

Environmental and host history shape microbiota-mediated growth responses of native perennial grasses.

Eleonora Egidi

E.Egidi@westernsydney.edu.au

Western Sydney University Hawkesbury Institute for the Environment <https://orcid.org/0000-0002-1211-2355>

Research Article

Keywords:

Posted Date: October 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11503612/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Background

Plant establishment and growth are strongly influenced by rhizosphere microbial communities, which are shaped by environmental filtering and host-mediated selection. These processes may generate microbial legacies of past environmental conditions and host associations, yet whether such legacies influence plant growth and whether their effects transfer across plant species remains unclear.

Methods

We tested whether rhizosphere communities assembled under contrasting environments retain growth-promotion effects under common conditions and whether these effects extend across host species. Soils associated with *Themeda triandra*, a keystone Australian perennial grass, were collected from sites from contrasting climates (dry sub-humid, semiarid and arid) and used as inocula to grow conspecific (*T. triandra*) and heterospecific (*Austrostipa scabra*, *Chloris truncata*, and *Dichanthium sericeum*) grasses in glasshouse conditions. Effects of inoculation on plant biomass and rhizosphere microbial recruitment were subsequently assessed.

Results

Themeda-derived inocula produced contrasting plant responses depending on environmental origin and host. The inoculum from the dry sub-humid region significantly increased root and shoot biomass and length in *T. triandra*, while inocula from more arid regions had weak or neutral effects on *Themeda* growth. Growth benefits were associated with enrichment of Mortierellomycota and Pseudomonadota in the rhizosphere. The growth promoting effects of the dry sub-humid inoculum was extended to root biomass and length in *A. scabra*, but not the other hosts.

Conclusions

Rhizosphere microbiomes retain signatures of both environmental history and host identity, and their consequences on plant growth are strongly context-dependent.

1. Introduction

Plant germination, establishment and growth in terrestrial ecosystems is increasingly recognised as an emergent property resulting from the interactions between plants and their associated microbial communities (Trivedi et al., 2020; Bai et al., 2022; Compant et al., 2025). Microbes dwelling in the rhizosphere (that is, soils in close proximity of roots) play a particularly important role in influencing key ecological processes, including growth, nutrient acquisition and tolerance to biotic and abiotic stress,

with consequences that scale from individual plants to population dynamics and ecosystem functioning (Khan et al., 2021; Ge et al., 2023). Further, by modifying plant responses to environmental change, microbes shape plant distribution, competitive interactions, and ecosystem resilience under changing climatic conditions (Kandlikar et al., 2019; Delgado-Baquerizo et al., 2020). Plants on the other hand actively structure their associated microbial communities in the rhizosphere by allocating carbon to roots, releasing chemically diverse exudates, modulating immune thresholds, and using specific signalling pathways (Sasse, Martinoia and Northen, 2018; Trivedi et al., 2020).

Despite strong evidence of these ecological relationships, we know remarkably little about how stable or portable beneficial interactions are across environmental and host contexts. In the rhizosphere, root exudate quantity and composition are highly plastic and responsive to environmental conditions (Williams et al., 2022), resulting in shifts in microbial community composition and functions that might affect plant performance (de Vries et al., 2020). Under conditions of reduced water availability, for example, reduced nutrients and altered root exudation patterns lead to restructuring of rhizosphere microbial communities (Chai and Schachtman, 2022; Pan et al., 2024; Silva, Gamage and Thapa, 2025), favouring microbes supporting stress-tolerant traits, sometimes at the expense of plant-growth functions (Hartmann et al., 2017). Critically, the matching between soil microbes and plant environmental histories have been found to influence plant fitness (Lau and Lennon, 2012), suggesting that environmentally-driven rhizosphere selection may influence which microbes persist and which functions are expressed in the rhizosphere (Petipas et al., 2023). These dynamics raise a fundamental question on whether the functional outcomes of plant-microbial interactions in the rhizosphere are contingent to the specific conditions under which they originally formed (Hannula et al., 2021; Nannipieri et al., 2023).

A second unresolved question concerns the transferability of microbiome-derived benefits across host species. Rhizosphere assembly is strongly mediated by host identity through differences in root architecture, exudate chemistry, and immune filtering (Sasse, Martinoia and Northen, 2018; Hawkes, Bull and Lau, 2020; Yue et al., 2023). Experimental evolution studies have shown that mutualistic relationships can be strengthened when they are associated with host genotypes they evolved with (Batstone et al., 2020). Similarly, root commensal bacteria carry host colonisation preferences and are better competitors on their preferred host (Wippel et al., 2021). As such, microbial communities may be locally adapted not only to environmental conditions but also to specific host plants (Petipas et al., 2023), implying that the impact of microbes on plant growth might be strongly host-dependent (Liu et al., 2021; Soumare et al., 2021). However, experimental evidence also indicates that microbial selection can be decoupled from the host over time (Morella et al., 2020), suggesting that, if microbial functions are broadly compatible, benefits may be transferable across hosts (Zhang et al., 2022). Disentangling host specificity from general functional effects is critical for predicting when microbiome-derived benefits can be generalised beyond the plant-microbe combinations in which they originated. These uncertainties have practical implications for applying plant-microbe interactions in managed ecosystems, such as restoration, where microbial effects on plant establishment may heavily depend on environmental and host contexts (Peddle et al., 2025).

Here, we sought to evaluate whether environmentally assembled rhizosphere communities differ in their plant growth promoting effects and whether these effects are transferable across plant species. To specifically test whether the environmental origin leaves a persistent microbial signature that affects plant growth and rhizosphere assembly, we sourced rhizosphere communities associated with *Themeda triandra* from three sites along an aridity gradient (based on the Aridity Index, AI, that is, the ratio of precipitation:evapotranspiration) and evaluated their effect on the growth of *Themeda triandra* under glasshouse conditions. *T. triandra*, also known as kangaroo grass, is a dominant C₄ perennial tussock grass found in grasslands and grassy woodlands across the southern hemisphere, including Australia, with a wide geographical distribution (Snyman, Ingram and Kirkman, 2013; Dunning et al., 2017; Ahrens et al., 2020) and extensive use in restoration (Cole et al., 2004; Prober and Thiele, 2005). Recent studies show that aridity shapes its rhizosphere bacterial communities, while host filtering further structures the microbes colonising its roots (Hodgson et al., 2024, 2025b). Arid soil legacies have also been shown to increase *T. triandra* biomass under both drought and well-watered conditions (Hodgson et al., 2025a). However, whether the environmental history of *T. triandra* rhizosphere microbial communities influences plant growth remains untested. To further determine whether inocula effects on growth persisted across different host species, we examined the influence of *Themeda*-derived rhizosphere inocula on three Australian native grass species that co-occur with *T. triandra* across much of its range: *Dichanthium sericeum* (C₄), *Chloris truncata* (C₄), and *Austrostipa scabra* (C₃). We hypothesised that growth traits of *T. triandra* inoculated with conspecific donor soil will be modulated by the environmental history of the inoculum, with inocula sourced from contrasting environments inducing distinct growth responses, consistent with environmentally contingent microbial function. Further, we expected these microbial effects on plant growth to be host-specific, with *T. triandra* exhibiting stronger responses to inoculation than other grasses, reflecting co-evolutionary relationships between *T. triandra* and its microbial associates.

2. Methods

2.1 Soil collection

Between November and December 2021, rhizosphere soils were collected from three grassland communities supporting natural populations of Australian native grasses, including *Themeda triandra*, *Dichanthium sericeum*, *Chloris truncata*, and *Austrostipa scabra* (Fig. 1A). Three sites located along a 1000 km longitudinal transect from coastal to inland zones of New South Wales, Australia, were selected to represent a gradient spanning high (M1; AI = 0.6; dry sub-humid), medium (M2; AI = 0.25; semi-arid), and low (M3; AI = 0.12; arid) (source: WorldClim - Global Climate Data, v2, <https://agdss.com/>) (Fig. 1B). *T. triandra* rhizosphere-associated soils (donor soils) were sampled from beneath four patches of *T. triandra* occurring within a 20x20 m plot at each site by excavating the roots of one plant from each patch and collecting the soil 10 cm beneath the roots. Plants at similar vegetative stage (flowering) were selected and the samples were collected using sterile tools and subsequently pooled to create a

composite sample representative of each site and sieved to remove coarse debris; samples were subsequently kept at 4°C until the experiment was set up (~ 3 months).

