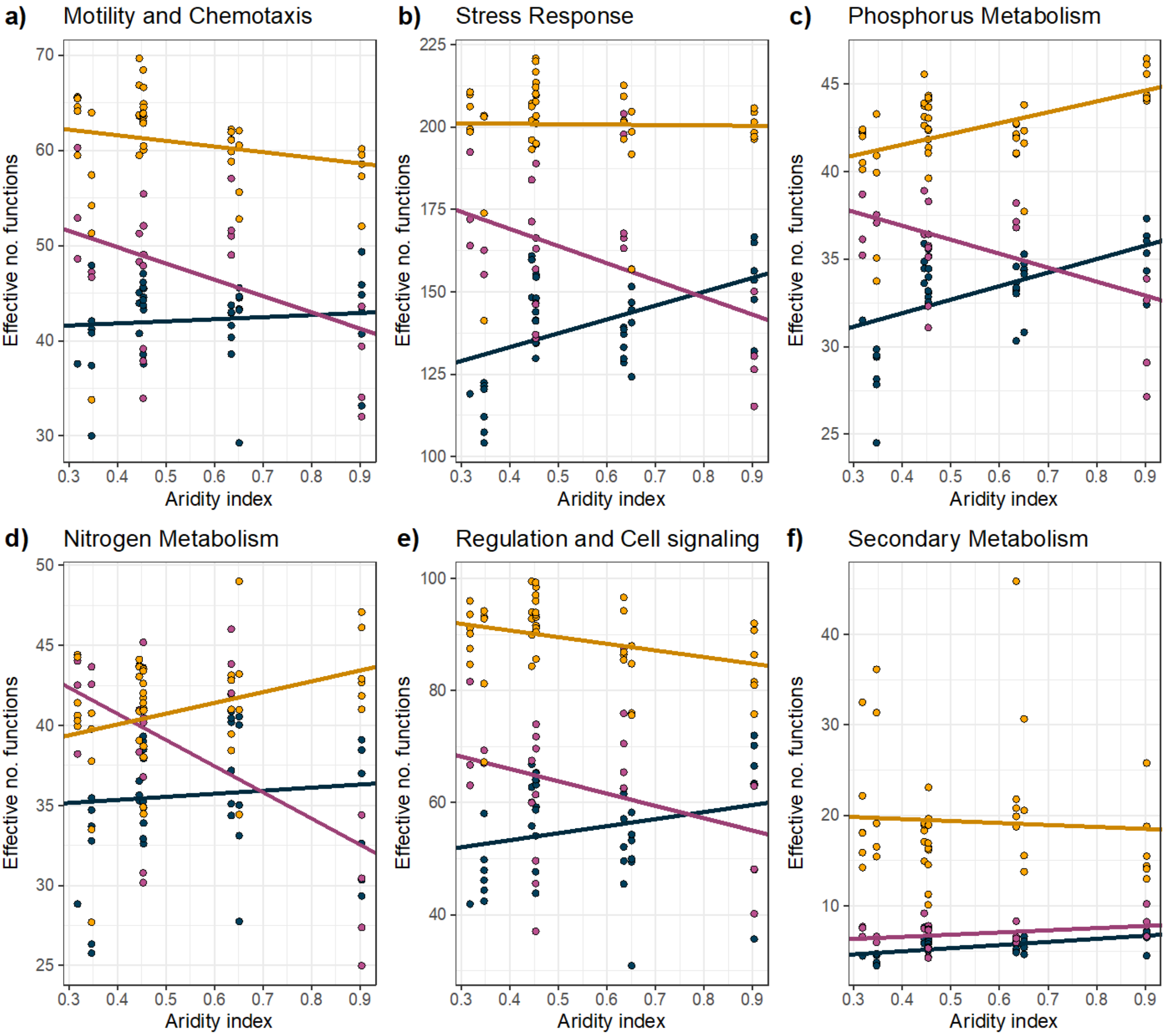

Figure S4.3. Alpha diversity with aridity index. Effective number of functions across plant compartment (bulk soil=blue, rhizosphere=pink, endosphere=yellow). Functional annotations include: (a) motility and chemotaxis, (b) stress genes, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) regulation and cell signalling, and (f) secondary metabolism. For full statistical output, see Table S4.2

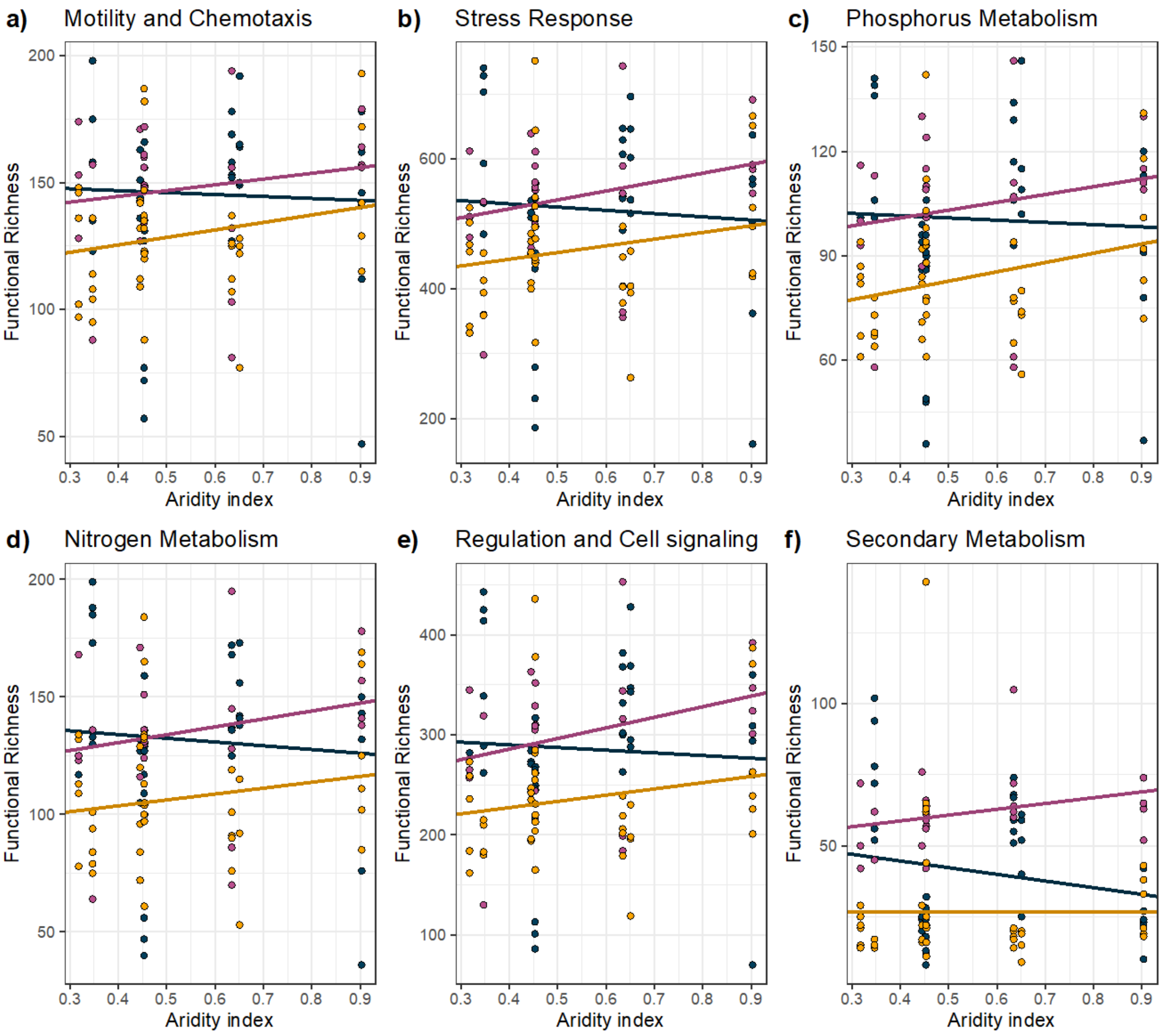

Figure S4.4. Functional richness across mean aridity index. Colour represents plant compartment (bulk soil=blue, rhizosphere=pink, endosphere=yellow) across several key functional gene categories. Functional annotations include: (a) motility and chemotaxis, (b) stress genes, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) regulation and cell signalling, and (f) secondary metabolism. For full statistical output, see Table S4.2

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Figure S4.5. The alpha diversity of bacterial species of samples is correlated with the alpha diversity of microbial functions. (a) Effective number of species for each plant compartment (bulk soil=blue, rhizosphere=pink, endosphere=yellow) increases with their effective number of functions. (b) An increase in effective number of species for all samples is correlated with a general decrease in the effective number of functions. Effective number of species/functions represents the exponential transformation of Shannon's diversity index. Density plots above the x and y axes, represent the distribution of samples showing the differences in median values across each compartment. For full statistical output, see Table S4.11.

