

RESEARCH ARTICLE

Contrasting drought responses in two grassland plant-microbe systems under climate change

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Abstract

1. Drought strongly affects plant performance and their associated microbiomes, which can play crucial roles in plant stress resistance. Yet, how drought shapes plant-microbiome interactions under global change—particularly elevated temperature and CO₂—remains insufficiently understood, especially across distinct plant compartments (leaf endosphere, root endosphere, rhizosphere) that host functionally different microbial assemblages.
2. We examined plant and microbiome responses to a drought intensity gradient under current and future climate scenarios (warming and elevated CO₂) in two widespread Central European grasses with contrasting ecological strategies: the stress-tolerant *Festuca rubra* and the competitive *Lolium perenne*. Plants were grown in mesocosms subjected to controlled watering and climate treatments, and we quantified plant biomass, functional traits and composition of bacterial and fungal communities using DNA amplicon sequencing.
3. *Festuca* showed greater responsiveness to drought, exhibiting higher trait plasticity and more pronounced shifts in its microbial communities—particularly in bacteria—than *Lolium*. In contrast, *Lolium* displayed limited trait plasticity, relatively stable microbiomes and consistently higher fungal richness in the rhizosphere. The two species hosted distinct bacterial and fungal compositions, indicating strong species-specific microbiome structuring.
4. Bacterial richness declined markedly with increasing drought intensity, especially in the root endosphere and rhizosphere, whereas fungal richness remained largely stable. Warming and elevated CO₂ partly mitigated drought-induced declines in bacterial richness, particularly in the *Festuca* rhizosphere. Conversely, bacterial community composition in the root endosphere showed stronger moisture-related differentiation under warming and elevated CO₂ than under current climate.
5. *Synthesis*. By comparing two contrasting plant strategies across a pronounced soil moisture gradient and two global change scenarios, our study demonstrates how

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drought and climate jointly shape plant performance and associated microbiomes. These findings highlight the importance of integrating plant functional strategies with microbiome dynamics to better understand and predict grassland responses to shifting precipitation regimes under climate warming and rising atmospheric CO₂.

KEYWORDS

climate change, drought gradient, grassland FACE experiment, grassland microbiome, leaf endosphere, microbiome–climate interactions, plant functional traits, rhizosphere, root endosphere, warming and elevated CO₂

1 | INTRODUCTION

Drought is one of the most pressing threats to ecosystems, and its frequency and intensity are projected to increase under climate change (Ault, 2020; IPCC, 2023). Grasslands, which provide both biodiversity and forage services, are particularly sensitive to changes in water availability. A central challenge in these systems is the trade-off between productivity and drought resistance: While highly productive, nutrient-rich species maximize yield, more conservative species endure prolonged droughts but produce less biomass (Roscher et al., 2004; Volaire, 2018). Understanding how grassland species balance this trade-off is crucial for predicting future ecosystem functioning.

Plants may cope with drought through two complementary pathways: intrinsic trait adjustments and associations with microbial partners. At the plant level, trait plasticity in characteristics such as specific leaf area (SLA) captures strategies along the spectrum from acquisitive to conservative resource use (Berg & Ellers, 2010; Famiglietti et al., 2024). Stress-tolerant species tend to exhibit low SLA, high leaf dry matter content (LDMC) and dense roots, often coupled with greater plasticity that enables adjustment to fluctuating water supply (Kramp et al., 2022; Mount et al., 2024; Münzbergová et al., 2025). In contrast, competitive species are characterized by high SLA, high leaf nitrogen concentration, and low root tissue density—traits that maximize productivity under favorable conditions but limit drought resistance (Chieppa et al., 2022; Pérez-Ramos et al., 2019).

Beyond trait adjustments, plants often rely on microbial partners that support drought resistance through multiple mechanisms: enhancing nutrient and water acquisition, modulating phytohormone production and influencing carbon allocation (Abdelaal et al., 2021; Xu & Coleman-Derr, 2019). Plant-associated bacteria and fungi differ in their ecological strategies. Bacteria are typically fast-growing and responsive to changes in plant exudation, but their communities are often sensitive to drought stress (Naylor & Coleman-Derr, 2018). Fungi, in contrast, maintain more stable functioning under stress through hyphal networks that bridge soil microsites and enzymatic capacity to access complex carbon sources (De Vries et al., 2018; Guhr et al., 2015). These differences imply that plant strategies may be linked not only to trait plasticity but also to the relative reliance on bacterial versus fungal partners.

Microbial communities differ among the phyllosphere, root endosphere and rhizosphere, each contributing uniquely to plant drought responses (Compant et al., 2021; Santos-Medellín et al., 2017; van der Heijden & Schlaeppi, 2015). In the phyllosphere, microbes can enhance drought tolerance by modulating stomatal regulation and producing protective metabolites, with foliar endophytes such as *Epichloë* spp. providing well-documented examples in grasses (Nagabhyru et al., 2013; Saikkonen et al., 2016; Vanani et al., 2025). In the root endosphere, microbes may influence phytohormone and antioxidant production, shaping host stress physiology (Xu & Coleman-Derr, 2019). Finally, in the rhizosphere, microbial taxa, especially mycorrhizal fungi, often mobilize water and nutrients to the host under drought (Bandopadhyay et al., 2024; Liu et al., 2025; Parniske, 2008).

Empirical studies suggest that microbes residing in different plant compartments vary in their sensitivity to drought. For instance, rhizosphere communities often show pronounced compositional shifts, with declines in diversity and enrichment of drought-tolerant taxa. In contrast, phyllosphere responses are typically more variable and strongly dependent on the host species (Naylor & Coleman-Derr, 2018; Santos-Medellín et al., 2017; Sun et al., 2021). Despite these compartment-specific roles, little is known about how phyllosphere, root endosphere and rhizosphere microbiomes jointly respond to drought in grassland species with contrasting ecological strategies. In this study, we therefore examined all three compartments, but for the above-ground component, we focused on the leaf endosphere, since endophytic communities are more stably associated with the host than surface microbes and have clearer functional links to plant stress responses (Ameen et al., 2024; Giauque et al., 2019).

Global change introduces additional factors that interact with drought, most notably warming and elevated CO₂. Elevated CO₂ can stimulate photosynthesis and alter plant carbon allocation, leading to shifts in root exudation and microbial nutrient demand (Liu et al., 2021; van der Putten et al., 2016). Warming can accelerate microbial activity and decomposition, not only enhancing nutrient cycling but also increasing soil carbon losses (Osanai et al., 2015). Together, elevated CO₂ and warming may buffer drought impacts by favouring drought-tolerant taxa and maintaining microbial processes that support nutrient supply (Canarini et al., 2024; Metze et al., 2023). Yet their interactions remain complex: warming may

exacerbate water limitation, while CO₂ enrichment can to some extent offset drought by improving water-use efficiency (Gebrechorkos et al., 2025; Roy et al., 2016). How drought, warming and elevated CO₂ jointly shape plant trait variation and plant-microbe associations across plant species and compartments is, however, still poorly understood, leaving major uncertainties in predicting grassland resistance to future climate.

To address these gaps, we conducted a field experiment manipulating drought intensity under two climate scenarios: a current scenario (ambient conditions) and a future scenario (warming and elevated CO₂). The experiment was established with seed mixtures representing stress-tolerant and competitive grassland community types. Within these communities, we focused on two representative and abundant species—*Festuca rubra* (stress-tolerant, conservative traits, highly plastic) and *Lolium perenne* (competitive, acquisitive traits, highly productive)—to test how plant traits and microbial partners jointly mediate plant drought responses.