2.2 Experimental setup

In March 2022, a controlled glasshouse experiment was set-up to evaluate the effect of the *Themeda* donor soil on the growth and traits of *T. triandra* and three co-occurring grass species. The experiment thus included four different species of native Australian grasses, comprising one C₃ (*Austrostipa scabra*) and three C₄ plants (*Themeda triandra*, *Dichanthium sericeum* and *Chloris truncata*) (Fig. 1A). These species are widespread across Australia and have a broad climatic and edaphic envelope (Harradine and Whalley, 1979; Garden et al., 1996). Seeds were procured from Greening Australia and originated from the Sydney Region to control for provenance effects (Larson, Venette and Larson, 2022). Seeds were germinated in 13x19 cm pots filled with 1.5 kg of sterile potting mix inoculated at a 1:10 ratio with the following *Themeda* donor soil inocula: four 'live' inocula derived from M1, M2, M3; a composite inoculum (M4) mixing M1, M2, and M3; and a sterile version of M4 (M5) as an uninoculated control (Fig. 1B). M5 was sterilised with two cycles of autoclaving at 121°C for 100 minutes. Sterility was subsequently confirmed by plate counts on nutrient agar and potato dextrose agar following serial dilution. Briefly, 5 g of autoclaved soil was suspended in sterile water and serially diluted to 10⁻⁶, with triplicate plates for each dilution incubated for 7 days at 25 and 28°C. As no microbial growth was detected, we considered the sterilisation successful.

Seedlings were maintained under glasshouse conditions (28/18°C day/night temperature cycles) for 23 weeks under well-watered conditions (70–90% field capacity) using sterile water (Fig. 1C). Twenty grams of each *Themeda*-derived rhizosphere inoculum were re-applied to the soil surface as a soil slurry (i.e., donor soil mixed with 100 ml of sterile water) in week 6 of the experiment. Each inoculum treatment included ten pots per grass species, giving a total of 200 pots. Pots were arranged in five spatial blocks, each containing two pots per species–inoculum combination.

At harvest, plant material was collected to assess changes in plant growth, and dry root and shoot biomass and root and shoot lengths were measured (Fig. 1C). Shoot length was measured from the base to the tip of the longest leaf. Root length was measured as length of the longest root within the fibrous root system. The roots were agitated in 10mL sterile phosphate buffer for 2 mins to collect the soil that adhered to them (rhizosphere soil). The resulting slurry was then centrifuged (3000 g for 5 mins), the supernatant was discarded, and the pellets were subsequently used for genomic DNA extraction. The roots and shoots were oven-dried at 40°C for 48 h and weighed to assess dry root and shoot biomass.

2.3 DNA extraction and bioinformatics

The original 'live' inocula derived from *T. triandra* donor soil (M1, M2, M3, and M4; in three technical replicates) were profiled at the start of the experiment to evaluate the initial diversity and composition of the inoculated community. To evaluate changes in microbial community composition, rhizosphere soils

from *T. triandra* and *A. scabra*, both of which showed a significant response to M1 treatments in the greenhouse, were also profiled.

DNA was extracted using the DNeasy PowerSoil Pro DNA Isolation Kit (Qiagen Inc.) following the manufacturer's instructions, except for cell lysis. A FastPrep bead-beating system (speed of 5.5 m/s for 30 s) was used to facilitate cell lysis. The quality and quantity of the DNA were assessed using a NanoDrop spectrophotometre. PCR assays were conducted using primers 799F and 1193R (Bodenhausen, Horton and Bergelson, 2013; Alivisatos et al., 2015) for bacteria and ITS2 (ITS7F/ITS4R) (Ihrmark et al., 2012) for fungi. Sequencing was performed on an Illumina MiSeq platform at the Next Generation Sequencing facility of Western Sydney University. Downstream amplicon sequence processing using the DADA2 platform. Primers were removed from the ITS sequences using the Cutadapt function in Conda. All further amplicon sequence processing was performed using the DADA2 pipeline in the R software (Callahan et al., 2016). A total of 16,383 bacterial and 3,121 fungal Amplicon Sequence Variants (ASVs) were identified, and taxonomy was assigned using the SILVA v138.1 (Quast et al., 2013) database for 16S and the UNITE database (SH, v10.0) for ITS (Abarenkov et al., 2024). Samples were rarefied to an even depth. Amplicon Sequence Variants and taxonomy tables were used for downstream analyses using Phyloseq (McMurdie and Holmes, 2013). Relative abundances were calculated at the phylum level, normalized within each sample and averaged across inocula for each plant species.

2.4 Statistical analyses

After bioinformatics analysis, the composition of the 'live' inocula originating from the *Themeda* rhizosphere donor soils was characterised using the phyloseq package (McMurdie and Holmes, 2013). The relative abundances of ASVs agglomerated at the phylum level were also calculated using the microbiome (Shetty and Lahti, 2019) and microViz packages (Barnett, Arts and Penders, 2021) and plotted as faceted stacked bar graphs. The community beta diversity was calculated using the Bray-Curtis dissimilarity metric with the vegan package (Oksanen et al., 2025) and compositional differences among inocula were visualised as a principal coordinate analysis (PCoA). However, given the low replication, no statistical analysis was performed on the live inocula.

Linear mixed-effects models were used to test the effect of the *Themeda*-derived inocula on each of the plant traits for the four grass species using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova, Brockhoff and Christensen, 2017) packages. Each trait was the response variables, each inoculum (M1-M4) was the fixed effect and the sterile control (M5) was the reference level and replicate block was the random intercept. Models were fit using the restricted maximum likelihood (REML). Where the block variance was zero, the model was refit as a linear model (lm) without the random term. Effect size was extracted with 95% confidence intervals and *p* values using broom.mixed (Bolker et al., 2026)

To assess the composition of the microbial community recruited in the rhizosphere of *T. triandra* and *A. scabra* grown in the glasshouse, beta diversity was calculated using the Bray-Curtis dissimilarity metric with the vegan package. A principal coordinate analysis (PCoA) was performed in the vegan package to

visualise the differences in microbial community composition. The effects of the inoculum treatments (M1-M4) on the microbial community structure were further examined using permutational multivariate analysis of variance (PERMANOVA) with the *adonis2* function from the *vegan* package (Oksanen et al., 2025). The model tested the effect of grass species, inoculum, and their interaction (species*inoculum) and the marginal sum of squares (999 permutations). Further pairwise comparisons between the inoculum treatments for each grass species were performed using the *pairwiseAdonis* package (Martinez Arbizu 2020) with 999 permutations and Benjamini-Hochberg (BH) correction for multiple testing.

To investigate structural differences in rhizosphere microbial communities, the mean relative abundance of bacterial and fungal ASVs was aggregated at the phylum level using the *microbiome* package. Differences in the relative abundance of the top five bacterial and fungal phyla across the inocula were assessed using Kruskal-Wallis tests, followed by pairwise Wilcoxon tests with Benjamini-Hochberg correction for multiple comparisons. Boxplots were produced, and statistical analysis was conducted to analyse differences between the grasses and within each inoculum.

Relationships between the five most abundant bacterial and fungal phyla and plant traits were assessed separately for each plant species using redundancy analysis (RDA; *vegan* package in R). Phylum abundances were used as predictors and plant traits as response variables, with both sets of variables standardised before analysis. Model significance was assessed using 9,999 permutations, and the associated p-values were reported. Forward selection (*ordiR2step*) was used to identify phyla for inclusion in the final RDA models. Pearson's correlations were used to assess pairwise relationships between individual plant traits and phylum abundances.

Finally, to identify the microbial signatures associated with *T. triandra* and *A. scabra*, an indicator species analysis was performed separately for the two species using the *indicspecies* package (De Cáceres, Legendre and Moretti, 2010). Indicator species analyses allow to identify taxa that are significantly associated with particular groups or treatments, based on how specifically and consistently they occur within those groups. To identify specific associations between ASVs, species and inocula, the *IndVal.g* function with 999 permutations was used. Significant indicator ASVs ($p \leq 0.05$) were identified and grouped at the phylum level to visualise compositional differences in microbial associations.

3. Results

3.1 Composition of the original inocula

Comparison across *Themeda*-derived inocula revealed consistent patterns in the five most dominant phyla (Figure S1). Bacillota dominated across all inocula (42–51% of total reads), with Pseudomonadota being more abundant in M2 (25% of total reads) and Actinomycetota in M4 (17% of total reads). Fungi were dominated by Ascomycota (66–77% of total reads), while the abundance of secondary phyla varied: Mortierellomycota was highest in M4 (11% of total reads), Basidiomycota was lowest in M2 (8.8%

of total reads), and Rozellomycota was only found in M3. The ordination showed that the inocula tended to cluster separately based on their origin.