Figure S4.6. Relative abundance of microbial functional processes pertaining to motility and chemotaxis genes across all bulk soil, rhizosphere, endosphere samples. Sample labels are coloured by aridity index of sampling sites, whereas bar labels indicate plant compartment (bulk soil = blue, rhizosphere = pink, and endosphere = yellow).

Figure S4.7. Relative abundance of microbial functional processes pertaining to nitrogen metabolism across all bulk soil, rhizosphere, endosphere samples. Sample labels are coloured by aridity index of sampling sites, whereas bar labels indicate plant compartment (bulk soil = blue, rhizosphere = pink, and endosphere = yellow).

Figure S4.8. Relative abundance of microbial functional processes pertaining to phosphorus metabolism across all bulk soil, rhizosphere, endosphere samples. Sample labels are coloured by aridity index of sampling sites, whereas bar labels indicate plant compartment (bulk soil = blue, rhizosphere = pink, and endosphere = yellow).

Figure S4.9. Relative abundance of microbial functional processes pertaining to secondary metabolism across all bulk soil, rhizosphere, endosphere samples. Sample labels are coloured by aridity index of sampling sites, whereas bar labels indicate plant compartment (bulk soil = blue, rhizosphere = pink, and endosphere = yellow).

Figure S4.10. Relative abundance of microbial functional processes pertaining to regulation and cell signalling across all bulk soil, rhizosphere, endosphere samples. Sample labels are coloured by aridity index of sampling sites, whereas bar labels indicate plant compartment (bulk soil = blue, rhizosphere = pink, and endosphere = yellow).

Figure S4.11. Relative abundance of microbial functional processes pertaining to stress responses across all bulk soil, rhizosphere, endosphere samples. Sample labels are coloured by aridity index of sampling sites, whereas bar labels indicate plant compartment (bulk soil = blue, rhizosphere = pink, and endosphere = yellow).

Figure S4.12. Principal coordinates analysis with Bray-Curtis distances for six different functional gene categories annotated to subsystem 1, showing principal coordinates 1 (axis.1) and 2 (axis.2). Point shape and hull colours represent the samples belonging to the different plant compartments (bulk soil =blue, rhizosphere = pink, endosphere =yellow), whereas point colour shows the mean annual aridity index. Functional annotations include: (a) motility and chemotaxis, (b) stress genes, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) regulation and cell signalling, and (f) secondary metabolism (see Table S4.15 for statistical output on distance to centroid estimates of samples to their respective compartments).

Figure S4.13. Principal coordinates analysis with Bray-Curtis distances for six different functional gene categories annotated to subsystem 1, showing principal coordinates 2 (axis.2) and 3 (axis.3). Point shape and hull colours represent the samples belonging to the different plant compartments (bulk soil =blue, rhizosphere = pink, endosphere =yellow), whereas point colour shows the mean annual aridity index. Functional annotations include: (a) motility and chemotaxis, (b) stress genes, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) regulation and cell signalling, and (f) secondary metabolism (see Table S4.15 for statistical output on distance to centroid estimates of samples to their respective compartments).

Figure S4.14. Distance to centroid estimates for functional genes annotated to subsystem 1, based on principle coordinates analyses (PCoA). Colour represent the samples belonging to the different plant compartments (bulk soil =blue, rhizosphere = pink, endosphere =yellow). Functional annotations include: (a) motility and chemotaxis, (b) stress genes, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) regulation and cell signalling, and (f) secondary metabolism. See Table S4.15 for full statistical output.

Figure S4.15. Counts of unique and shared bacterial species across each *T. triandra* compartment and site. (a) Venn diagram showing unique and overlapping bacterial species across bulk soils rhizospheres and endospheres**.** Petal diagram showing counts that reveal which species are unique or common to each sampling site across: (b) bulk soils, (c) rhizospheres and (d) endospheres.

Figure S4.16. Petal diagram showing counts for motility and chemotaxis functions. Counts show which functions are unique within each site or common to all sites across: (a) bulk soils, (b) rhizospheres and (c) endospheres.

Figure S4.17. Petal diagram showing counts for nitrogen metabolism functions. Counts show which functions are unique within each site or common to all sites across: (a) bulk soils, (b) rhizospheres and (c) endospheres.

Figure S4.18. Petal diagram showing counts for phosphorus metabolism functions. Counts show which functions are unique within each site or common to all sites across: (a) bulk soils, (b) rhizospheres and (c) endospheres.

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Figure S4.19. Petal diagram showing counts for secondary metabolism functions. Counts show which functions are unique within each site or common to all sites across: (a) bulk soils, (b) rhizospheres and (c) endospheres.

Figure S4.20. Petal diagram showing counts for regulation and cell signalling functions. Counts show which functions are unique within each site or common to all sites across: (a) bulk soils, (b) rhizospheres and (c) endospheres.

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Figure S4.21. Petal diagram showing counts for stress response functions. Counts show which functions are unique within each site or common to all sites across: (a) bulk soils, (b) rhizospheres and (c) endospheres.

Figure S4.22. Heatmap of differentially abundant motility and chemotaxis functions annotated at subsystem level 3. Comparisons show the differences across plant compartments (bulk soils, rhizospheres and endospheres). See Table S4.16.

Figure S4.23. Heatmap of differentially abundant nitrogen metabolism functions annotated at subsystem level 3. Comparisons show the differences across plant compartments (bulk soils, rhizospheres and endospheres). See Table S4.16.

Figure S4.24. Heatmap of differentially abundant phosphorus metabolism functions annotated at subsystem level 3. Comparisons show the differences across plant compartments (bulk soils, rhizospheres and endospheres). See Table S4.16

Figure S4.25. Heatmap of differentially abundant secondary metabolism functions annotated at subsystem level 3. Comparisons show the differences across plant compartments (bulk soils, rhizospheres and endospheres). See Table S4.16.

Figure S4.26. Heatmap of differentially abundant regulation and cell signalling functions annotated at subsystem level 3. Comparisons show the differences across plant compartments (bulk soils, rhizospheres and endospheres). See Table S4.16

Figure S4.27. Heatmap of differentially abundant stress response functions annotated at subsystem level 3. Comparisons show the differences across plant compartments (bulk soils, rhizospheres and endospheres). See Table S4.16.

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Figure S4.28. Heatmap of differentially abundant motility and chemotaxis across aridity groups annotated at subsystem level 3. Intercept differences compare the low aridity functions to a grand mean.

Figure S4.29. Heatmap of differentially abundant nitrogen metabolism functions across aridity groups annotated at subsystem level 3. Intercept differences compare the low aridity functions to a grand mean.

Figure S4.30. Heatmap of differentially abundant phosphorus metabolism functions across aridity groups annotated at subsystem level 3. Intercept differences compare the low aridity functions to a grand mean.