Our overall aim was to determine whether these two contrasting plant species exhibit distinct strategies of plant traits and associated microbes under drought and climate change. Specifically, we aimed to (A1) compare plant functional traits and associated microbial communities in *Festuca* and *Lolium*; (A2) assess how drought intensity alters plant performance and microbial community composition in both species; and (A3) examine whether warming and elevated CO₂ modify these effects across plant compartments (leaf endosphere, root endosphere, rhizosphere).

We hypothesize that contrasting ecological strategies of *Festuca rubra* and *Lolium perenne* translate into distinct coordination between plant traits and associated microbes under drought and climate change. Specifically, (H1) *Festuca*, as a stress-tolerant species, will cope with stress through conservative traits and higher trait plasticity, supported by flexible microbial adjustments, particularly among bacteria. In contrast, *Lolium*, as a competitive species, will depend on acquisitive traits and more stable microbial partnerships, particularly fungi, that help sustain productivity under stress. (H2) Under drought, *Lolium* will experience stronger biomass reductions than *Festuca*. By contrast, *Festuca* will exhibit greater trait shifts that buffer biomass decline, and these adjustments will be accompanied by larger microbial reassembly, particularly among bacterial communities. (H3) Warming and elevated CO₂ will mitigate drought impacts by enhancing microbial functioning and supporting plant performance, with microbial responses the strongest in the rhizosphere, intermediate in the root endosphere and the weakest in the leaf endosphere.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up and climate manipulation

The experiment was conducted at the Agricultural Research and Education Centre (AREC) Raumberg-Gumpenstein, Austria (47°29'38" N, 14°06'03" E), within a long-term multifactorial global

change experiment (Piepho et al., 2017; Radolinski et al., 2025; Simon et al., 2020). No specific permits or licenses were required to conduct the fieldwork for this study.

The site has a mean annual precipitation of 1077 mm and a mean annual temperature of 8.5°C. Since 2014, atmospheric CO₂ concentrations and canopy temperatures have been manipulated using a free-air CO₂ enrichment (FACE) system and infrared heaters, respectively (Maxwell et al., 2022; Meeran et al., 2021). The 'future climate' treatment elevated CO₂ by +300 ppm and temperature by +3°C, based on IPCC RCP8.5 projections, while the 'current climate' treatment reflected ambient conditions. Each climate scenario was represented by two plots (diameter 4 m; see Figure S1a for experimental design). Replication at the plot level (warming and CO₂) was therefore limited ($N=2$), which constrains statistical power and introduces potential pseudoreplication. We explicitly acknowledge this limitation when interpreting our results.

2.2 | Watering treatments

Each plot contained 12 bottomless mesocosms (30 cm diameter × 60 cm deep) (Meeran et al., 2021). Automatic rain-out shelters excluded natural precipitation from all the plots from late May to late July in 2022 and 2023. A lysimeter installed at the plot centre (Figure S1b) was used to determine the timing of watering events: when soil moisture in the lysimeter dropped below 50% of field capacity, all surrounding mesocosms were watered. During each event, the lysimeter mesocosm was rewatered to 100% field capacity, while the surrounding mesocosms received different water volumes corresponding to six target levels of field capacity (Table S1), ranging from completely dry (0 mL, ~5% field capacity) to ~80% field capacity (1600 mL, equivalent to 2.3 mm precipitation per watering event). Thus, some mesocosms experienced progressive and severe drought while others remained relatively well watered. Watering events occurred approximately twice per week. Watering regimes were randomly assigned to mesocosms within each plot, with two mesocosm per watering treatment (one for each plant community, see below), to minimize potential bias from small-scale heterogeneity (e.g. soil or microclimate).

Following the gradient-design approach (Kreyling et al., 2018), the full moisture gradient was interpreted as a continuous moisture gradient rather than fixed categorical irrigation levels, and the same gradient was represented across the two replicate plots for each climate treatment ('future climate': A and C; 'current climate': B and D, Figure S1a). Thus, each climate scenario contained the complete range of watering levels, avoiding any confounding of moisture with plot identity.

Continuous soil-moisture measurements were not available for individual mesocosms because installed sensors did not provide reliable time series. Soil moisture was therefore monitored continuously only in the central lysimeter, which triggered watering events but did not capture variation among mesocosms. To confirm that the watering gradient produced real differences in soil moisture, we measured

gravimetric water content (GWC) for all mesocosms at peak drought period in July 2023, which was closely related to the actual amount of water provided (Figure S2).

2.3 | Plant communities and focal species

Two seed mixtures representing contrasting grassland community types were sown into the mesocosms in 2021. Each seed mixture was sown into one of the two mesocosms of each watering level in each plot. The stress-tolerant community mixture contained *Festuca rubra* (15% by weight), *Arrhenatherum elatius* (20%), *Dactylis glomerata* (50%) and *Trifolium repens* (15%). The competitive community mixture contained *Alopecurus pratensis* (15%), *Festuca pratensis* (20%), *Lolium perenne* (50%) and *Trifolium pratense* (15%). Vegetation was managed with a three-cut system common to this region. From these communities, *Festuca rubra* (stress-tolerant) and *Lolium perenne* (competitive) were selected as focal species because they were consistently abundant across their respective communities after 2 years of watering treatments. Their contrasting ecological strategies, with *Festuca* classified as a stress tolerator and *Lolium* as a competitor under Grime's CSR framework (Grime et al., 1988), provided a basis for testing species-level responses to drought and climate change.

2.4 | Plant performance measurements

At the end of the experiment (July 2023), above-ground biomass was harvested at 3 cm stubble height from each mesocosm. Biomass was sorted by species, oven-dried at 60°C for at least 48 h and weighed. Species-specific and total community biomass were then calculated (g/m^2). Species' biomass proportions, expressed as each species' percentage of total community biomass within the mesocosm, were also calculated. For trait measurements, fully expanded leaves of *Festuca* and *Lolium* were collected prior to harvest. Leaves were rehydrated, scanned for leaf area, weighed fresh and then oven-dried at 60°C for 48 h. Specific leaf area (SLA; leaf area/dry mass, mm^2/mg) and leaf dry matter content (LDMC; dry mass/fresh mass, mg/g) were calculated for each species. Leaf trait measurements were based on two replicate individuals per species in each mesocosm, giving a total of 96 plants (4 plots $\times$ 12 mesocosms $\times$ 2 replicates). These same individuals were also used for microbiota sampling described below.

2.5 | Microbial sampling and processing

At the same time as biomass harvest (July 2023), the same *Festuca* and *Lolium* individuals were sampled for microbiota. For each plant, three compartments were collected: (i) rhizosphere soil, defined as soil adhering within $\sim 2\text{mm}$ of the root; (ii) root endosphere; and (iii) leaf endosphere. Roots with adhering soil were placed in sterile paper bags with silica gel for desiccation and transport, a

method shown to preserve microbial community DNA profiles in soil and root-associated samples (Allison et al., 2021; Smenderovac et al., 2024). In the laboratory, rhizosphere soil was separated from roots, and roots were rinsed in sterile deionized water and used for root endosphere DNA extraction. For leaf endosphere samples, three fully expanded leaves per plant were pooled into sterile 1.5 mL tubes, transported on ice and stored at -20°C until processing. Prior to DNA extraction, leaves were surface-sterilized (75% ethanol for 1 min, 3.25% NaClO for 3 min, triple rinse in sterile H_2O) following Sahu et al. (2022). The surface-sterilized leaves then were kept in sterilized 1.5 mL tubes and stored in the freezer before the extraction of the total DNA of leaf endosphere microbes. We thus focused on internal leaf endophytes, while excluding surface-associated microbes, which are difficult to preserve consistently in field settings and are strongly influenced by transient environmental deposition.