3.2 Plant response to inoculum treatment

Linear mixed-effects models revealed that the responses to microbial inoculation varied among species and traits (Figure S2). In *T. triandra*, inoculum M1 (dry sub-humid) significantly enhanced shoot length, root length, root, and shoot biomass. Inoculum M2 (semi-arid) increased root length and root biomass. Inocula M3 (arid) and M4 (composite) had no significant effects on *T. triandra* growth. In *A. scabra*, inoculum M1 significantly augmented root length and root biomass, while inocula M2, M3, and M4 did not affect any traits. In the case of *C. truncata*, M2 has a significant but very weak positive effect on root biomass, while in *D. sericeum*, none of the inocula had a significant impact on the traits (Fig. 2, Figure S2, Table S1).

3.3 Microbial composition of rhizosphere communities in *T. triandra* and *A. scabra*

Ordination plots based on PCoA revealed clear differences in both bacterial and fungal community compositions between *T. triandra* and *A. scabra* (Fig. 3, Table S2). For bacteria, the first two coordinates explained 8.6% and 5.7% of the variation, whereas for fungi, they accounted for 13.5% and 8.7%, respectively. The rhizosphere communities tended to cluster by plant species and both bacterial and fungal community compositions were significantly influenced by plant species, donor soil inoculum origin, and their interactions ($p < 0.05$; Fig. 3, Table S2). Pairwise comparisons further supported significant compositional differences in bacterial communities between all inoculum pairs for *T. triandra* ($p \leq 0.030$), whereas no pairwise differences were significant for *A. scabra*. Similarly, fungal communities of *T. triandra* differed significantly between all pairs except M2 vs. M4 ($p = 0.112$), while no pairwise differences were significant for *A. scabra* (Table 1).

Table 1

Pairwise PERMANOVA comparisons of bacterial and fungal community composition among microbial inoculum treatments in *Themeda triandra* and *Austrostipa scabra*. Inocula originated from dry sub-humid (M1), semi-arid (M2) and arid (M3) environments, while M4 comprised a composite mixture of the three source inocula. For each pairwise comparison, the table reports the pseudo-F statistic, R^2 (proportion of variation in community composition explained by inoculum treatment) and Benjamini–Hochberg-adjusted p-value (Padj). Significance is indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, $p < 0.10$ and ns, $p \geq 0.05$.

Microbial group	Comparison	<i>T. triandra</i>			<i>A. scabra</i>		
		F-value	R^2	Padj	F-value	R^2	Padj
Bacteria	M1 vs M2	2.57	0.125	0.002(**)	1.02	0.068	0.262(ns)
	M1 vs M3	1.99	0.099	0.002(**)	1.07	0.082	0.262(ns)
	M1 vs M4	1.7	0.116	0.002(**)	1.2	0.107	0.141(ns)
	M2 vs M3	2.36	0.116	0.002(**)	1.05	0.08	0.262(ns)
	M2 vs M4	1.52	0.105	0.03(*)	1.05	0.095	0.262(ns)
	M3 vs M4	1.64	0.112	0.002(**)	1.3	0.139	0.132(ns)
Fungi	M1 vs M2	4	0.182	0.003(**)	1.18	0.077	0.377(ns)
	M1 vs M3	3.21	0.151	0.003(**)	1.34	0.1	0.204(ns)
	M1 vs M4	1.98	0.132	0.02(*)	1.33	0.117	0.204(ns)
	M2 vs M3	1.84	0.093	0.015(*)	1.01	0.078	0.49(ns)
	M2 vs M4	1.42	0.098	0.112(ns)	0.99	0.09	0.49(ns)
	M3 vs M4	1.96	0.131	0.01(*)	1.18	0.129	0.377(ns)

Analysis of the five major bacterial and fungal phyla recruited in the rhizosphere showed significant differences in the relative abundances of Actinomycetota ($p = 0.002$), Pseudomonadota ($p = 0.048$), and Verrucomicrobiota ($p = 0.006$) among the inoculation treatments in *T. triandra*, with Actinomycetota abundance differing significantly between groups (M1 vs M2, M2 vs M4, M2 vs M3). In fungi, significant differences were observed for Ascomycota (M1 vs M2) and Mortierellomycota (M1 vs M2, M1 vs M3). However, there were no significant differences in the relative abundance of the top five bacterial and fungal phyla in the rhizosphere of *A. scabra* (Fig S3).

Indicator species analysis, which identified ASVs significantly associated with individual inoculum treatments, revealed distinct phylum-level patterns across inoculum treatments across hosts, with *T. triandra* associated with higher number of indicator ASVs as compared to *A. scabra*. There were 329 and 88 bacterial indicator ASVs, along with 95 and 47 fungal species, associated with *T. triandra* and *A. scabra*, respectively. Among these, there were two bacterial and three fungal indicator ASVs common to both grasses. Bacillota dominated the bacterial indicator ASVs across both grasses, accounting for nearly all indicator ASVs in *A. scabra* and most of the indicator ASVs in M1 and M3. Pseudomonadota

accounted for the bacterial ASVs associated with M2 and M4 in *T. triandra* but was absent in *A. scabra*. Myxococcota was present only in M4 treatment of *A. scabra*. ASVs also varied between hosts, with *T. triandra* showing greater diversity (31–47 ASVs per inoculum) compared to *A. scabra* (2–28 ASVs per inoculum). Ascomycota dominated both hosts but were consistently more numerous in *T. triandra*. *A. scabra* was associated with minor phyla, such as *Chytridiomycota* (M4) and *Glomeromycota* (M3) (Fig. 4).

Associations between microbial phylum abundances and plant traits differed between the two grass species. In *T. triandra*, the bacterial RDA model was significant and explained 11.5% of the variation in plant traits (Table S3). Forward selection retained Pseudomonadota ($p = 0.008$), which explained 9.6% of the variation and was positively associated with root length and root biomass (Fig. 5). The fungal RDA model was significant and explained 12.1% of the variation (Table S3). Forward selection retained Mortierellomycota ($p = 0.044$), which explained 5.3% of the variation and was positively associated with root biomass. Neither the bacterial nor the fungal RDA models were significant in *A. scabra*.

4. Discussion

This study investigated the interaction between environmental history and host identity on rhizosphere microbial communities and the growth of four commonly co-occurring native Australian grasses. Although plant-microbe interactions are crucial for plant productivity and resilience, their persistence, transferability, and context-dependent nature are insufficiently understood. Our results demonstrate that both environmental history and host identity shape the microbial composition of rhizosphere communities, with implications for plant growth and microbial recruitment in the rhizosphere.

4.1 Environmental legacies of the inoculum shape plant traits but the effects are expressed unevenly

In line with our predictions, our results support the notion that contrasting microbial source environments can influence subsequent plant responses (Kaisermann et al., 2017; Ginnan et al., 2025). The *Themeda*-derived inocula, which originated from contrasting environments, supported distinct communities that resulted in contrasting expression of *Themeda* plant traits under common glasshouse conditions. Inoculation with M1 (dry sub-humid origin) resulted in increased biomass of in *T. triandra* compared to uninoculated control. In contrast, inoculation with M2 (semi-arid origin) induced greater root length and root biomass without increasing shoot traits, indicating a shift in biomass allocation to the root rather than a uniform growth enhancement; conversely, inoculation with M3 (arid) and composite (M4) inocula did not result in any detectable changes in the measured traits compared to non-inoculated controls. Differences between root and shoot responses to inoculum source have been reported in another perennial grass (Khasanova et al., 2023). Interestingly, previous studies reported that arid soil legacies increased *T. triandra* biomass under both drought and well-watered conditions (Hodgson et al., 2025a); the lack of detectable growth benefits in our arid-source inoculum suggests that knowing the climatic origin of the inoculum alone is insufficient to predict inoculum benefits.

These source-specific responses may reflect differences in the organisms and functions introduced by each inoculum, together with differences in their ability to establish. Microbial populations can retain legacies of past environmental conditions (Chase et al., 2021), and community composition and activity can also change rapidly when environmental conditions change (Lau and Lennon, 2012; Broderick et al., 2025; Ginnan et al., 2025). Glasshouse conditions could therefore have filtered each source community differently, altering which microorganisms colonised roots and influenced plant traits. In particular, for M1, which originated from sites with higher average rainfall than the other source environments, the absence of water limitation in the glasshouse may have favoured microorganisms adapted to wetter conditions, potentially contributing to its growth-promoting effect. Consequently, our results point at plant-microbe interactions as outcomes of environmentally structured microbial communities whose influence depends on compatibility with current conditions (Friesen et al., 2011; Friesen, 2013). This explanation, however, remains untested, as the relative contributions of the source communities and their reassembly after transfer was not directly resolved in this study. Further, the seeds used here came from a single Sydney Basin provenance, and interactions between seed provenance and inoculum origin may also have contributed to the detected responses (Lumibao et al., 2022). Similarly, our design comprised of one donor inoculum per source environment, which did not allow to capture within-site variation or distinguish environmental history from other differences among sites. Further studies explicitly evaluating establishment and activity of the introduced communities under matching or mismatching conditions, and across plant genotypes, will be useful to clarify the role of environmental filtering in rhizosphere plant-microbial interactions.