Figure S4.31. Heatmap of differentially abundant secondary metabolism functions across aridity groups annotated at subsystem level 3. Intercept differences compare the low aridity functions to a grand mean.

Figure S4.32. Heatmap of differentially abundant regulation and cell signalling functions across aridity groups annotated at subsystem level 3. Intercept differences compare the low aridity functions to a grand mean.

Figure S4.33. Heatmap of differentially abundant stress response functions across aridity groups annotated at subsystem level 3. Intercept differences compare the low aridity functions to a grand mean.

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Figure S4.34. Canonical correspondence analysis (CCA) on bacterial taxonomic community structure. (a) Covariates with r < 0.75 were removed from CCA analyses. CCAs were constructed against environmental predictor variables in (b) bulk soil, (c) rhizosphere, and (d) endosphere communities. Sample points are coloured by sampling site.

Figure S4.35. Canonical correspondence analysis on microbial functional community structure against environmental predictor variables in bulk soils. Functional gene categories include (a) motility and chemotaxis, (b) stress response, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) secondary metabolism, and (f) regulation and cell signalling**.** Coloured points represent samples belonging to each sampling site, black vectors indicate significant environmental variable associations.

Figure S4.36. Canonical correspondence analysis on microbial functional community structure against environmental predictor variables in rhizospheres. Functional gene categories include (a) motility and chemotaxis, (b) stress response, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) secondary metabolism, and (f) regulation and cell signalling. Coloured points represent samples belonging to each sampling site, black vectors indicate significant environmental variable associations.

Figure S4.37. Canonical correspondence analysis on microbial functional community structure against environmental predictor variables in endospheres. Functional gene categories include (a) motility and chemotaxis, (b) stress response, (c) nitrogen metabolism, (d) phosphorus metabolism, (e) regulation and cell signalling**.** Coloured points represent samples belonging to each sampling site, black vectors indicate significant environmental variable associations.

Supplementary references

Blair, G., N. Chinoim, R. Lefroy, G. Anderson, and G. Crocker. 1991. A soil sulfur test for pastures and crops. Soil Research **29**:619-626.

Bonham, C. D. 2013. Measurements for terrestrial vegetation. 2nd edition. Wiley-Blackwell, West Sussex, England.

Colwell, J. D. 1965. An automatic procedure for the determination of Phosphorus in sodium hydrogen carbonate extracts of soils. Chemistry Industry **22**:893-895.

McPherson, M. R., P. Wang, E. L. Marsh, R. B. Mitchell, and D. P. Schachtman. 2018. Isolation and Analysis of Microbial Communities in Soil, Rhizosphere, and Roots in Perennial Grass Experiments. JoVE:e57932.

Walkley, A., and B. I. Armstrong. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science **37**:29-38.

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Supplementary tables

Table S5.1. Sampling sites for high and low aridity soil microbiota. Aridity index data values were extracted from the mean annual aridity index data layer (ADM) and annual precipitation data layer (Clim_PTA) were sourced from the Soil and Landscape Grid of Australia (Searle et al. 2022), where aridity index is calculated via annual precipitation/annual potential evaporation.

Sampling site	Latitude, Longitude	Mean annual aridity index	Aridity category	Annual precipitation (mm)	Provenance
Quorn Floral Reserve	32.3434°S, 138.0182°E	0.227	High aridity	355.2	Soil microbiota
Kuitpo Forest Reserve	35.2279°S, 138.7199°E	0.658	Low aridity	850.5	Soil microbiota and *T. triandra* seed

Table S5.2. *T. triandra* germination rates analysed using generalised linear mixed models (GLMM), with statistical output. '*' denotes p values <0.05, '**' denotes p values <0.01, '***' denotes p values <0.001. †Only output related to aridity variables is reported.

Formula	Fixed effects	Estimate	SE	z-statistic	P-Value
Germination rate ~ Soil aridity + Sterilisation + Seed weight + (1|Pot ID)	(Intercept)	−4.472	0.465	−9.61	<0.001
	Soil aridity (Quorn)	0.087	0.206	0.42	0.67
	Sterilisation (yes)	−0.088	0.206	−0.43	0.67
	Seed weight (g)	397.87	39.91	9.97	
	Random Effects	**Group**	**Variance**	**Standard Deviation**	-
	Intercept	Pot ID	0.195	0.442	-

Table S5.3. *T. triandra* growth traits analysis using randomised linear mixed effects models, with statistical output. Fixed effects estimates, and standard error represent the initial model outputs, prior to our permutations. '*' denotes p values <0.05, '**' denotes p values <0.01, '***' denotes p values <0.001. †Only output related to aridity variables is reported.

Formula (response and predictors)	Random effects	Permutations	Fixed effects	t-statistic	P-Value
Total biomass ~ Sterilisation x Water stress	Aridity	10,000	Sterilisation	-6.877	<0.001***
			Water stress	6.566	<0.001***
			Sterilisation x Water stress	-4.183	<0.001***
Total biomass~ Aridity† x Sterilisation x Water stress	Sterilisation + Water stress	10,000	Aridity	6.246	<0.001***
			Aridity x Sterilisation	-1.015	0.311
			Aridity x Water stress	2.323	0.022*
			Aridity x Sterilisation x Water stress	-0.910	0.364
Aboveground biomass ~ Sterilisation x Water stress	Aridity	10,000	Sterilisation	-5.549	<0.001***
			Water stress	5.700	<0.001***
			Sterilisation x Water stress	-3.539	<0.001***
Aboveground biomass~ Aridity† x	Sterilisation + Water stress	10,000	Aridity	6.983	<0.001***

Sterilisation x Water stress					
			Aridity x Sterilisation	-2.221	0.032*
			Aridity x Water stress	4.252	<0.001***
			Aridity x Sterilisation x Water stress	-2.046	0.046*
Belowground biomass ~ Sterilisation x Water stress	Aridity	10,000	Sterilisation	-4.864	<0.001***
			Water stress	3.67	<0.001***
			Sterilisation x Water stress	-2.484	0.014*
Belowground biomass~ Aridity† x Sterilisation x Water stress	Sterilisation + Water stress	10,000	Aridity	3.586	<0.001***
			Aridity x Sterilisation	0.470	0.6368
			Aridity x Water stress	-0.900	0.3627
			Aridity x Sterilisation x Water stress	0.834	0.4033
Root-mass fraction ~ Sterilisation (High aridity only)	Water	10,000	Sterilisation	3.239	0.003**
Root-mass fraction ~ Sterilisation (Low aridity only)	Water	10,000	Sterilisation	-3.373	0.0016**
Root-mass fraction ~ Aridity x Sterilisation	NA (General linear model)	10,000	Aridity	-3.305	0.0016**

(Control soils only)					
			Sterilisation	-3.259	0.0026**
			Aridity x Sterilisation	3.527	0.001**
Root-mass fraction ~ Aridity x Sterilisation (Water stress soils)	NA (General linear model)	10,000	Aridity	-1.664	0.104
			Sterilisation	-2.303	0.026*
			Aridity x Sterilisation	2.860	0.0071**
Root-mass fraction ~ Aridity† x Sterilisation x Water stress	Sterilisation + Water stress	10,000	Aridity	3.586	<0.001***
			Aridity x Sterilisation	0.470	0.6368
			Aridity x Water stress	-0.900	0.3627
			Aridity x Sterilisation x Water stress	0.834	0.4033

Table S5.4. Linear mixed model output for *T. triandra* growth traits. Random and fixed effects from REML-fitted models. Estimates and standard errors represent unpermuted model outputs.