2.6 | DNA extraction, amplification and sequencing

DNA was extracted from rhizosphere soil, roots and leaves using commercial kits (MACHEREY-NAGEL NucleoSpin® Soil Kit for soil, QIAGEN DNeasy® Plant Mini Kit for roots and leaves), following manufacturer protocols. The bacterial 16S rRNA gene (V4 region) was amplified using primers 515F/806R, and the fungal ITS2 region was amplified using primers ITS3/ITS4, both with Illumina adapters. PCR products were pooled and sequenced on an Illumina NovaSeq 6000 platform ($2 \times 250\text{bp}$), with 40,000,000 reads/all pools (SEQme, Dobříš, Czech Republic). Negative controls from DNA extraction and PCR were included to monitor potential contamination. Detailed procedures are provided in Method S1.

2.7 | Bioinformatics and sequence processing

Raw sequences were processed in QIIME2 (Bolyen et al., 2019) using the DADA2 plugin to denoise, remove chimeras and infer amplicon sequence variants (ASVs). Taxonomic assignment was performed against the SILVA database (v138) for bacteria and the UNITE database (v9) for fungi (Abarenkov et al., 2024; Quast et al., 2013). Archaeal reads were excluded because they accounted for $<0.1\%$ of the dataset; chloroplast, mitochondrial and unassigned ASVs were also removed. Negative controls from DNA extraction and PCR were sequenced in parallel, and potential contaminants were removed using the *decontam* R package (Davis et al., 2018). In total, 8405 bacterial ASVs and 1949 fungal ASVs were retained across the six datasets; read depth distributions are summarized in Table S2. Rarefaction curves for all datasets are shown in Figure S3. Rarefaction thresholds were set to the minimum read depth per dataset to maximize sample retention, as higher thresholds would have excluded a large proportion of samples (Table S3). This conservative approach allowed us to retain sample size for comparisons while still capturing the majority of observed diversity. The bacteria-leaf

endosphere dataset had extremely low sequencing depth (median 21 reads) and was therefore excluded from alpha diversity analyses. A full description of the sequence-processing pipeline is provided in Method S2.

2.8 | Statistical analyses

All analyses were performed in R v4.3.2 (R Core Team, 2023). For simplicity, watering treatments are hereafter referred to as moisture in the analyses. Linear models tested the effects of moisture, climate scenario and plant species on biomass production, and beta regression was used for species' biomass proportions. Plant traits (SLA, LDMC) and microbial richness were analysed with linear mixed-effects models including mesocosm as a random effect. This structure allowed us to test the fixed effects of moisture, climate scenario and plant species while controlling for the non-independence of samples within mesocosms.

Determinants of microbial community composition were tested with redundancy analysis (RDA) and permutation tests constrained by mesocosm identity. To evaluate the relative importance of experimental factors across all model types, variance explained (%) by each fixed effect was calculated based on F - or χ^2 -statistics, normalized within models and scaled by the corresponding model R^2 (or adjusted R^2 for RDA).

Indicator taxa of bacterial and fungal communities were identified with indicator species analysis in *indicspecies* (De Cáceres et al., 2011) and differential abundance models in *DESeq2* (Love et al., 2014). Bacterial functional profiles were inferred with Tax4Fun2 (Wemheuer et al., 2020) and fungal functional groups with FUNGuild (Nguyen et al., 2016) and FungalTraits (Pölme et al., 2020).

Finally, we used structural equation models (SEMs) to assess direct and indirect effects of moisture and climate on plant biomass through plant traits and microbial communities in *Festuca* and *Lolium*. Moisture, climate and their interaction were treated as exogenous predictors. SEMs were implemented using the *piecewiseSEM* package (Lefcheck et al., 2024), combining linear and mixed-effects components with mesocosm included as a random factor. This integrative approach allowed us to quantify causal relationships among environmental drivers, plant functional traits, microbial attributes and plant biomass across both focal species. Full model formulas, preprocessing steps and SEM details are provided in Method S3.

3 | RESULTS

3.1 | Plant performance and trait responses

Plant performance varied primarily between plant species and climatic conditions, while responses to the moisture gradient were weak (Table S5; Figure 1). *Lolium* produced higher above-ground biomass and contributed a greater biomass proportion than *Festuca* (Figure 1a), consistent with their relative representation

in the original seed mixtures. Specific leaf area (SLA) did not differ significantly between species ($p=0.055$), whereas leaf dry matter content (LDMC) was clearly higher in *Festuca* than in *Lolium* (Table S5; Figure 1a).

Across the full watering gradient (0–1600 mL per event), moisture effects were generally weak (Table S5; Figure S6a), but *Festuca* showed two significant responses. Across climate treatments, *Festuca* biomass increased and *Festuca* LDMC decreased significantly with increasing moisture (Figure 1b). When moisture–trait relationships were examined separately for each climate, the same two responses were evident under the current climate, whereas no moisture effects were detected for any trait under the future climate (Figure 1c; Figure S6b). *Lolium* did not exhibit significant moisture responses for any trait in either climate (Table S5).

Climatic conditions also shaped plant performance independently of moisture (Table S5; Figure S7a). Biomass was higher under the current climate than under the future climate, whereas SLA was higher under the future climate. LDMC and biomass proportion did not vary significantly between climates. A significant climate $\times$ species interaction was detected for biomass, reflecting that *Lolium* exhibited a climate-driven decline in biomass while *Festuca* remained unchanged (Figure S7b). No other interactions involving climate were statistically supported (Table S5).

3.2 | Microbial richness responses

Microbial richness differed markedly between plant species and compartments (Table S6; Figure 2). For bacteria, richness was consistently higher in *Festuca* than in *Lolium* in both the root endosphere and rhizosphere. For fungi, *Festuca* exhibited higher richness in the leaf endosphere. Fungal richness did not differ between species in the root endosphere. *Lolium* supported higher fungal richness in the rhizosphere (Figure 2a).

Moisture strongly influenced bacterial richness but had little effect on fungal richness (Table S6; Figure 2b–e; Figure S8a). In the root endosphere, bacterial richness increased significantly with moisture for both *Festuca* and *Lolium*. In the rhizosphere, this positive response was significant only for *Lolium* (Figure 2b). When analysed separately for each climatic condition, these moisture responses were most pronounced under the current climate (Figure S8b). Bacterial richness in the root endosphere increased significantly with moisture for both species, while in the rhizosphere, only *Festuca* exhibited a significant positive response (Figure 2d). Under the future climate, moisture responses were generally weak; the only significant effect occurred for fungal richness in the rhizosphere of *Festuca* (Figure 2e; Figure S8c).

Climatic conditions did not significantly affect microbial richness in any compartment (Figure S9). Bacterial and fungal richness were similar under current and future climates, species differences remained consistent across climates, and no species $\times$ climate interactions were detected (Table S6).