4.2 Host identity shapes rhizosphere assembly and the transferability of inoculum effects

Comparisons between conspecific and heterospecific hosts supported the hypothesis that microbial benefits on plant growth are host specific. *Themeda* consistently gained the greatest growth benefits (higher shoot and root biomass and length) from M1; among the heterospecific hosts, *A. scabra* showed higher root growth and length, while *C. truncata*, and *D. sericeum* displayed weak or neutral responses. These results suggest that *T. triandra*-conditioned rhizosphere microbial assemblage provides the largest number of benefits to its own host, resulting in a limited transfer of beneficial traits across hosts (Hannula et al., 2021; Idbella et al., 2024; Lopes et al., 2026). These findings point at possible host-specific assembly processes that shape both microbial composition and functional expression (Pickles et al., 2015; Revillini, Gehring and Johnson, 2016; Adedayo and Olorunkosebi, 2025).

Consistent with the notion that host identity plays a central role in microbial recruitment and function (Wippel et al., 2021), the inoculated rhizosphere communities differed markedly between conspecific (*T. triandra*) and heterospecific (*A. scabra*) hosts. Host filtering has also been reported during rhizosphere-to-endosphere microbial colonisation in natural populations of *T. triandra* (Hodgson et al., 2025b). In our study, *T. triandra*, which was grown in conspecific rhizosphere soil, hosted a greater number of indicator ASVs (329 bacteria, 95 fungi), and supported stronger association between bacterial composition and plant traits variance, suggesting a distinctive microbial assemblage more closely associated with host

phenotypic variation. Specifically, in *Themeda*, the relative abundance of Mortierellomycota was specifically associated with higher root biomass, whereas Pseudomonadota had positive associations with root biomass and length. These relationships are consistent with experimental evidence that members of Mortierellomycota can promote plant biomass accumulation and modify plant hormone responses (Vandepol et al., 2022). Similarly, inoculation with Pseudomonadota has been associated with root-system development in the perennial grass *Panicum virgatum* (Chen et al., 2022). This earlier evidence supports potential microbial contributions of members from these microbial phyla to growth responses in *Themeda*, although our study cannot directly identify the organisms or processes.

In contrast, *A. scabra* supported fewer indicator ASVs, exhibited weaker clustering, and showed non-significant correspondence between microbial community composition and plant trait variation. The stronger correspondence between microbial composition and growth in *T. triandra* overall indicates that communities derived from its rhizosphere retained greater functional relevance to their original host (Semchenko et al., 2022; Wang et al., 2025). This is consistent with studies showing that microbiomes shaped by a single host show weaker host-filtering and growth benefits when transplanted to a different host (Meyer et al., 2023).

Interestingly, the mixed rhizosphere inoculum (M4), which combined communities shaped by contrasting environmental histories, showed evidence of loss of growth-promoting effects. This suggests that the positive response to M1 depended on community properties that were not conserved, or were diluted, following mixing. Such responses are consistent with community coalescence outcomes being contingent on the identity, relative abundance and compatibility of the source communities (Rillig et al., 2015; Wubs et al., 2019; Castledine et al., 2020; Pacheco, Osborne and Segrè, 2021; Rocca et al., 2021). For restoration, these results may imply that pooling rhizosphere material from several sources should not be assumed to retain the benefits observed for an individual source, and candidate inocula - and any mixtures intended for deployment - need to be assessed for their effects on the target plant.

4.3 Conclusions

Our findings elucidate the relationships between environmental filtering, host identity, and microbial composition in rhizosphere microbiome assembly and provision of host functions. Here we show that environmental and host history exert a persistent influence on microbial communities, which in turn impacts plant growth. The host and context specificity observed in this study supports the notion that the benefits of microbes are not universally transferable among different species. These findings highlight the complexity of predicting plant responses to microbial effects and emphasize the need to integrate microbial legacies while understanding plant microbial interactions in the rhizosphere. Critically, the effects observed here were assessed under a single watering regime, so whether inocula retain, lose or strengthen their effects under climatically variable conditions remains unresolved. It is also possible that the three months storage might have affected the functional activity of the inoculated microbiomes. Further, the relatively small pots might have constrained growth responses, and their strength under field conditions may differ. Field studies will be useful to resolve these relationships under restoration-relevant conditions.

Declarations

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

AI declaration

ChatGPT was used debug and optimise the R code and Paperpal was used to edit the manuscript. All outputs were validated by the authors.

Funding:

E.E. acknowledges support from the Australian Research Council through a Discovery Early Career Researcher Award (DECRA; DE210101822). D.S. is supported by a HDR scholarship from Western Sydney University.

Author's contribution:

D.S.: Methodology; Formal analysis; Data curation; Writing - original draft

Acknowledgements:

E.E. acknowledges support from the Australian Research Council through a Discovery Early Career Researcher Award (DECRA; DE210101822). D.S. is supported by a HDR scholarship from Western Sydney University. The authors would like to thank Sarah McDonald for providing access to *Themeda* collection sites.

Data availability:

The datasets generated and analysed during the current study will be made available upon acceptance.

References

1. Abarenkov K et al (2024) 'The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered', *Nucleic Acids Research*, 52(D1), pp. D791–D797. Available at: <https://doi.org/10.1093/nar/gkad1039>
2. Adedayo AA, Olorunkosebi MT (2025) 'Coevolution Dynamics of Beneficial and Pathogenic Microbes in Plant–Microbe Interactions', *Biology*, 14(11). Available at:

<https://doi.org/10.3390/biology14111505>

3. Ahrens CW et al (2020) 'Spatial, climate and ploidy factors drive genomic diversity and resilience in the widespread grass *Themeda triandra*', *Molecular Ecology*, 29(20), pp. 3872–3888. Available at: <https://doi.org/10.1111/mec.15614>
4. Alivisatos AP et al (2015) 'A unified initiative to harness Earth's microbiomes', *Science*, 350(6260), pp. 507–508. Available at: <https://doi.org/10.1126/science.aac8480>
5. Arbizu PM (2025) 'pmartinezarbizu/pairwiseAdonis'. Available at: <https://github.com/pmartinezarbizu/pairwiseAdonis> (Accessed: 7 November 2025)
6. Bai B et al (2022) 'The root microbiome: Community assembly and its contributions to plant fitness', *Journal of Integrative Plant Biology*, 64(2), pp. 230–243. Available at: <https://doi.org/10.1111/jipb.13226>
7. Barnett DJM, Arts IC, w, Penders J (2021) 'microViz: an R package for microbiome data visualization and statistics', *Journal of Open Source Software*, 6(63), p. 3201. Available at: <https://doi.org/10.21105/joss.03201>
8. Bates D et al (2015) 'Fitting Linear Mixed-Effects Models Using lme4', *Journal of Statistical Software*, 67, pp. 1–48. Available at: <https://doi.org/10.18637/jss.v067.i01>
9. Batstone RT et al (2020) 'Experimental evolution makes microbes more cooperative with their local host genotype', *Science*, 370(6515), pp. 476–478. Available at: <https://doi.org/10.1126/science.abb7222>
10. Bodenhausen N, Horton MW, Bergelson J (2013) 'Bacterial Communities Associated with the Leaves and the Roots of *Arabidopsis thaliana*', *PLOS ONE*, 8(2), p. e56329. Available at: <https://doi.org/10.1371/journal.pone.0056329>
11. Bogati KA, Sewerniak P, Walczak M (2025) 'Unraveling the Effect of Soil Moisture on Microbial Diversity and Enzymatic Activity in Agricultural Soils', *Microorganisms*, 13(6), p. 1245. Available at: <https://doi.org/10.3390/microorganisms13061245>
12. Bolker B, Robinson (2026) 'broom.mixed: Tidying Methods for Mixed Models'. Available at: <https://cran.r-project.org/web/packages/broom.mixed/index.html> (Accessed: 30 September 2026)
13. Broderick CM et al (2025) 'Long-term climate establishes functional legacies by altering microbial traits', *The ISME Journal*, 19(1), p. wraf005. Available at: <https://doi.org/10.1093/ismejo/wraf005>
14. Callahan BJ et al (2016) 'DADA2: High-resolution sample inference from Illumina amplicon data', *Nature Methods*, 13(7), pp. 581–583. Available at: <https://doi.org/10.1038/nmeth.3869>
15. Castledine M et al (2020) 'Community coalescence: an eco-evolutionary perspective', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1798), p. 20190252. Available at: <https://doi.org/10.1098/rstb.2019.0252>
16. Chai YN, Schachtman DP (2022) 'Root exudates impact plant performance under abiotic stress', *Trends in Plant Science*, 27(1), pp. 80–91. Available at: <https://doi.org/10.1016/j.tplants.2021.08.003>