Model	Effect	Type	Estimate	Std. Dev.
Total biomass ~ sterilisation * water + (1 | sterilisation) + (1 | water)	(Intercept)	Fixed	1.8363	-
	sterilisation (sterilised)	Fixed	-1.1609	-
	water (available)	Fixed	1.0939	-
	sterilisation (sterilised) × water (available)	Fixed	-0.9922	-
	Intercept (soil.source)	Random	-	1.0558
	Residual	Random	-	0.5268
Total biomass ~ soil.source * sterilisation * water + (1 | sterilisation) + (1 | water)	(Intercept)	Fixed	1.141	-
	soil.source (Quorn)	Fixed	1.3905	-
	sterilisation (sterilised)	Fixed	-0.9877	-
	water (available)	Fixed	0.7282	-
	soil.source (Quorn) × sterilisation (sterilised)	Fixed	-0.3241	-
	soil.source (Quorn) × water (available)	Fixed	0.7314	-
	sterilisation (sterilised) × water (available)	Fixed	-0.7993	-

	soil.source (Quorn) × sterilisation (sterilised) × water (available)	Fixed	-0.4079	-
	Intercept (sterilisation random effect)	Random	-	1.0772
	Intercept (water random effect)	Random	-	0.1434
	Residual	Random	-	0.4978
Aboveground biomass ~ sterilisation * water + (1 | sterilisation) + (1 | water)	(Intercept)	Fixed	1.1219	-
	sterilisation (sterilised)	Fixed	0.7439	-
	water (available)	Fixed	-0.7338	-
	sterilisation (sterilised) × water (available)	Fixed	-0.6575	-
	Intercept (soil.source)	Random	-	0.7646
	Residual	Random	-	0.4127
Aboveground biomass ~ soil.source * sterilisation * water + (1 | sterilisation) + (1 | water)	(Intercept)	Fixed	0.6109	-
	soil.source (Quorn)	Fixed	1.022	-
	sterilisation (sterilised)	Fixed	-0.4871	-
	water (available)	Fixed	0.3039	-

	soil.source (Quorn) × sterilisation (sterilised)	Fixed	-0.466	-
	soil.source (Quorn) × water (available)	Fixed	0.88	-
	sterilisation (sterilised) × water (available)	Fixed	-0.3697	-
	soil.source (Quorn) × sterilisation (sterilised) × water (available)	Fixed	-0.603	-
	Intercept (sterilisation random effect)	Random	-	0.06148
	Intercept (water random effect)	Random	-	0.092
	Residual	Random	-	0.32724
Belowground biomass ~ sterilisation * water + (1 | sterilisation) + (1 | water)	(Intercept)	Fixed	0.7652	-
	sterilisation (sterilised)	Fixed	0.3408	-
	water (available)	Fixed	-0.4514	-
	sterilisation (sterilised) × water (available)	Fixed	-0.326	-
	Intercept (soil.source)	Random	-	0.3398
	Residual	Random	-	0.2935
Belowground biomass ~ soil.source * sterilisation *	(Intercept)	Fixed	0.53009	-

water + (1 | sterilisation) + (1 | water)				
	soil.source (Quorn)	Fixed	0.47022	-
	sterilisation (sterilised)	Fixed	-0.49492	-
	water (available)	Fixed	0.4243	-
	soil.source (Quorn) × sterilisation (sterilised)	Fixed	0.08708	-
	soil.source (Quorn) × water (available)	Fixed	-0.16691	-
	sterilisation (sterilised) × water (available)	Fixed	-0.4353	-
	soil.source (Quorn) × sterilisation (sterilised) × water (available)	Fixed	0.21865	-
	Intercept (sterilisation random effect)	Random	-	0.23496
	Intercept (water random effect)	Random	-	0.06838
	Residual	Random	-	0.29321
Root-mass fraction ~ sterilisation + (1 | water) (high aridity soil data only)	(Intercept)	Fixed	0.3366	-
	sterilisation (sterilised)	Fixed	0.1089	-
	Intercept (water random effect)	Random	-	0.03362

	Residual	Random	-	0.10627
Root-mass fraction ~ sterilisation + (1 | water) (Low aridity soil data only)	(Intercept)	Fixed	0.4932	-
	sterilisation (sterilised)	Fixed	-0.1774	-
	Intercept (water random effect)	Random	-	0
	Residual	Random	-	0.1641
Root-mass fraction ~ sterilisation * soil source (water control data only)	(Intercept)	Fixed	0.5168	-
	sterilisation (sterilised)	Fixed	-0.2069	-
	soil.source (Quorn)	Fixed	-0.2097	-
	soil.source (Quorn) × sterilisation (sterilised)	Fixed	0.3166	-
Root-mass fraction ~ sterilisation * soil source (water stress data only)	(Intercept)	Fixed	0.4697	-
	sterilisation (sterilised)	Fixed	-0.1473	-
	soil.source (Quorn)	Fixed	-0.1035	-
	soil.source (Quorn) × sterilisation (sterilised)	Fixed	0.2552	-
Root-mass fraction ~ sterilisation * soil source * water + (1|sterilisation) + (1|water)	(Intercept)	Fixed	0.46967	-

	soil.source (Quorn)	Fixed	-0.10355	-
	sterilisation (sterilised)	Fixed	-0.14727	-
	water (available)	Fixed	0.04709	-
	soil.source (Quorn) × sterilisation (sterilised)	Fixed	0.25524	-
	soil.source (Quorn) × water (available)	Fixed	-0.1062	-
	sterilisation (sterilised) × water (available)	Fixed	-0.0596	-
	soil.source (Quorn) × sterilisation (sterilised) × water (available)	Fixed	0.06136	-
	Intercept (sterilisation random effect)	Random	-	0.01499
	Intercept (water random effect)	Random	-	0.01042
	Residual	Random	-	0.14057

Table S5.5. Statistical output for *T. triandra* Plant-soil feedback (PSF) ratios for biomass and root-mass fractions with bootstrapped 95% confidence intervals at 10,000 permutations. Significant differences between pairwise groups were interpreted when upper or lower confidence intervals did not alight with means in the comparative treatment (See Figure S8).

Growth trait	Comparison group 1	Comparison group 2	Mean difference	Lower CI	Upper CI	Significance
Total Biomass ~	High aridity-Stress	Low aridity-Stress	-6.264	-13.136	-1.740	*
	High aridity-Stress	High aridity-Control	-0.688	-1.970	0.262	
	High aridity-Stress	Low aridity-Control	-21.138	-29.971	-14.546	*
	Low aridity-Stress	High aridity-Control	5.576	0.927	12.424	*
	Low aridity-Stress	Low aridity-Control	-14.874	-25.247	-5.189	*
	High aridity-Control	Low aridity-Control	-20.450	-29.328	-13.754	*
Aboveground Biomass~	High aridity-Stress	Low aridity-Stress	-3.546	-9.109	0.270	
	High aridity-Stress	High aridity-Control	-0.830	-2.518	0.443	
	High aridity-Stress	Low aridity-Control	-13.655	-20.457	-8.410	*
	Low aridity-Stress	High aridity-Control	2.716	-1.429	8.439	
	Low aridity-Stress	Low aridity-Control	-10.109	-18.259	-2.251	*
	High aridity-Control	Low aridity-Control	-12.824	-19.669	-7.394	*
Belowground Biomass~	High aridity-Stress	Low aridity-Stress	-14.610	-28.628	-7.284	*
	High aridity-Stress	High aridity-Control	-0.320	-1.358	0.585	
	High aridity-Stress	Low aridity-Control	-39.186	-61.129	-22.930	*

	Low aridity-Stress	High aridity-Control	14.290	6.973	28.433	*
	Low aridity-Stress	Low aridity-Control	-24.576	-47.994	-3.071	*
	High aridity-Control	Low aridity-Control	-38.867	-60.890	-22.576	*
Root-mass Fraction~	High aridity-Stress	Low aridity-Stress	-0.744	-1.599	-0.185	*
	High aridity-Stress	High aridity-Control	0.036	-0.212	0.298	
	High aridity-Stress	Low aridity-Control	-0.924	-1.641	-0.281	*
	Low aridity-Stress	High aridity-Control	0.780	0.252	1.624	*
	Low aridity-Stress	Low aridity-Control	-0.179	-1.057	0.820	
	High aridity-Control	Low aridity-Control	-0.959	-1.658	-0.356	*

Table S5.6. Statistical output for randomised linear mixed effects models, and randomised ANOVAs. The effect of each treatment variables on *T. triandra* associated with bacterial alpha diversity under live and sterile communities. '*' denotes p values <0.05, '**' denotes p values <0.01, '***' denotes p values <0.001. †Only output related to aridity variables is reported.