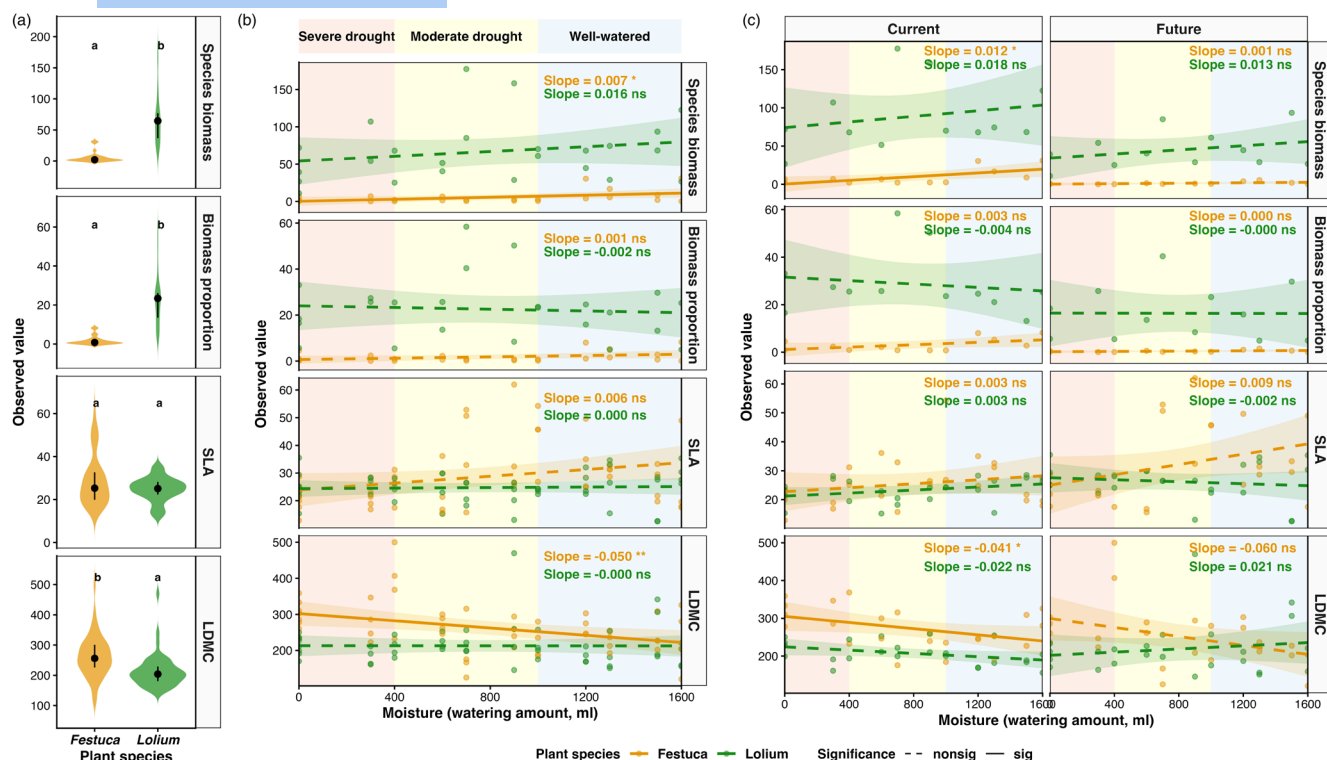


FIGURE 1 Plant performance across plant species, moisture gradient and climatic conditions. (a) Species-level differences in species biomass, biomass proportion, specific leaf area (SLA) and leaf dry matter content (LDMC), shown as violin plots. Black points denote medians and vertical bars denote interquartile ranges. Different letters above violins represent significantly different means at $p < 0.05$. (b) Moisture-response patterns of *Festuca* and *Lolium* across the watering gradient (0–1600 mL per event). Colours indicate plant species (*Festuca* in orange and *Lolium* in green). Fitted regression lines and 95% confidence ribbons are derived from linear models. Solid lines indicate significant moisture effects ($p < 0.05$), and dashed lines indicate non-significant effects. Slope estimates printed in each panel correspond to the regression line of the same colour and quantify the moisture response of each species; significance is indicated by $^{(*)}$ ($p < 0.05$), $^{(**)}$ ($p < 0.01$) and $^{(***)}$ ($p < 0.001$) and 'ns' ($p \geq 0.05$). (c) Moisture–trait relationships separated by climatic conditions (current vs. future). Panels and line styles follow those in (b).

3.3 | Microbial community composition

Microbial community composition differed strongly between plant species and across compartments (Table S7; Figure 3). Bacterial communities of *Festuca* and *Lolium* formed distinct but partly overlapping clusters across compartments, while fungal communities showed much clearer species-level partitioning in the leaf and root endosphere and the rhizosphere (Figure 3a–f). These patterns were supported by significant plant species effects in all bacterial and fungal compartments (Table S7).

Moisture effects on microbial community composition were the strongest for bacteria, with significant responses in the root endosphere and rhizosphere, and weaker but still significant effects for fungi in the root endosphere (Table S7). No moisture $\times$ species interactions were detected, indicating similar moisture-related shifts in *Festuca* and *Lolium* (Table S7).

Climatic conditions significantly affected bacterial community composition in the leaf and root endosphere but not in the rhizosphere (Table S7). For fungi, climate effects were significant across all compartments, with the strongest response in the rhizosphere. Despite these main effects, climate-based separation in the PCA plots was

limited, with current and future climate samples largely overlapping (Figure S10). Species $\times$ climate interactions were generally absent, except for a weak effect in the bacterial rhizosphere (Table S7).

3.4 | Taxonomic responses of microbial communities

Relative abundances of dominant bacterial and fungal taxa were shaped primarily by compartment, with strong differences between the leaf endosphere, root endosphere and rhizosphere (Table S13; Figure S11). Bacterial phyla were broadly consistent across treatments, with Proteobacteria and Bacteroidota dominating the rhizosphere, while Actinobacteriota was the most abundant in the root endosphere. Fungal phyla were strongly dominated by Ascomycota across all factors, with smaller contributions from Basidiomycota. Treatment effects of moisture, climate or plant species were generally weak at the phylum level, although Ascomycota and Basidiomycota showed significant moisture responses, and Ascomycota varied modestly between plant species (Table S13). At finer taxonomic resolution, compartment