17. Chase AB, Weihe C, Martiny JBH (2021) 'Adaptive differentiation and rapid evolution of a soil bacterium along a climate gradient', *Proceedings of the National Academy of Sciences*, 118(18), p. e2101254118. Available at: <https://doi.org/10.1073/pnas.2101254118>
18. Chen Z et al (2022) 'Plant growth-promoting rhizobacteria with ACC deaminase activity enhance maternal lateral root and seedling growth in switchgrass', *Frontiers in Plant Science*, 12, p. 800783. Available at: <https://doi.org/10.3389/fpls.2021.800783>
19. Cole I et al (2004) 'Effects of soil disturbance, weed control and mulch treatments on establishment of *Themeda triandra* (Poaceae) in a degraded white box (*Eucalyptus albens*) woodland in central western New South Wales', *Australian Journal of Botany*, 52(5), pp. 629–637. Available at: <https://doi.org/10.1071/BT04010>
20. Compant S et al (2025) 'Harnessing the plant microbiome for sustainable crop production', *Nature Reviews Microbiology*, 23(1), pp. 9–23. Available at: <https://doi.org/10.1038/s41579-024-01079-1>
21. De Cáceres M, Legendre P, Moretti M (2010) 'Improving indicator species analysis by combining groups of sites', *Oikos*, 119(10), pp. 1674–1684. Available at: <https://doi.org/10.1111/j.1600-0706.2010.18334.x>
22. Delgado-Baquerizo M et al (2020) 'Increases in aridity lead to drastic shifts in the assembly of dryland complex microbial networks', *Land Degradation & Development*, 31(3), pp. 346–355. Available at: <https://doi.org/10.1002/ldr.3453>
23. Dunning LT et al (2017) 'The recent and rapid spread of *Themeda triandra*', *Botany Letters*, 164(4), pp. 327–337. Available at: <https://doi.org/10.1080/23818107.2017.1391120>
24. Fan J et al (2021) 'Grazing intensity induced alternations of soil microbial community composition in aggregates drive soil organic carbon turnover in a desert steppe', *Agriculture, Ecosystems & Environment*, 313, p. 107387. Available at: <https://doi.org/10.1016/j.agee.2021.107387>
25. Friesen ML et al (2011) 'Microbially Mediated Plant Functional Traits', *Annual Review of Ecology, Evolution, and Systematics*, 42(1), pp. 23–46. Available at: <https://doi.org/10.1146/annurev-ecolsys-102710-145039>
26. Friesen ML (2013) 'Microbially Mediated Plant Functional Traits', *Molecular Microbial Ecology of the Rhizosphere*. John Wiley & Sons, Ltd, pp. 87–102. Available at: <https://doi.org/10.1002/9781118297674.ch8>
27. Garden D et al (1996) 'Regional research on native grasses and native grass-based pastures', *New Zealand Journal of Agricultural Research*, 39(4), pp. 471–485. Available at: <https://doi.org/10.1080/00288233.1996.9513209>
28. Ge J et al (2023) 'Microbial coexistence in the rhizosphere and the promotion of plant stress resistance: A review', *Environmental Research*, 222, p. 115298. Available at: <https://doi.org/10.1016/j.envres.2023.115298>
29. Ginnan NA et al (2025) 'Precipitation legacy effects on soil microbiota facilitate adaptive drought responses in plants', *Nature Microbiology*, 10(11), pp. 2823–2844. Available at: <https://doi.org/10.1038/s41564-025-02148-8>

30. Hannula SE et al (2021) 'Persistence of plant-mediated microbial soil legacy effects in soil and inside roots', *Nature Communications*, 12(1), p. 5686. Available at: <https://doi.org/10.1038/s41467-021-25971-z>
31. Harradine A, Whalley R (1979) 'Relative drought tolerance of three grass species from the Northwestern slopes of New South Wales.', *The Rangeland Journal*, 1(3), pp. 244–247. Available at: <https://doi.org/10.1071/RJ9790244>
32. Hartman K, Tringe SG (2019) 'Interactions between plants and soil shaping the root microbiome under abiotic stress', *Biochemical Journal*, 476(19), pp. 2705–2724. Available at: <https://doi.org/10.1042/BCJ20180615>
33. Hartmann M et al (2017) 'A decade of irrigation transforms the soil microbiome of a semi-arid pine forest', *Molecular Ecology*, 26(4), pp. 1190–1206. Available at: <https://doi.org/10.1111/mec.13995>
34. Hawkes CV, Bull JJ, Lau JA (2020) 'Symbiosis and stress: how plant microbiomes affect host evolution', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1808), p. 20190590. Available at: <https://doi.org/10.1098/rstb.2019.0590>
35. Hodgson RJ et al (2024) 'Increasing aridity strengthens the core bacterial rhizosphere associations in the pan-palaeotropical C4 grass, *Themeda triandra*', *Applied Soil Ecology*, 201, p. 105514. Available at: <https://doi.org/10.1016/j.apsoil.2024.105514>
36. Hodgson RJ, Cando-Dumancela C, Davies T et al (2025a) 'Arid Soil Bacterial Legacies Improve Drought Resilience of the Keystone Grass, *Themeda triandra*', *Molecular Ecology*, 34(18), p. e70062. Available at: <https://doi.org/10.1111/mec.70062>
37. Hodgson RJ, Cando-Dumancela C, Liddicoat C et al (2025b) 'Strong Host Modulation of Rhizosphere-to-Endosphere Microbial Colonisation in Natural Populations of the Pan-Palaeotropical Keystone Grass Species, *Themeda triandra*', *Ecology and Evolution*, 15(6), p. e71595. Available at: <https://doi.org/10.1002/ece3.71595>
38. Idbella M et al (2024) 'Impact of soil legacy on plant–soil feedback in grasses and legumes through beneficial and pathogenic microbiota accumulation', *Frontiers in Microbiology*, 15. Available at: <https://doi.org/10.3389/fmicb.2024.1454617>
39. Ihrmark K et al (2012) 'New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities', *FEMS Microbiology Ecology*, 82(3), pp. 666–677. Available at: <https://doi.org/10.1111/j.1574-6941.2012.01437.x>
40. Kaisermann A et al (2017) 'Legacy effects of drought on plant–soil feedbacks and plant–plant interactions', *New Phytologist*, 215(4), pp. 1413–1424. Available at: <https://doi.org/10.1111/nph.14661>
41. Kandlikar GS et al (2019) 'Winning and losing with microbes: how microbially mediated fitness differences influence plant diversity', *Ecology Letters*, 22(8), pp. 1178–1191. Available at: <https://doi.org/10.1111/ele.13280>
42. Khan N et al (2021) 'Insights into the Interactions among Roots, Rhizosphere, and Rhizobacteria for Improving Plant Growth and Tolerance to Abiotic Stresses: A Review', *Cells*, 10(6). Available at:

<https://doi.org/10.3390/cells10061551>

43. Khasanova A et al (2023) Quantitative genetic-by-soil microbiome interactions in a perennial grass affect functional traits. *Proceedings of the Royal Society B: Biological Sciences*, 290(1991), 20221350
44. Kuznetsova A, Brockhoff PB, Christensen RHB (2017) 'lmerTest Package: Tests in Linear Mixed Effects Models', *Journal of Statistical Software*, 82, pp. 1–26. Available at: <https://doi.org/10.18637/jss.v082.i13>
45. Larson JL, Venette RC, Larson DL (2022) 'Restoration for Resilience: The Role of Plant–Microbial Interactions and Seed Provenance in Ecological Restoration', *Natural Areas Journal*, 42(2), pp. 152–159. Available at: <https://doi.org/10.3375/21-42>
46. Lau JA, Lennon JT (2012) 'Rapid responses of soil microorganisms improve plant fitness in novel environments', *Proceedings of the National Academy of Sciences*, 109(35), pp. 14058–14062. Available at: <https://doi.org/10.1073/pnas.1202319109>
47. Liu T-Y et al (2021) 'Drought stress and plant ecotype drive microbiome recruitment in switchgrass rhizosphere', *Journal of Integrative Plant Biology*, 63(10), pp. 1753–1774. Available at: <https://doi.org/10.1111/jipb.13154>
48. Lopes ACA et al (2026) 'Do plants remember their microbial partners?', *Trends in Plant Science*, 31(2), pp. 132–135. Available at: <https://doi.org/10.1016/j.tplants.2025.07.005>
49. Lumibao CY et al (2022) 'Microbial mediation of salinity stress response varies by plant genotype and provenance over time', *Molecular Ecology*, 31(17), pp. 4571–4585. Available at: <https://doi.org/10.1111/mec.16603>
50. McMurdie PJ, Holmes S (2013) 'phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data', *PLOS ONE*, 8(4), p. e61217. Available at: <https://doi.org/10.1371/journal.pone.0061217>
51. Meyer KM et al (2023) 'Conspecific versus heterospecific transmission shapes host specialization of the phyllosphere microbiome', *Cell Host & Microbe*, 31(12), pp. 2067–2079.e5. Available at: <https://doi.org/10.1016/j.chom.2023.11.002>
52. Morella NM et al (2020) 'Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection', *Proceedings of the National Academy of Sciences*, 117(2), pp. 1148–1159. Available at: <https://doi.org/10.1073/pnas.1908600116>
53. Nannipieri P et al (2023) 'Legacy effects of rhizodeposits on soil microbiomes: A perspective', *Soil Biology and Biochemistry*, 184, p. 109107. Available at: <https://doi.org/10.1016/j.soilbio.2023.109107>
54. Oksanen J et al (2025) 'vegan: Community Ecology Package'. Available at: <https://cran.r-project.org/web/packages/vegan/index.html> (Accessed: 19 November 2025)
55. Pacheco AR, Osborne ML, Segrè D (2021) 'Non-additive microbial community responses to environmental complexity', *Nature Communications*, 12(1), p. 2365. Available at: <https://doi.org/10.1038/s41467-021-22426-3>