Formula	Random effects	Permutations	Fixed effect	T-statistic	P-value
Effective No. ASVs (Live only) ~ Aridity	Compartment + Water stress	10,000	Aridity	-0.156	0.875
Effective No. ASVs (Live only) ~ Water stress	Aridity + Compartment	10,000	Water stress	-1.049	0.312
Effective No. ASVs (Sterilised only) ~ Aridity	Compartment + Water stress	10,000	Aridity	-1.774	0.086
Effective No. ASVs (Sterilised only) ~ Water stress	Aridity + Compartment	10,000	Water stress	0.211	0.833
Effective No. ASVs ~ Water stress (Initial sampling)	Water stress + Aridity	10,000	Sterilisation	-8.760	<0.001***
Effective No. ASVs ~ Water stress (Harvest)	Compartment + Water stress + Aridity	10,000	Sterilisation	-17.296	<0.001***

Table S5.7. Statistical output for alpha beta diversity (bacterial community composition) across *T. triandra* bulk soils, rhizospheres and endospheres using permuted multivariate analysis of variance (PERMANOVA). ‘*’ denotes p values <0.05, ‘**’ denotes p values <0.01, ‘***’ denotes p values <0.001. †Only output related to aridity variables is reported.

Compartment	Predictor variable	Degrees of Freedom	F-statistic	R squared	P-Value
Soil~	Aridity	1 and 102	22.129	0.153	p<0.001***
	Sterilisation	1 and 102	18.117	0.126	p<0.001***
	Water Stress	1 and 102	2.116	0.015	p=0.013*
Rhizosphere~	Aridity	1 and 36	11.737	0.191	p<0.001***
	Sterilisation	1 and 36	11.630	0.189	p<0.001***
	Water Stress	1 and 36	2.215	0. 036	p<0.023*
Endosphere~	Aridity	1 and 36	10.522	0.172	p<0.001***
	Sterilisation	1 and 36	12.696	0.207	p<0.001***
	Water Stress	1 and 36	1.976	0.032	P=0.033*

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Table S5.8. Differential abundance output showing log fold change in bacterial phyla across each plant timepoint and compartment (soil, rhizosphere and endosphere), and each treatment (microbial aridity, sterilisation and water stress). All comparisons are made to high aridity, live, control watering treatments. Reference comparisons represent LFC change from grand mean. 'NS' indicate non-significant LFC results. '*' represents p<0.05, '**' represents p<0.01, and '***' represents p<0.001.

Compartment	Bacterial phylum	Reference (High aridity: Live: Control)	~Low aridity	~Sterile	~Water-stress
Initial sampling: Soil~	Actinobacteriota	0.523*	-0.668**	-1.332**	0.208 NS
	Firmicutes	-0.185 NS	-1.209**	2.161**	0.214 NS
	Verrucomicrobiota	0.553*	-1.021**	-0.941 NS	0.205 NS
	Bacteroidota	-0.676*	0.739 NS	0.839 NS	0.467 NS
	Gemmatimonadota	0.535 NS	-0.668*	-1.499 NS	0.279 NS
	Myxococcota	-0.145 NS	0.887**	-0.58 NS	-0.084 NS
	Cyanobacteria	0.441 NS	-1.171*	-1.364*	0.51 NS
	Armatimonadota	0.336 NS	-1.549**	-0.076 NS	0.485 NS
	RCP2-54	-1.775***	2.26***	2.408**	0.032 NS
	Nitrospirota	0.294 NS	-1.554**	0.017 NS	0.327 NS
	WPS-2	1.35**	-2.489***	-0.735 NS	-0.439 NS
	Methylomirabilota	-2.001***	2.544***	0.895 NS	0.444 NS
	Dependentiae	-1.175**	0.832 NS	2.427**	1.14 NS
	Deinococcota	-0.553 NS	-0.377 NS	2.325*	-0.19 NS
	WS2	-0.706 NS	-0.371 NS	3.278**	0.831 NS

	Entotheonellaeota	0.45 NS	-1.108*	0.084 NS	-0.088 NS
Harvest: Soil~	Actinobacteriota1	0.028 NS	-0.047 NS	-0.757***	0.773***
	Fibrobacterota	-0.421*	0.788***	0.958***	-0.662***
	Firmicutes1	-1.14***	-0.263 NS	2.853***	0.124 NS
	Verrucomicrobiota1	-0.255 NS	-0.037 NS	0.754*	0.02 NS
	Acidobacteriota	0.714***	-0.058 NS	-0.9***	-0.524**
	Bacteroidota1	-0.692*	0.721*	1.476***	-0.425 NS
	Myxococcota1	-0.101 NS	0.506*	0.157 NS	-0.273 NS
	Sumerlaeota	0.436 NS	-1.308***	2.008***	-1.101***
	Desulfobacterota	-0.251 NS	0.664*	1.363***	-1.192***
	Dadabacteria	-0.811***	-0.312 NS	2.132***	-2.077***
	Armatimonadota1	0.54 NS	-0.721*	0.367 NS	-0.657*
	Patescibacteria	0.75*	0.129 NS	-0.524 NS	-1.028**
	RCP2-541	0.858**	-0.469 NS	-0.428 NS	-0.736*
	Bdellovibrionota	-0.385 NS	0.078 NS	1.64***	-0.353 NS
	Hydrogenedentes	-0.1 NS	0.443 NS	1.071***	-1.533***
	Nitrospirota1	0.515*	-0.534*	-0.146 NS	-0.414 NS
	WPS-21	0.683*	-0.466 NS	0.195 NS	-1.076***
	Methylomirabilota1	-0.811***	1.979***	-1.154***	-0.4*
	Latescibacterota	-1.434***	2.576***	0.047 NS	-0.38 NS
	Dependentiae1	0.404 NS	-0.62*	1.081***	-0.858**

Harvest: Rhizosphere~	Deinococcota1	-0.721***	0.205 NS	1.592***	-0.27 NS
	MBNT15	0.37 NS	-0.057 NS	0.696**	-1.152***
	WS21	0.933***	-0.85***	0.025 NS	-0.931***
	NB1-j	0.518**	0.614**	-1.226***	-1.09***
	Entotheonellaeota1	0.318 NS	-0.526*	-0.093 NS	0.003 NS
	Planctomycetota	-0.188 NS	0.336 NS	0.669*	-0.352 NS
	SAR324_clade (Marine_group_B)	-0.748***	0.648**	1.09***	-1.18***
	Elusimicrobiota	0.514*	-0.549*	0.017 NS	-0.535**
	Actinobacteriota2	-0.398 NS	0.06 NS	-0.024 NS	0.916***
	Fibrobacterota1	-1.378***	1.057**	0.95**	0.726 NS
	Firmicutes2	-1.217**	0.079 NS	1.741***	0.562 NS
	Chloroflexi	0.559**	-0.15 NS	-0.543**	-0.187 NS
	Acidobacteriota1	0.616***	0.476*	-1.217***	-0.218 NS
	Bacteroidota2	-0.225 NS	0.166 NS	0.486*	-0.024 NS
	Gemmatimonadota1	0.344 NS	-0.826***	-0.041 NS	0.364 NS
	Myxococcota2	-0.737 NS	0.806*	-0.54 NS	1.367***
	Sumerlaeota1	0.994**	-0.952*	0.703 NS	-1.499***
	Desulfobacterota1	-0.471 NS	0.712**	0.576*	-0.165 NS
	Dadabacteria1	-3.156***	0.943***	3.805***	-1.41***
	Armatimonadota2	0.914***	-0.959***	-0.253 NS	-0.384 NS
	Patescibacteria1	0.71 NS	-0.237 NS	-1.821***	0.885 NS
	RCP2-54	1.277***	-0.562 NS	-1.352***	-0.355 NS