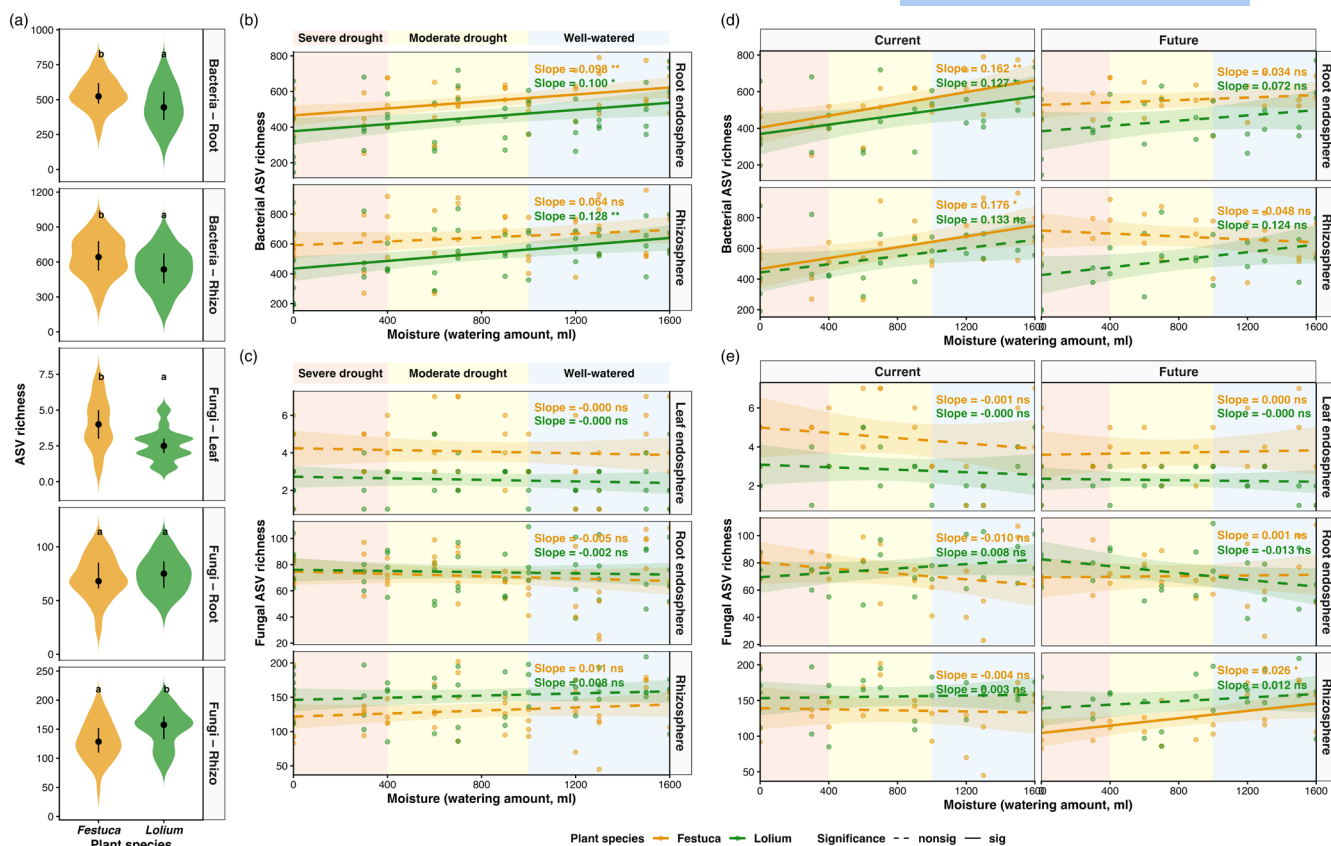


FIGURE 2 Microbial richness across plant species, moisture gradient and climatic conditions. (a) Differences in bacterial and fungal ASV richness between *Festuca* and *Lolium* across leaf, root and rhizosphere compartments. Violin plots show the distribution of individual samples; black points denote medians and vertical bars denote interquartile ranges. Different letters above violins represent significantly different means at $p < 0.05$. (b, c) Relationships between microbial richness and the moisture gradient for bacteria (b) and fungi (c). Linear model fits are shown separately for plant species (*Festuca* in orange; *Lolium* in green); solid lines indicate significant moisture effects ($p < 0.05$); and dashed lines indicate non-significant effects. Slope estimates printed in each panel correspond to the regression line of the same colour and quantify the moisture response of each species; significance is indicated by (*) ($p < 0.05$), (**) ($p < 0.01$) and 'ns' ($p \geq 0.05$). (d, e) Moisture–richness relationships for bacteria (d) and fungi (e) shown separately for current and future climatic conditions. Points and regression lines are coloured by plant species as in (b, c). Line styles and shaded backgrounds follow those in (b, c).

remained the strongest determinant of bacterial and fungal order-level composition, with only limited evidence for treatment-driven shifts (Table S13).

Predicted bacterial functions were largely stable across plant species, moisture levels and climate treatments (Figure S12). Across all factors, the most abundant predicted pathway was 'metabolic pathways', followed by other core functions such as 'microbial metabolism in diverse environments' and 'ABC transporters', and no treatment produced clear shifts in pathway composition. Because Tax4Fun predictions are inferred from 16S rRNA data and reference genomes, they should be interpreted as coarse functional tendencies rather than direct measurements.

Fungal trophic modes varied strongly by compartment but showed no effects of moisture, climate or plant species (Figure S13; Table S14). Pathotroph-associated guilds were most abundant in the rhizosphere, while saprotrophs and symbiotrophs were more common in the root endosphere. Overall, functional patterns reflected strong compartmental structuring and minimal responses to experimental treatments (Table S14).

3.5 | Indicator ASVs

Moisture-responsive ASVs were taxonomically diverse, but their number was different between bacteria and fungi. In both *Festuca* and *Lolium*, bacterial moisture responders were dominated by Actinobacteriota, Proteobacteria and Bacteroidota, whereas fungal responders were mainly Ascomycota, followed by Basidiomycota (Figure 4; Table S15). Positive responses (increasing abundance with moisture) were more common than negative responses across domains (Figure S14a). *Festuca* generally hosted more moisture-responsive ASVs than *Lolium*, but overlap between host species was minimal: only three ASVs (two bacterial and one fungal) showed consistent moisture responses in both species (Figure S15).

Beyond moisture, many ASVs were identified as indicators for compartment- and host-specific patterns. The rhizosphere contained far more indicator ASVs than the leaf or root endosphere for both bacteria and fungi (Figure S16a). Host identity also structured indicator communities: *Festuca* harboured more bacterial indicators, whereas *Lolium* supported more fungal indicators. Fungal indicators