56. Pan J et al (2024) 'Soil microbial richness and community composition are primarily mediated by functional trait diversity of fine roots in subtropical forests', *Plant and Soil*, 497(1), pp. 485–501. Available at: <https://doi.org/10.1007/s11104-023-06408-6>
57. Peddle SD et al (2025) 'Practical applications of soil microbiota to improve ecosystem restoration: current knowledge and future directions', *Biological Reviews*, 100(1), pp. 1–18. Available at: <https://doi.org/10.1111/brv.13124>
58. Petipas R et al (2023) *Habitat, plant height, and soil nutrients are important determinants of the Hypericum perforatum microbiome*. preprint. In Review. Available at: <https://doi.org/10.21203/rs.3.rs-2859556/v1>
59. Pickles BJ et al (2015) 'Local adaptation in migrated interior Douglas-fir seedlings is mediated by ectomycorrhizas and other soil factors', *New Phytologist*, 207(3), pp. 858–871. Available at: <https://doi.org/10.1111/nph.13360>
60. Põlme S et al (2020) 'FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles', *Fungal Diversity*, 105(1), pp. 1–16. Available at: <https://doi.org/10.1007/s13225-020-00466-2>
61. Prober SM, Thiele KR (2005) 'Restoring Australia's temperate grasslands and grassy woodlands: integrating function and diversity', *Ecological Management & Restoration*, 6(1), pp. 16–27. Available at: <https://doi.org/10.1111/j.1442-8903.2005.00215.x>
62. Quast C et al (2013) 'The SILVA ribosomal RNA gene database project: improved data processing and web-based tools', *Nucleic Acids Research*, 41(D1), pp. D590–D596. Available at: <https://doi.org/10.1093/nar/gks1219>
63. Revillini D, Gehring CA, Johnson NC (2016) 'The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems', *Functional Ecology*, 30(7), pp. 1086–1098. Available at: <https://doi.org/10.1111/1365-2435.12668>
64. Rillig MC et al (2015) 'Interchange of entire communities: microbial community coalescence', *Trends in Ecology & Evolution*, 30(8), pp. 470–476. Available at: <https://doi.org/10.1016/j.tree.2015.06.004>
65. Rocca JD et al (2021) 'Guided by Microbes: Applying Community Coalescence Principles for Predictive Microbiome Engineering', *mSystems*, 6(4), p. 10.1128/msystems.00538 – 21. Available at: <https://doi.org/10.1128/msystems.00538-21>
66. Sasse J, Martinoia E, Northen T (2018) 'Feed Your Friends: Do Plant Exudates Shape the Root Microbiome?', *Trends in Plant Science*, 23(1), pp. 25–41. Available at: <https://doi.org/10.1016/j.tplants.2017.09.003>
67. Semchenko M et al (2022) 'Deciphering the role of specialist and generalist plant–microbial interactions as drivers of plant–soil feedback', *New Phytologist*, 234(6), pp. 1929–1944. Available at: <https://doi.org/10.1111/nph.18118>
68. Shetty SA, Lahti L (2019) 'Microbiome data science', *Journal of Biosciences*, 44(5), p. 115. Available at: <https://doi.org/10.1007/s12038-019-9930-2>

69. Silva SD, Gamage LKH, Thapa VR (2025) 'Impact of Drought on Soil Microbial Communities', *Microorganisms*, 13(7). Available at: <https://doi.org/10.3390/microorganisms13071625>
70. Snyman HA, Ingram LJ, Kirkman KP (2013) Themeda triandra: a keystone grass species. *African Journal of Range & Forage Science*, 30(3), pp.99–125. Available at: <https://doi.org/10.2989/10220119.2013.831375>
71. Soumare A et al (2021) 'Potential Role and Utilization of Plant Growth Promoting Microbes in Plant Tissue Culture', *Frontiers in Microbiology*, 12. Available at: <https://doi.org/10.3389/fmicb.2021.649878>
72. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant–microbiome interactions: from community assembly to plant health. *Nature reviews microbiology*, 18(11), pp.607–621. Available at: <https://doi.org/10.1038/s41579-020-0412-1>
73. Vandepol N, Liber J, Yocca A, Matlock J, Edger P, Bonito G (2022) Linnemannia elongata (Mortierellaceae) stimulates Arabidopsis thaliana aerial growth and responses to auxin, ethylene, and reactive oxygen species. *PLoS One.* ;17(4): e0261908. Available at: <https://doi.org/10.1371/journal.pone.0261908>
74. de Vries FT et al (2020) 'Harnessing rhizosphere microbiomes for drought-resilient crop production', *Science*, 368(6488), pp. 270–274. Available at: <https://doi.org/10.1126/science.aaz5192>
75. Wang H et al (2025) 'Rhizosphere priming effects and trade-offs among root traits, exudation and mycorrhizal symbioses', *Soil Biology and Biochemistry*, 202, p. 109690. Available at: <https://doi.org/10.1016/j.soilbio.2024.109690>
76. Williams A et al (2022) 'Root functional traits explain root exudation rate and composition across a range of grassland species', *Journal of Ecology*, 110(1), pp. 21–33. Available at: <https://doi.org/10.1111/1365-2745.13630>
77. Wippel K, Tao K, Niu Y, Zgadzaj R, Kiel N, Guan R, Dahms E, Zhang P, Jensen DB, Logemann E, Radutoiu S (2021) Host preference and invasiveness of commensal bacteria in the Lotus and Arabidopsis root microbiota. *Nat Microbiol* 6(9):1150–1162
78. Wubs ERJ et al (2019) 'Soil Inoculation Steers Plant-Soil Feedback, Suppressing Ruderal Plant Species', *Frontiers in Ecology and Evolution*, 7. Available at: <https://www.frontiersin.org/articles/10.3389/fevo.2019.00451> (Accessed: 6 June 2023)
79. Yue H et al (2023) 'Plant domestication shapes rhizosphere microbiome assembly and metabolic functions', *Microbiome*, 11(1), p. 70. Available at: <https://doi.org/10.1186/s40168-023-01513-1>
80. Zhang Z et al (2022) 'Cross-inoculation of rhizobiome from a congeneric ruderal plant imparts drought tolerance in maize (Zea mays) through changes in root morphology and proteome', *The Plant Journal*, 111(1), pp. 54–71. Available at: <https://doi.org/10.1111/tpj.15775>