Harvest: Endosphere~	Hydrogenedentes1	0.045 NS	1.075**	0.509 NS	-1.645***
	Methylomirabilota2	-0.348 NS	1.109***	-1.815***	0.244 NS
	Latescibacterota1	-0.819**	3.518***	-0.682*	-0.554 NS
	Dependentiae2	0.396 NS	-0.141 NS	0.565*	-1.004***
	WS22	0.805**	-0.361 NS	-1.867***	0.131 NS
	NB1-j1	1.459***	0.052 NS	-2.722***	-0.612*
	Spirochaetota	1.105***	-1.185**	-0.861**	-1.042**
	SAR324_clade(Marine_group_B)1	-0.685*	0.639*	0.861**	-0.447 NS
	Fibrobacterota2	-1.827***	0.272 NS	3.456***	0.06 NS
	Firmicutes3	-1.726***	0.071 NS	3.49***	0.032 NS
	Proteobacteria	-0.36*	0.327 NS	0.833***	-0.298 NS
	Chloroflexi1	0.455 NS	0.223 NS	-0.415 NS	-0.577*
	Verrucomicrobiota2	-0.553 NS	0.336 NS	1.768***	-0.856*
	Bacteroidota3	0.083 NS	-0.322 NS	0.795**	-0.498 NS
	Gemmatimonadota2	0.493 NS	-0.969*	-0.544 NS	0.668 NS
	Myxococcota3	-0.287 NS	0.452 NS	0.591*	-0.328 NS
	Sumerlaeota2	0.747*	-1.847***	0.674*	-0.73 NS
	Desulfobacterota2	-1.484***	1.074***	2.049***	-0.24 NS
	Cyanobacteria1	-0.68*	0.128 NS	1.706***	-0.333 NS
	Armatimonadota3	0.585*	-1.053**	0.323 NS	-0.332 NS
	Patescibacteria2	1.289***	-0.663 NS	-1.966***	-0.107 NS
	Bdellovibrionota1	-0.913***	0.736*	1.883***	-0.651 NS

Hydrogenedentes2	-0.581 NS	-0.707*	1.493***	-0.179 NS
Nitrospirota2	-0.213 NS	0.124 NS	-0.682**	0.239 NS
WPS-22	-0.406 NS	-0.176 NS	1.323***	-0.561 NS
Dependentiae3	-0.099 NS	-0.3 NS	1.169***	-0.602 NS
Deinococcota2	-1.232***	1.959***	-0.726*	-0.131 NS
MBNT151	-1.384***	0.652*	1.303***	-0.272 NS
WS23	-0.49*	0.53*	0.909***	-0.071 NS
NB1-j2	-1.739***	2.091***	-1.114***	-0.031 NS
Spirochaetota1	-0.333 NS	0.545*	0.795**	-2.101***
Planctomycetota1	-0.801**	0.636*	1.288***	-0.562 NS
SAR324_clade(Marine_group_B)2	0.049 NS	-0.501*	-0.103 NS	0.47 NS

Table S5.9. Differential abundance output showing log fold change in bacterial phyla across each control pot (plant-absent) across and each treatment (microbial aridity, sterilisation and water stress). All comparisons are made to high aridity, live, control watering treatments. Reference comparisons represent LFC change from grand mean. 'NS' indicate non-significant LFC results, '*' represents p<0.05, '**' represents p<0.01, and '***' represents p<0.001.

Compartment	Bacterial phylum	Reference (High aridity: Live: Control)	~Low aridity	~Sterile	~Water-stress
Initial sampling:					
Soil~	Actinobacteriota	0.015 NS	-0.182 NS	-0.662*	0.318 NS
	Firmicutes	-0.508 NS	-0.925 NS	1.95*	0.356 NS
	Chloroflexi	-0.568**	0.466 NS	0.739*	0.195 NS
	Bacteroidota	-0.872**	0.567 NS	1.372*	0.37 NS
	Gemmatimonadota	0.13 NS	-0.597 NS	-0.581*	0.334 NS
	Myxococcota	-0.15 NS	1.262*	-1.13 NS	-0.122 NS
	Cyanobacteria	-0.667 NS	-0.834 NS	1.306*	1.199*
	RCP2-54	-2.273**	2.828*	-0.268 NS	0.854 NS
	WPS-2	0.886*	-1.927**	-1.235 NS	-0.17 NS
	Methylomirabilota	-1.548**	3.001**	0.722 NS	0.138 NS
	Entotheonellaeota	-0.642 NS	0.274 NS	-0.435 NS	1.197*
	Planctomycetota	-1.294**	0.902 NS	1.385 NS	1.002 NS
Harvest: Soil~	Actinobacteriota1	-0.247 NS	0.09 NS	-0.437 NS	1.03**
	Fibrobacterota	-0.752*	0.877 NS	1.233**	-1.013**
	Firmicutes1	-2.417**	-0.014 NS	3.701***	1.085 NS
	Proteobacteria	-0.099 NS	0.13 NS	0.657*	-0.414 NS
	Acidobacteriota	0.633 NS	0.416 NS	-0.476 NS	-1.097*
	Bacteroidota1	-1.128*	1.375**	2.027***	-0.878*
	Sumerlaeota	0.064 NS	-0.847 NS	2.013**	-1.142*
	Desulfobacterota	-0.493 NS	0.819 NS	1.484*	-1.481*
	Dadabacteria	1.258**	-0.005 NS	-0.021 NS	-2.353***
	Armatimonadota	0.369 NS	0.151 NS	0.589 NS	-1.382**
	Patescibacteria	0.914 NS	1.123 NS	-1.147*	-1.72**
	Bdellovibrionota	-0.676*	0.376 NS	1.381***	-0.176 NS
	Hydrogenedentes	-0.177 NS	1.28 NS	0.37 NS	-2.497***
	Nitrospirota	0.926*	-0.147 NS	-0.592 NS	-1.198**
	Latescibacterota	-2.879***	5.28***	0.161 NS	-1.641**
	Dependentiae	0.161 NS	0.051 NS	1.026**	-1.261**
	MBNT15	0.571 NS	0.207 NS	0.516 NS	-2.14***
	WS2	0.203 NS	0.346 NS	0.258 NS	-1.262**

	NB1-j	1.802**	-0.209 NS	-2.81***	-1.864**
	Planctomycetota1	0.069 NS	0.565 NS	0.632 NS	-1.184*
	SAR324_clade (Marine_group_B)	-0.634 NS	0.811 NS	0.559 NS	-2.007**

Supplementary information: Chapter five

Supplementary figures

Figure S1. Experimental design for *T. triandra* growth trial. a) 8 experimental treatments with microbiota sourced from high (sun) and low aridity (cloud) soils, plants are then raised in these soils under live (block colour) and sterilised (striped colour) conditions, then subjected to water stress and control treatments (10 pots per treatment). (b) After sowing of 8 plant seeds per pot, and thinning of seedlings (one per pot), plants across each treatment are grown in the glasshouse for a total of 5 months, before growth trait measurements are made during harvest. (c) An additional 24 soil-only pots were maintained across these treatments with not *T. triandra* seeds planted to monitor changes in microbial communities in the absence of growing plants (8 treatments, 3 pots per treatment).

Figure S5.2. The relative soil water content (RSWC) percentage of each pot throughout the duration of the growth trial. Each line represents a single pot, coloured by treatment group including label codes for: High (Q) and low (K) microbial aridity; live (L) and sterile (S)treatments; water stress (D) and control (W), as well as the plant absent, control pots (C). Dotted lines indicate watering levels throughout the experiment. Control treatments were watered to 100% RSWC throughout the duration, whereas the water stress treatments were gradually stressed first to 70%, then 50% and finally 40% RSWC. RSWC is based on the average of lowest RSWC value (before watering) and highest RSWC value (after watering), for each recorded day.