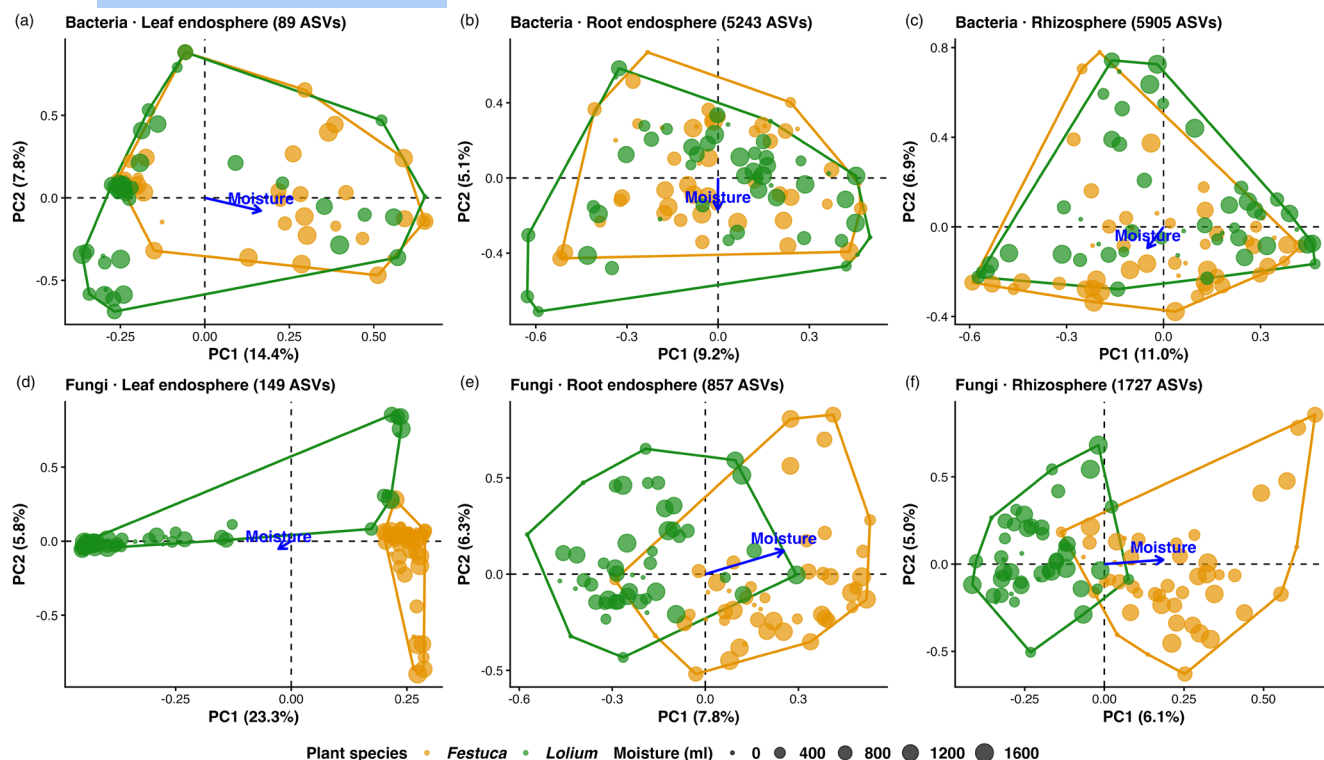


FIGURE 3 Principal component analysis (PCA) of bacterial (a–c) and fungal (d–f) community composition in the leaf endosphere (a, d), root endosphere (b, e) and rhizosphere (c, f). Each point represents one sample and is coloured by plant species (*Festuca* = orange, *Lolium* = green), with point size indicating the applied moisture level. Convex hulls enclose samples of the same plant species. Blue arrows indicate the direction of the moisture gradient based on fitted environmental vectors (envfit). Percentages on axes denote the proportion of variance explained by the first two principal components. Numbers in parentheses in panel titles represent the number of ASVs included in each dataset.

were dominated by Ascomycota, while bacterial indicators spanned a broad range of phyla (Figure S16b–d). Very few indicator ASVs were identified for climatic conditions (Figure S16d).

Across all experimental factors examined—moisture, compartment, host species and climate—overlap among indicator ASVs was limited. Most ASVs were linked to only one factor, and only a small subset showed associations across multiple factors (Figure S14b; Table S16).

3.6 | Relative importance of predictors

Across all plant and microbial responses, plant species identity consistently explained the largest share of variance, particularly for plant biomass, microbial richness and fungal community composition (Figure 5). Beyond these strong species effects, the relative contributions of moisture and climatic conditions varied among responses. Moisture accounted for more variation in bacterial richness in the root endosphere and rhizosphere, whereas climatic conditions contributed more strongly to plant biomass, SLA and several microbial compositional responses. Interaction terms generally explained little variance. In summary, predictor importance varied across responses, with plant species generally strongest and moisture or climate prevailing in different plant and microbial variables (Figure 5).

3.7 | Species-specific pathways of plant-microbe interactions

Species-specific SEMs revealed contrasting pathways through which moisture and climate influenced biomass production in *Festuca rubra* and *Lolium perenne* (Figure 6; Tables S17 and S18). For both species, moisture had a significant positive direct effect on biomass, while future climate (increased CO₂ and warming) consistently reduced biomass. The models explained substantial variance in biomass (*Festuca*: $R^2 = 0.59$; *Lolium*: $R^2 = 0.48$).

In *Festuca*, moisture effects propagated through several microbial components. Moisture increased bacterial richness in the root endosphere and altered both bacterial and fungal community composition in the root endosphere and rhizosphere, including strong negative effects on bacterial PC2 axes. Several moisture × climate interactions on microbial variables were also significant, showing climate-dependent microbial responses. Despite these indirect microbial pathways, direct effects of moisture and climate remained important contributors to variation in *Festuca* biomass (Figure 6).

In *Lolium*, fewer microbial variables were involved in the pathway structure. A significant moisture × climate interaction affected fungal richness in the root endosphere, but fungal richness and fungal community composition did not show significant paths to biomass. *Lolium* biomass was instead associated with direct effects of moisture and climate and with the biomass of neighbouring species (other