Figures

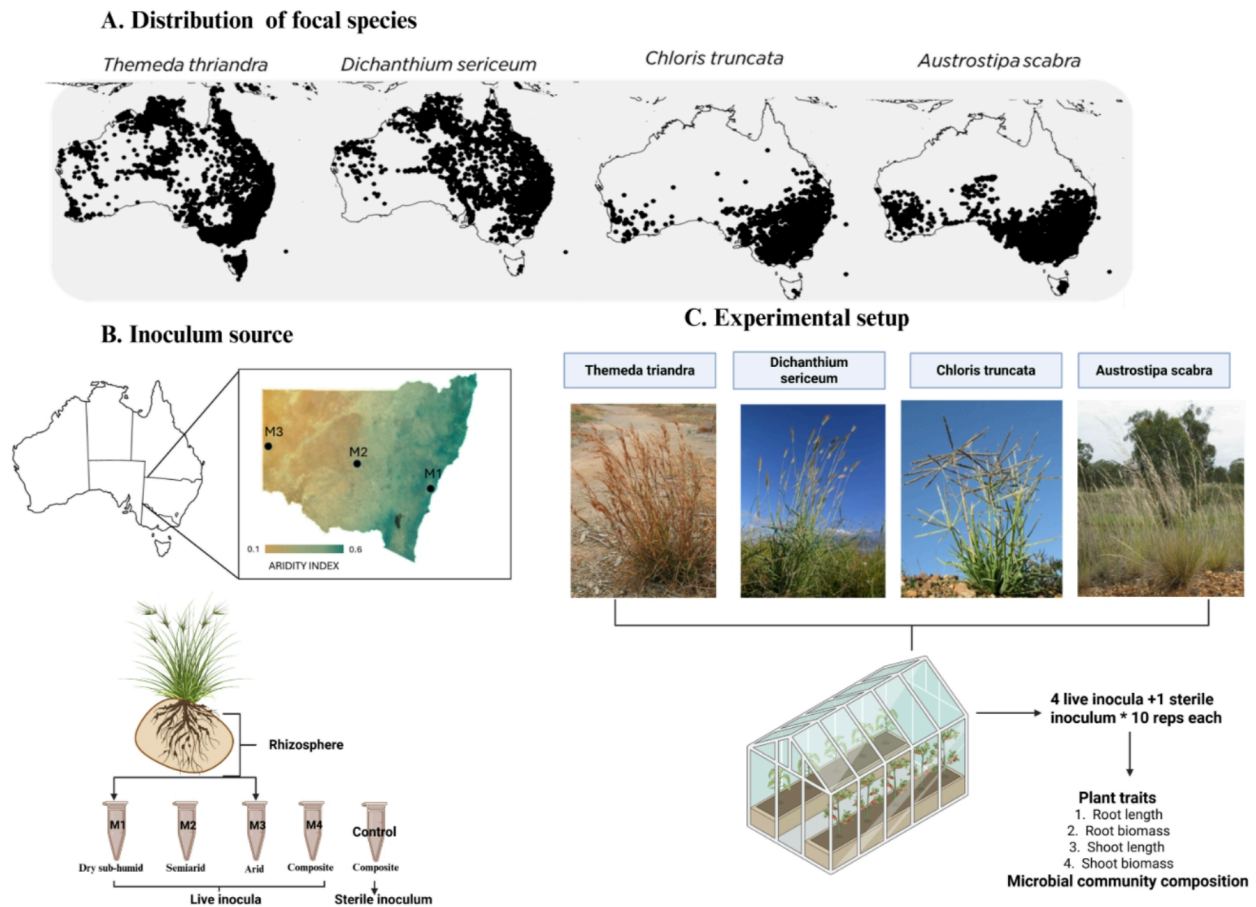


Figure 1

Experimental design to assess the influence of *T. triandra* rhizosphere inocula from different climatic regions on the plant growth and microbial communities of four native Australian grasses. (A) Distribution of focal species: Maps show the native distributions of the four grass species studied: *Themeda triandra*, *Dichanthium sericeum*, *Chloris truncata*, and *Austrostipa scabra* (sourced from the Atlas of Living Australia Spatial Portal). (B) Inoculum source: Donor soils were collected from the rhizosphere of *T. triandra* across three sites (M1- dry sub-humid, M2 - semi-arid, M3 - arid) along an aridity gradient. M4 was a composite inoculum derived from the mixing of M1, M2, and M3, and M5 was a sterile version of M4 (Control). (C) Experimental setup: Plants of the four focal species were grown in a glasshouse and subjected to five inoculation treatments (four 'live inocula' M1-M4, plus a sterile control) with 10 replicates each. At the end of the study four plant growth traits (root length, root biomass, shoot length, shoot biomass) were measures and rhizosphere microbial community composition was examined

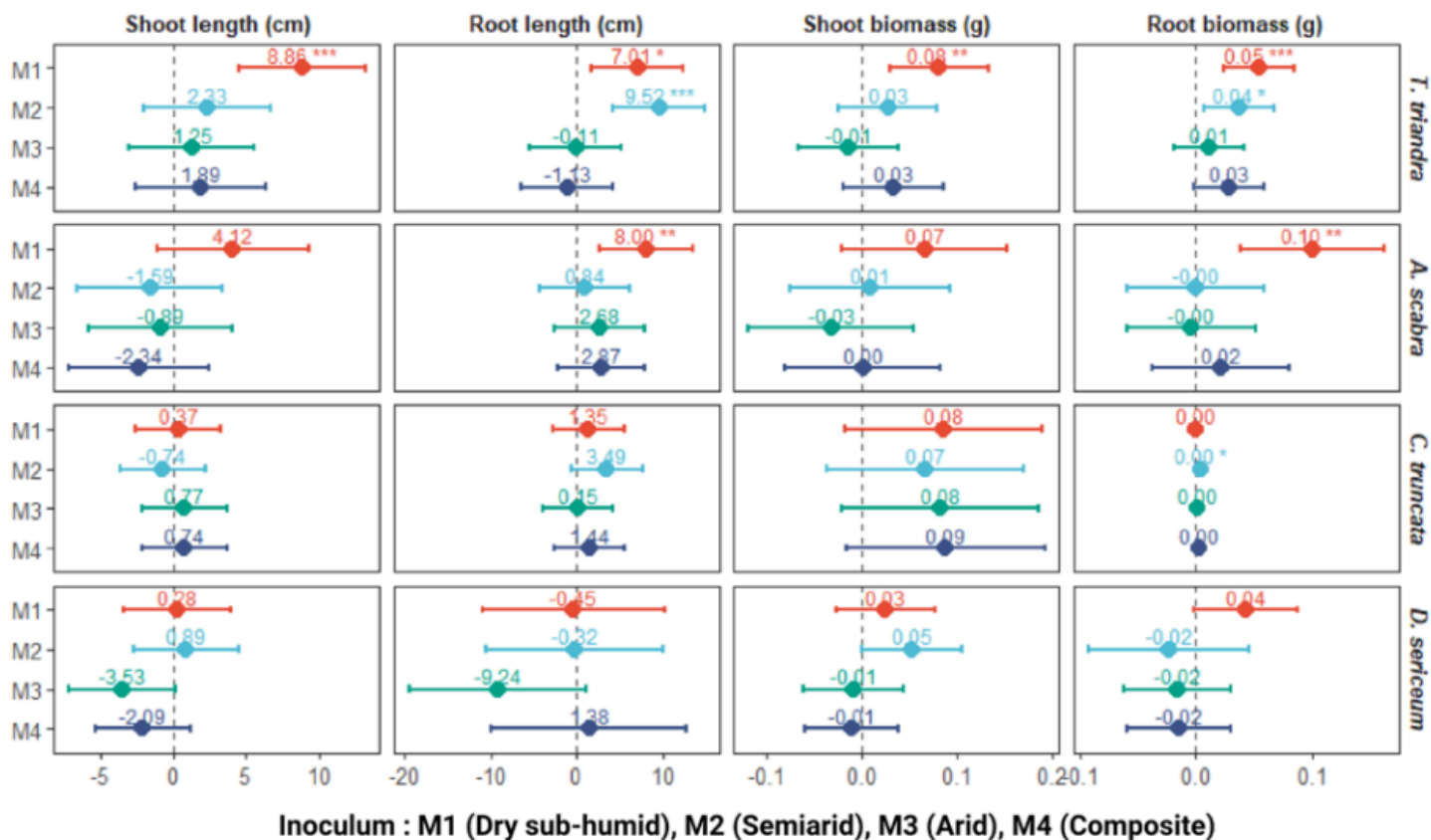


Figure 2

Effect of rhizosphere inocula on plant growth traits relative to a sterile control. Panels are arranged with plant traits in column and plant species in rows. Effect sizes were estimated using linear mixed models with inocula (M1-M4) compared against a sterile (M5). The points represent the effect size; the error bars represent 95% confidence intervals; significance was expressed as: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

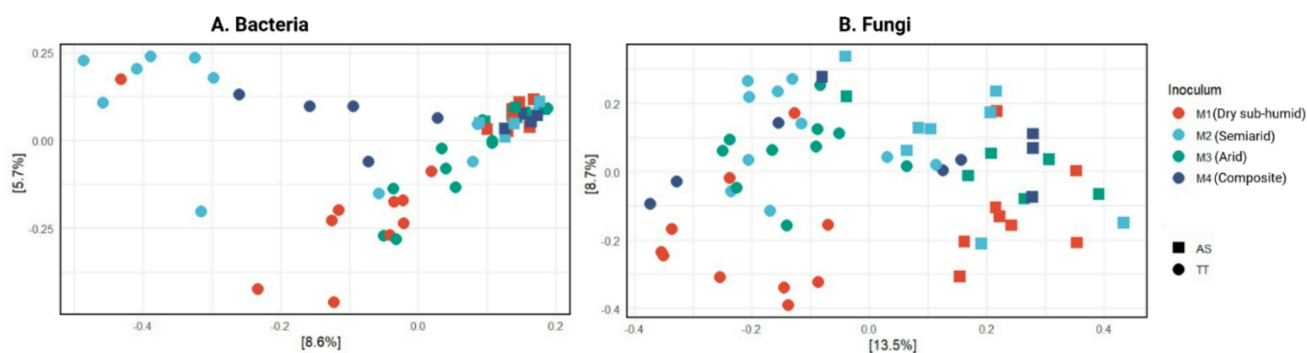


Figure 3

Principal coordinates analysis (PCoA) of the rhizosphere bacterial (A) and fungal (B) communities recruited in the rhizosphere of *Themeda triandra* (TT; circles) and *Austrostipa scabra* (AS; squares). Samples are coloured by inoculum (M1–M4).