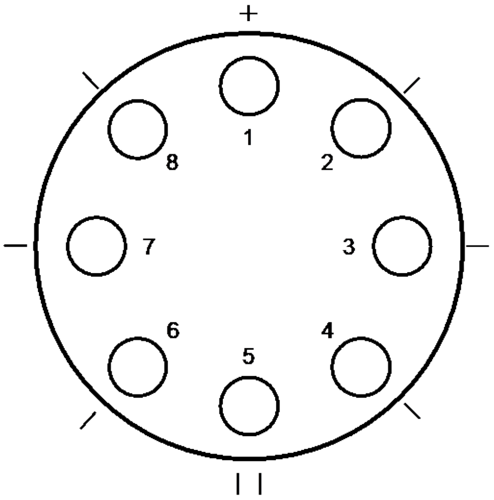

Figure S5.3. Seed planting template for pots during germination trial. Eight different seeds were planted radially with identifying marks on the lip of each part for traceability.

Supplementary information: Chapter five

Figure S5.4. Rarefaction plot showing number of reads against estimated number of species (ASVs). Each sample is coloured by plant compartment (soil, rhizosphere, and endosphere) at the initial soil sampling and the plant harvest. Samples were rarefied to 18,738 reads (black vertical line).

Figure S5.5. (a) Cumulative germination curve showing average recorded germination percentage per pot, across each soil treatment and (b) predicted effects plot showing predicted germination against seed weight. Treatments include: (Quorn, Kuitpo), and sterilisation treatment (Live, Sterile). Errorbars represent 95% confidence intervals.

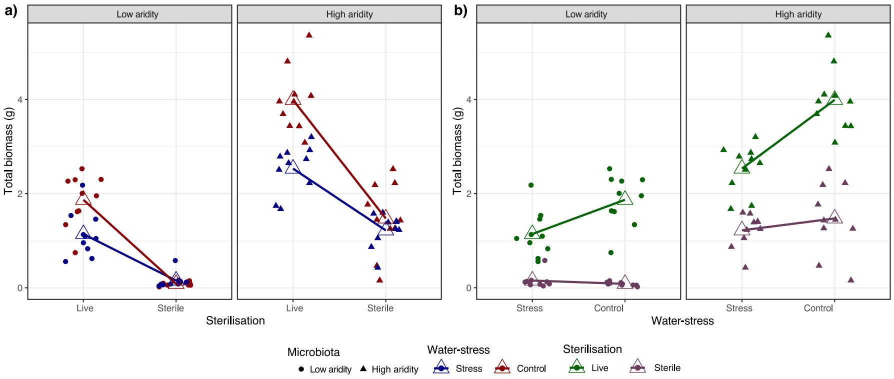

Figure S5.6. Interaction plot for *T. triandra* total biomass (g). Interaction plots show: a) low and high ardity microbiotas, showing how sterilisation impacts *T. triandra* growth under water stress versus control, and b) how low and high ardity microbiotas impact *T. triandra* growth under water stress conditions. Lines connect means across treatments to highlight interaction patterns between categorical levels; they are included for interpretive clarity and do not imply continuity.

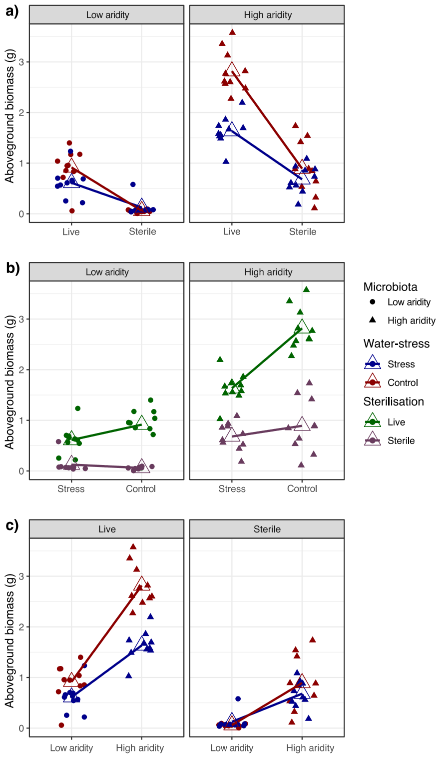

Figure S5.7. Interaction plot for aboveground *T. triandra* biomass (g). a) low and high ardity microbiotas, showing how sterilisation impacts *T. triandra* aboveground biomass under water stress versus control, and b) how low and high ardity microbiotas impact *T. triandra* aboveground biomass under water stress conditions, and c) *T. triandra* aboveground biomass response to water stress is impacted by microbial aridity across live and sterile conditions. Lines connect means across treatments to highlight interaction patterns between categorical levels; they are included for interpretive clarity and do not imply continuity.

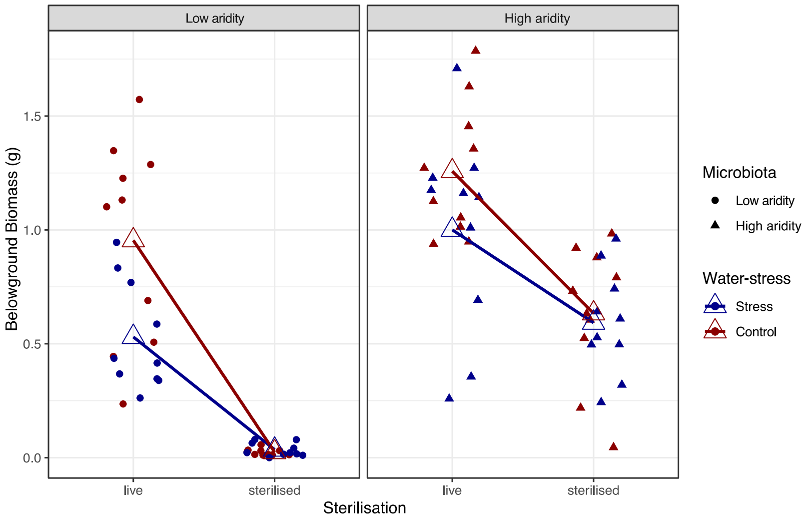

Figure S5.8. Interaction plot for belowground *T. triandra* biomass (g). a) low and high ardity microbiotas, showing how sterilisation impacts *T. triandra* beloggground biomass under water stress. Lines connect means across treatments to highlight interaction patterns between categorical levels; they are included for interpretive clarity and do not imply continuity.

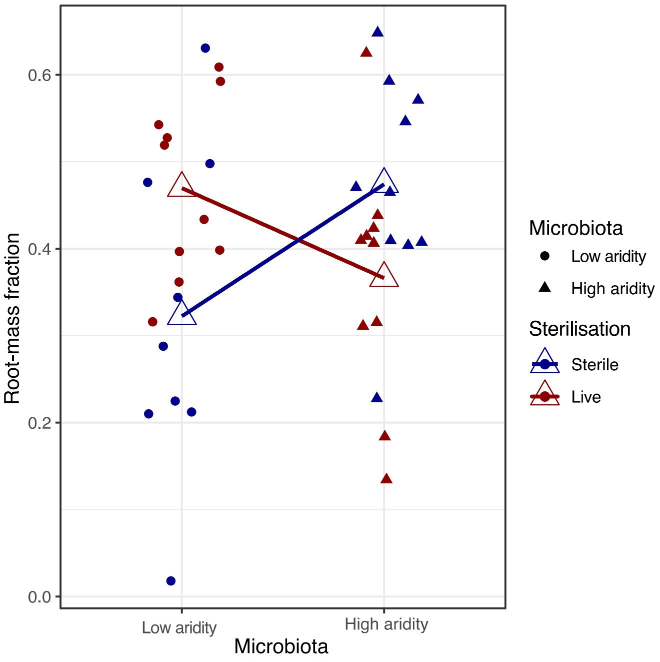

Figure S5.9. Interaction plot for *T. triandra* biomass root-mass fractions. This plot shows how sterilisation impacts *T. triandra* root investment across low and high aridty microbiotas. Lines connect means across treatments to highlight interaction patterns between categorical levels; they are included for interpretive clarity and do not imply continuity.