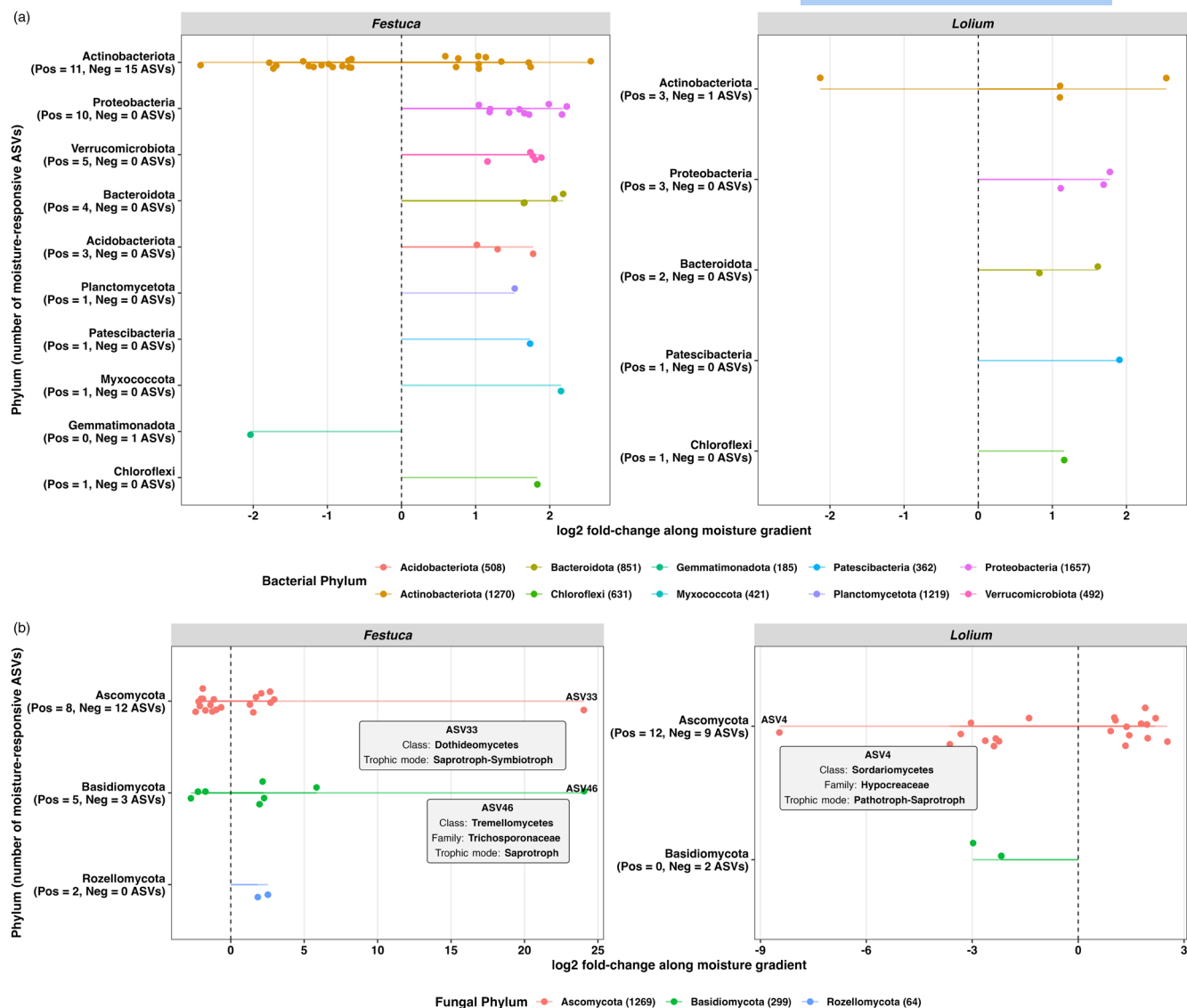


FIGURE 4 Moisture-responsive bacterial and fungal taxa associated with *Festuca* and *Lolium*. (a) Lollipop plots showing $\log_2$ fold-changes in bacterial ASV abundance along the experimental moisture gradient for each phylum in *Festuca* (left) and *Lolium* (right). Each point represents an individual ASV identified as moisture-responsive using differential abundance analysis in the DESeq2 package, and horizontal segments indicate the direction and magnitude of its response. Phylum labels include the number of ASVs that increased (Pos) or decreased (Neg) with increasing moisture. Colours denote phyla. (b) Equivalent lollipop plots for fungal ASVs. Insets highlight example ASVs with strong moisture responses in *Festuca* or *Lolium*, including their taxonomic classification and trophic mode. Positive $\log_2$ values indicate increasing abundance with increasing moisture, and negative values indicate declines. Legends report the total number of ASVs belonging to each phylum in the full dataset. Full taxonomic information for all moisture-responsive ASVs is provided in Table S15.

species biomass), whereas SLA and other microbial variables did not contribute significantly (Figure 6).

4 | DISCUSSION

4.1 | Species differences in plant traits and microbial communities

Our findings partially support H1, which predicted that the stress-tolerant *Festuca* would exhibit more conservative trait values and distinct microbial associations compared with the competitive

Lolium. As anticipated, *Festuca* showed a consistently more conservative leaf economic profile, most evident in its higher LDMC, whereas *Lolium* expressed traits associated with a more acquisitive growth strategy. SLA, however, did not differ significantly between species. This pattern suggests that SLA-LDMC relationships may not strictly follow classical leaf economics expectations under field conditions. SLA is a highly environmentally responsive trait and can vary independently of LDMC due to short-term adjustments in light capture, mesophyll structure or nutrient balance (Kramp et al., 2022; Mount et al., 2024). LDMC, in contrast, reflects tissue density, carbon investment and osmotic regulation, which are governed by physiological processes only partially coordinated with

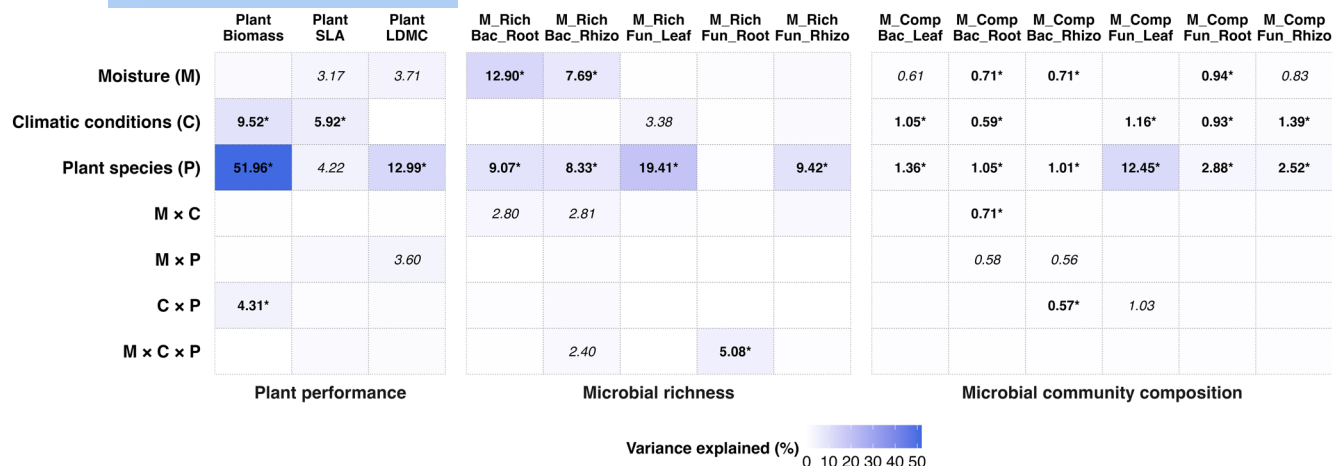


FIGURE 5 Variance explained (%) by experimental factors—moisture (M), climatic conditions (C), plant species (P) and their interactions—across plant and microbial responses. Panels show (i) plant performance (biomass, SLA, LDMC), (ii) microbial richness and (iii) microbial community composition in bacterial and fungal compartments. Numbers denote the percentage of variance explained by each factor, derived from the linear models, linear mixed-effects models and redundancy analyses (RDA) reported in [Tables S5–S7](#). Only significant or marginal effects are shown: Significant effects in bold with asterisks ($p < 0.05$) and marginal effects in italics ($0.05 \leq p < 0.1$). Bac, bacteria; Fun, fungi; LDMC, leaf dry matter content; Leaf, leaf endosphere; M_Comp, microbial community composition; M_Rich, microbial richness; Rhizo, rhizosphere; Root, root endosphere; SLA, specific leaf area.

lamina morphology (Wang et al., 2022). Such decoupling of SLA and LDMC has been widely observed in grasslands experiencing heterogeneous microenvironments and competition (Anderegg, 2023; Rolhauser et al., 2021), helping explain why *Festuca* and *Lolium* differed strongly in LDMC but not in SLA.

It is important to note that the two species were embedded in contrasting grassland community mixtures that reflect their typical ecological contexts. *Festuca* grew within a stress-tolerant mixture dominated by *Dactylis glomerata*, whereas *Lolium* was embedded in a competitive mixture in which it was initially the dominant sown species, together with species such as *Alopecurus pratensis*. These different neighbourhoods may shape the microenvironment, competition intensity and microbial pools experienced by each focal species. Consequently, the observed species differences likely reflect both inherent physiological strategies and the community contexts in which these strategies are realized. This interpretation aligns with the broader view that trait expressions measured in realistic multispecies settings integrate intrinsic species tendencies with neighbourhood-driven modification (La Pierre & Smith, 2015; Zheng et al., 2025).

Plant species identity also structured their associated microbial communities in consistent ways. In line with H1, *Festuca* supported higher bacterial richness in both the root endosphere and rhizosphere, indicating an association with taxonomically diverse bacterial assemblages that may provide functional redundancy and metabolic flexibility (Compant et al., 2010; Saleem et al., 2017). In contrast, *Lolium* harboured higher fungal richness in the rhizosphere, consistent with the well-documented reliance of competitive grasses on stable fungal partners that enhance nutrient acquisition and resource capture (Bever et al., 2012; Morella et al., 2020). These distinct microbial profiles likely arise from inherent differences between the two species in root morphology, chemical composition

and exudation patterns, which act as filters determining which microbes can colonize and persist. Recent evidence shows that plant community composition and trait syndromes modulate soil microbial structure and drought responses, highlighting the role of plant identity and neighbourhood context in shaping below-ground communities (Oram et al., 2025). Together, the trait and microbiome patterns demonstrate that *Festuca* and *Lolium* establish intrinsically different plant-microbe configurations, forming the ecological backdrop against which water availability and climate effects subsequently operate.