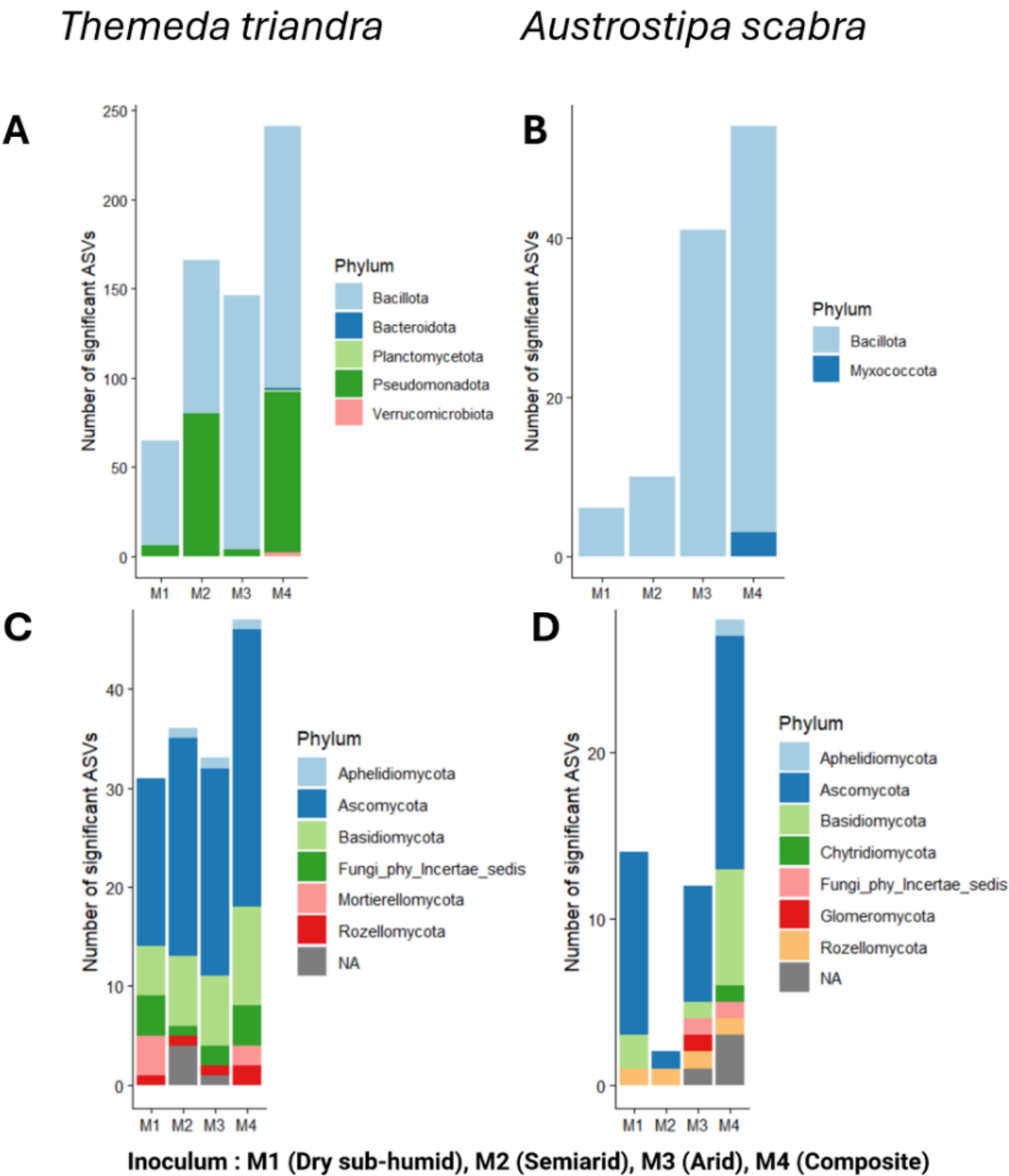


Figure 4

Stacked bar plots showing the number of significant indicator ASVs in *Themeda triandra* and *Austrostipa scabra*, grouped by phylum. Panels (A) and (C) represent bacterial and fungal indicator ASVs associated with *T. triandra*, respectively, while panels (B) and (D) correspond to bacterial and fungal ASVs associated with *A. scabra*, respectively.

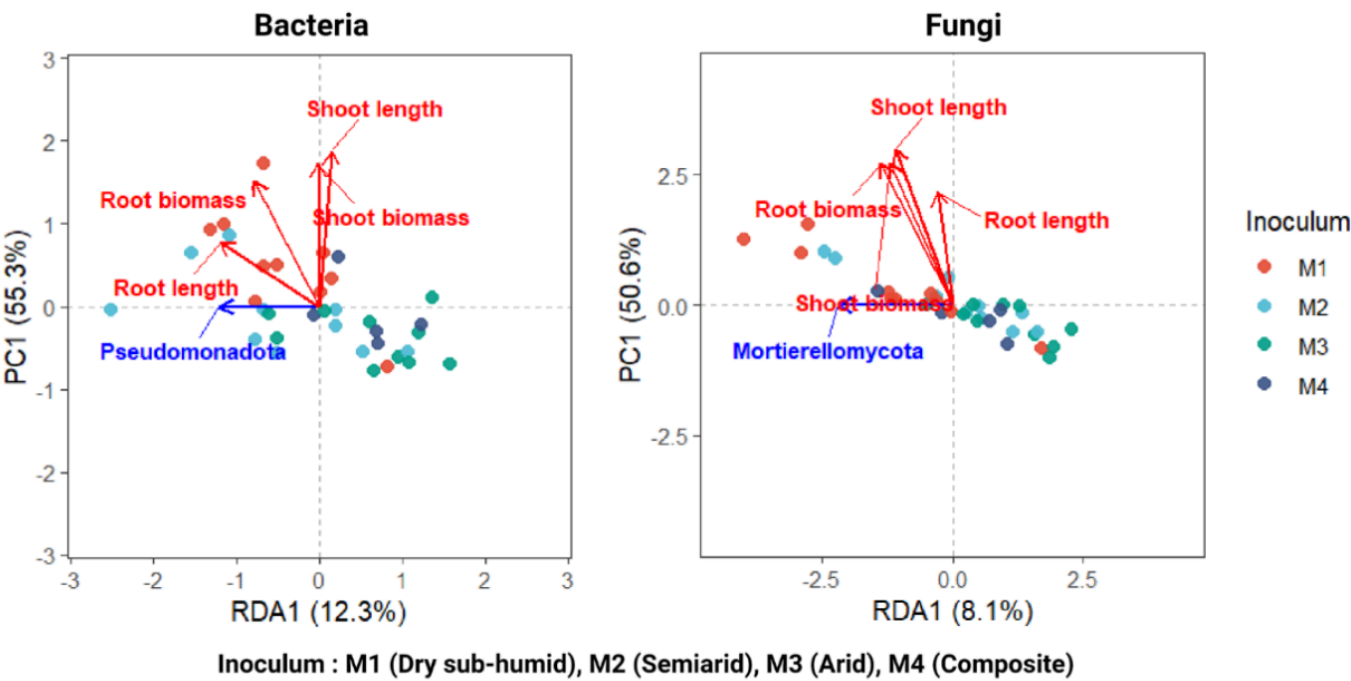


Figure 5

Redundancy analysis (RDA) showing the relationship between bacterial and fungal phyla retained after forward selection, and plant traits for *T. triandra*. Points represent individual samples coloured by inoculum. Red arrows indicate plant traits and blue arrows indicate microbial phyla; arrow direction and length reflect the correlation with the ordination axes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)