Arid soil bacteria legacies improve drought resilience of a keystone grass

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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 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.

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 & 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 & 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 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 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 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.

MATERIALS AND METHODS

Experimental design

We prepared a germination and five-month growth trial to test the influence of microbiota in soils collected from either high aridity or low aridity 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 soil aridity levels, 2 x sterilisation treatments, 2 x water availability) had 10 replicate pots, making 80 pots in total (see Figure S1a-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 S2). We included an additional 24 soil-only pots to account for changes in microbiota across each treatment in the absence of *T. triandra* (Figure S1c).

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 1a) with different levels of aridity: Kuitpo Forest Reserve at 35.2279^{o}S, 138.7199^{o}E (the mesic, low aridity site; aridity index = 0.658 – henceforth low aridity soil) and Quorn Floral Reserve at 32.3434^{o}S, 138.0182^{o}E (the semi-arid, high aridity site; aridity index = 0.227 – hereafter high aridity soil) on 14 and 16 November 2023, respectively (Table S1). 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 4^{o}C for one month

prior to setting up the growth trial. Half of the soil volumes were sterilised by autoclaving them twice at 121^{o}C, two days apart.

Germination and growth trial

The greenhouse was set at 16 h – 8 h day-night cycle, with temperatures set to 30^{o}C and 18^{o}C, 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 S3; 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 (6 February 2024), and 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).

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 & Armstrong 1934), nitrate, ammonium, electrical conductivity and pH ($CaCl_2$).

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 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.

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. Seed mass was included as a fixed effect to account for its potential effect on germination.

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 (for model details, see Table S2). Significance was determined by permuting each model 10,000 times and comparing observed test-statistics with those of the simulated random distributions.

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***x"*** *represents average plant biomass from the live or sterile treatment groups:*

$$PSF\ ratio = \frac{(?x\ Live - ?\text{x Sterile})}{?\text{X Sterile}}$$

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 S4). 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 & 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).

Bacterial communities were visualised using non-metric multidimensional scaling (NMDS) and principal coordinates analysis (PCoA) ordinations with Bray-Curtis distances. The effect of treatments on the bacterial communities were estimated via permutational multivariate analysis of variance (PERMANOVA) using the adonis2 () function in vegan (Oksanen J. et al. 2019).

RESULTS

Germination

We found no effects of high/low aridity soils (hereafter referred to as soil aridity) or sterilisation treatments on germination rates (Figure S5), however larger seeds germinated faster (GLMM; estimate =397.87, z =9.97, p <0.001).

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 1b; Table S2). 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 S2; Figure S6). Plants in the live high aridity soils had greater biomass than those in live low aridity soils (p<0.001; Figure 1b; Table S2), and the soil

aridity-sterilisation interaction was also significant, and showed plant biomass was higher under high aridity soil conditions (p=0.022; Table S2; Figure S6).

The aboveground biomass of *T. triandra* plants was lower in the water stress (p<0.001; Figure 2c; Table S2) and sterilisation treatments (p<0.001; Figure 2c; Table S2). 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 S2; Figure S7). 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 2c; Table S2). We also found significant interactions between soil aridity-sterilisation (p=0.032; Figure S7; Table S2), soil aridity-water stress (p<0.001; Table S2; Figure S7), and soil aridity-sterilisation-water stress (p=0.046; Table S2; Figure S7). 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 S7).

We found that belowground biomass decreased when under water stress (p<0.001; Figure 2d; Table S2) and sterilisation treatments (p<0.001; Figure 2c; Figure 2d; Table S2). High aridity soils also led to increased belowground biomass than low aridity soils (p<0.001; Figure 2d; Table S2). Belowground biomass was also affected by a water stress-sterilisation interaction (p= 0.014; Figure 2d; Table S2; Figure S8). 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 2e; Table S2) and sterilisation of high aridity soils reduced the root-mass fraction (p=0.002; Figure 2e; Table S2).

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 S3; Figure S10). 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 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 S3; Figure S10a-c).

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 2a). The soil-only pots had 8 bacterial phyla, which represented 96.9% of reads and had abundance estimates of

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 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 S4; Figure S12a) and these differences persisted until harvest (p<0.001; Table S4; Figure S12). We saw no effect of soil aridity (live p =0.875; sterile p = 0.086; Table S4; Figure S12) or water-stress treatment (live p = 0.312, sterile p =0.840; Table S4; Figure S12) 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 3b; Figure S13). Sterilisation

($p<0.001$, $p<0.001$, and $p=0.013$, respectively; Figure 4a; Table S5), soil aridity ($p<0.001$, $p<0.001$, and $p=0.023$, respectively; Figure 4a; Table S5), and water stress treatments ($p<0.001$, $p<0.001$, and $p=0.033$, respectively; Figure 4a; Table S5) 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 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 4b).

Soil aridity, sterilisation, and water-stress treatments had effects on differential abundance of bacterial phyla across the soils, rhizospheres and endospheres (Figure 1b; Tables S6-S7).

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). Biomass increased more with bacterial alpha diversity in high aridity soils (Figure 5).

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 S14). 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 S14). 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 S14).

DISCUSSION

We experimentally assessed the effects of high and low aridity soil legacies on the growth of the keystone grass species, *Themeda triandra* , under drought conditions. We show that microbiota from high aridity soils 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., soil aridity, sterilisation, and water stress) led to distinct bacterial assemblages in soils, rhizospheres and endospheres. This supports our second hypothesis by highlighting the conditional relationships that *T. triandra* forms with its bacterial communities that is dependent on its environment. 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.

Microbially mediated effects on biomass

We show that live soil communities enhanced plant growth in high and low aridity 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 soils, compared to the live high aridity 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 soils, where greater root investment occurred in the live low aridity soils compared to those grown in sterilised low aridity soils. As the bacterial communities were distinct, the low aridity soil microbiota may not provide the same functional benefits as those found in the high aridity 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 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 the interaction they form, may therefore depend on the aridity of the soil and plant growth environment (De Long *et al.* 2019).

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 soil aridity 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; He *et al.* 2024). Given the reduced *T. triandra* growth rates (biomass) in the low aridity sterilised soils (which was much lower than in high aridity 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). 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.

Conclusion

In our study, we sought to determine whether the widespread keystone grass*, Themada triandra* , relied on soil microbiota from arid locations to gain growth advantages when grown under drought conditions. We report that soil microbiota from more arid sources had strong positive effects on plant growth under drought conditions. We also show that soil aridity, 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 highlight that grassland decline by increased aridity due to climate change may be in part mitigated by the effects of healthy soil microbiota on keystone grass species. As such, we anticipate that soil-based

manipulations to enhance the restoration of climate-resilient grasslands will be increasingly applicable to future interventions in grassland ecosystems.

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CONFLICT OF INTEREST: The authors have no known conflicts of interest.

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FIGURES

Figure 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.

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Figure 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.

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Figure 3. Bacterial community differences across *T. triandra* compartments and timpoints (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.

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Figure 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.

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Figure 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).

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