Figure S5.10. Plant-soil feedback ratios from *T. triandra* high and low arid microbial communities under water stress versus control. These plot show differences in Plant-soil feedback ratios based on *T. triandra* (a) total biomass, (b) aboveground biomass, (c) belowground biomass, and (d) root-mass fractions. Significant differences are indicated by unique lettering (See table S3).

Figure S5.11. Mean relative abundance of major bacterial phyla across plant-absent control pots within *T. triandra* pot soils over time. (a) Compartment and timpeoint included were the initial soil sampling period (t_0: orange crosslabel), soils at plant harvest (t_1: red crosslabel). Treatmens include sterilisation (live, sterile), microbiome aridity (high, low arid sourced soil microbiotas), and watering regime (water-stress as red text labels, control as blue text labels). (b) Differential abundance analysis comparing changes in phyla within each timpoint and compartment across pot soils. Each category compares differences to a reference group (the high aridity, live, control treatment). Log fold changes for the reference groups show changes compared to the grandmean of each phyla.

Figure S5.12. Bacterial alpha diversity differences across *T. triandra* compartments and timpoints. Alpha diversity is indicated by the effective number of ASVs for high and low aridity soils, live and sterilised soils, and water stress and control plants.

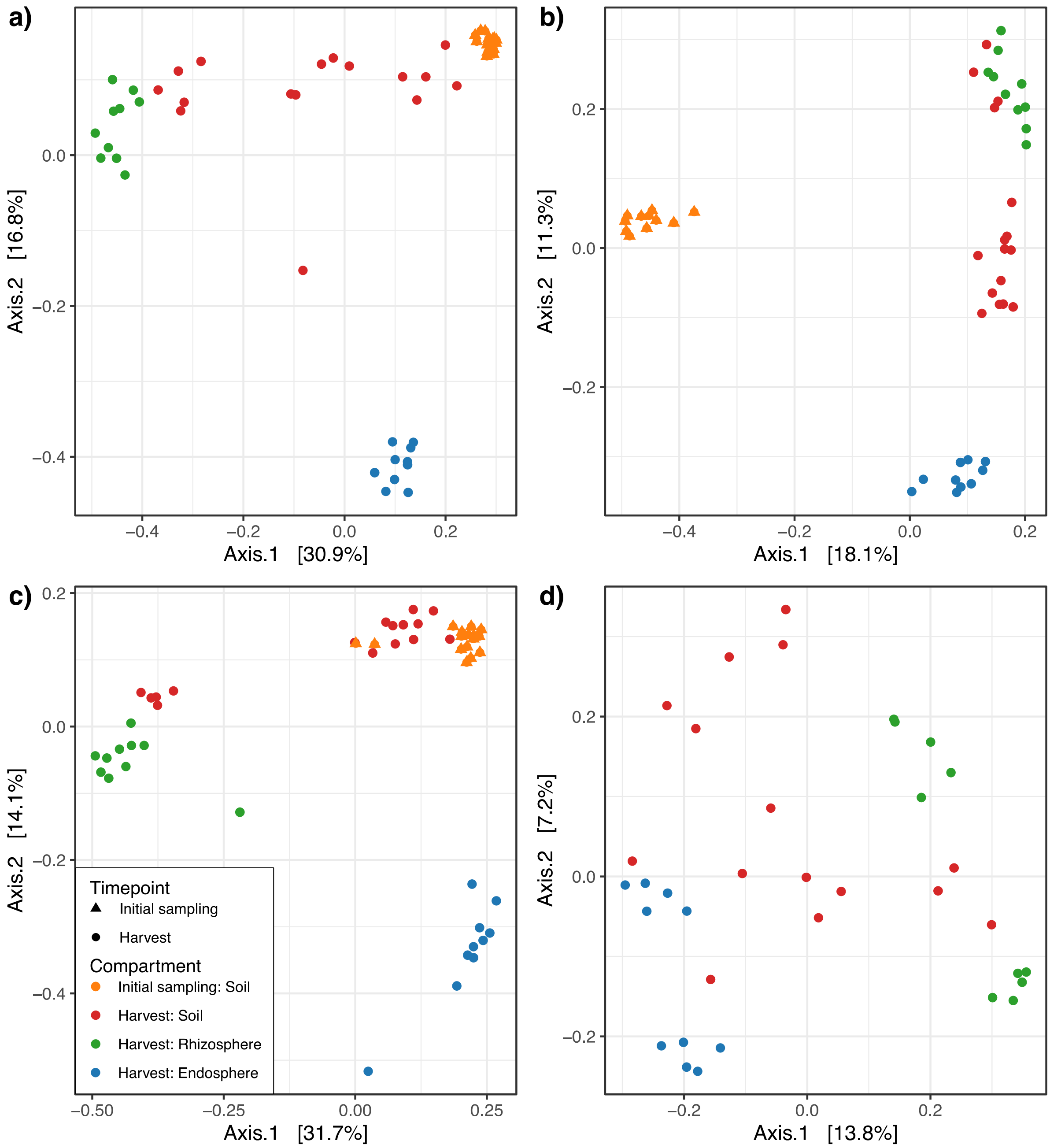

Figure S5.13. Principal co-ordinates analysis of bacterial communities associated with *T. triandra* bulk soils rhizospheres and endosphere. These orination plto show patterns of soil to endosphere community colonisation as per the two- step selection process (beta diversity). Axis 1 and 2 refer to the principal components 1 and 2, respectively.

Figure S5.14. Soil physicochemical differences across each plant treatment. Soil variables included (a) ammonium nitrogen, (b) nitrate nitrogen, (c) phosphorus, (d) potassium, (e) sulphur, (f) organic carbon, (g) electrical conductivity, and (h) pH ($CaCl_2$). Each point represents a sampled pot, coloured by treatment group. Treatment codes label codes are described by lettering with: High (Q) and low (K) microbial aridity; live (L) and sterile (S) treatments; water stress (D) and control (W), as well as the plant absent, control pots (C). Unique indicates significant differences between treatments at the 0.05 significance level using a Dunn test with Holm adjusted p-values.

Appendix A: Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions

Citation:

Shawn D. Peddle, Riley J. Hodgson, Ryan J. Borrett, Stella Brachmann, Tarryn C. Davies, Todd E. Erickson, Craig Liddicoat, Miriam Muñoz-Rojas, Jake M. Robinson, Carl D. Watson, Siegfried L. Krauss, Martin F. Breed 2023. Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions. *Biological Reviews,* ***100****, 1-18*

https://doi.org/10.1111/brv.13124

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Available online at https://doi.org/10.1111/brv.13124

(Peddle et al. 2024)

Appendix B: Opportunities and challenges for microbiomics in ecosystem restoration

Citation:

Jake M. Robinson, **Riley J. Hodgson**, Siegfried L. Krauss Craig Liddicoat, Ashish A. Malik, Belinda C. Martin, Jakki J. Mohr, David Moreno-Mateos, Miriam Muñoz-Rojas, Shawn D. Peddle, Martin F. Breed 2023. Opportunities and challenges for microbiomics in ecosystem restoration. *Trends in Ecology and Evolution* **38**(12), 1189-1202.

https://doi.org/10.1016/j.tree.2023.07.009

Please refer to the signed Co-authorship Approvals for Higher Degree by Research Thesis for Examination*, submitted alongside this thesis in accordance with Clauses 5, 7, and 8 of the* HDR Thesis Rules.

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Available online at https://doi.org/10.1016/j.tree.2023.07.009

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