4.2 | Effects of water availability on plant traits and microbial communities

In line with H1, which predicted greater trait plasticity in the stress-tolerant species, water availability produced clear but generally modest effects on plant performance and trait expression. Across the full watering gradient, *Festuca* showed two consistent moisture-related adjustments: biomass increased and LDMC decreased with higher water inputs. These patterns indicate that *Festuca* relaxes its conservative resource-use strategy when water becomes more abundant. By contrast, *Lolium* did not exhibit significant shifts in biomass, SLA or LDMC along the moisture gradient, suggesting limited trait plasticity in response to changes in water availability. The overall weak magnitude of trait responses is consistent with evidence that gradual soil-moisture gradients often elicit only small or inconsistent shifts in functional traits. For instance, Asefa et al. (2022) found that several above- and below-ground traits changed only weakly across low-medium-high soil moisture treatments, even under controlled conditions. Moisture-driven trait plasticity can also be constrained by neighbourhood

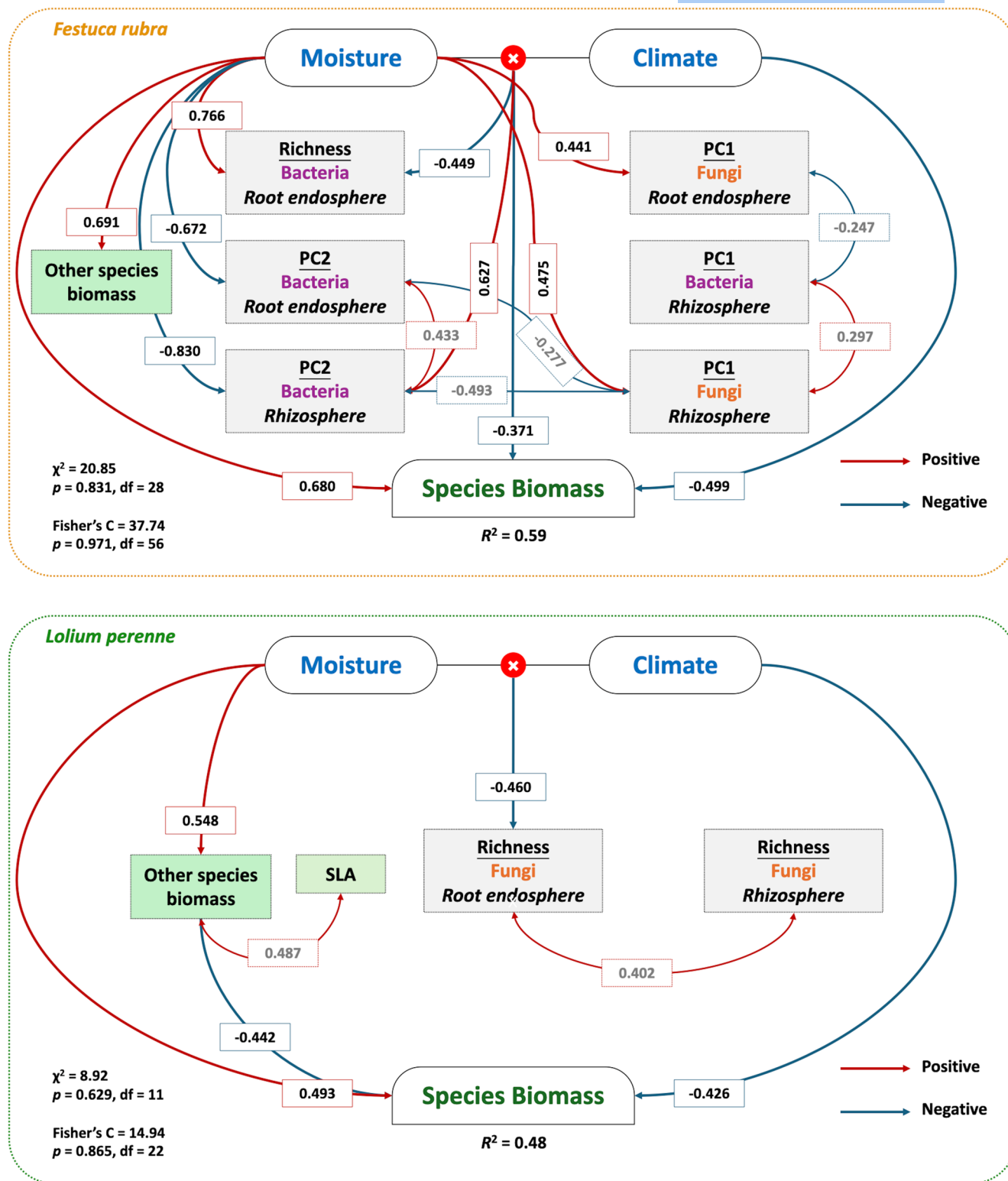


FIGURE 6 Structural equation models (SEMs) linking moisture, climate, plant traits, microbial communities and species biomass for *Festuca rubra* (top) and *Lolium perenne* (bottom). Arrows represent significant pathways identified in species-specific SEMs ($p < 0.05$), with red indicating positive and blue indicating negative standardized path coefficients. Moisture and climate were treated as exogenous predictors, and the moisture $\times$ climate interaction (red 'x') captures climate-dependent moisture effects. Boxes denote plant traits, microbial community attributes and biotic context variables. Black numbers next to arrows indicate standardized path coefficients ($p < 0.05$), while grey numbers represent significant covariances between variables. Non-significant pathways are omitted for clarity. Full path coefficients, covariances and R^2 values are provided in [Tables S17](#) and [S18](#).

effects: Wang and Callaway (2021) showed that competitive interactions can reduce or suppress plant trait responses to differences in water availability. These patterns parallel the modest moisture effects observed here, suggesting that gradual water treatments and multispecies neighbourhoods can diffuse strong plasticity in plant traits.

Consistent with our second hypothesis (H2), which anticipated stronger microbial reassembly under drought among bacterial than fungal partners, water availability strongly structured bacterial richness and these effects were broadly similar between *Festuca* and *Lolium*. Bacterial richness decreased with increasing moisture limitation in the root endosphere for both species and showed additional rhizosphere gains in *Lolium*, indicating that bacteria were consistently more drought-responsive than fungi across hosts. This pattern is consistent with broader evidence that bacterial growth and turnover are tightly coupled to soil moisture due to steep declines in hydration and resource mobility as soils dry (In 'T Zandt et al., 2025; Manzoni et al., 2012; Meisner et al., 2018). In contrast, fungal richness showed little change along the moisture gradient, with no consistent responses across compartments. This relative insensitivity aligns with studies showing that fungi maintain activity at lower water potentials through hyphal foraging, osmotic adjustment and the ability to bridge dry microsites (Canarini et al., 2024; Guhr et al., 2015). Together, these findings highlight that water availability acts as a stronger regulator of bacterial than fungal richness in plant-associated microbiomes.

Microbial compositional responses to water availability complemented the richness patterns, showing that drought reorganized plant-associated bacterial communities more readily than fungal assemblages. In both *Festuca* and *Lolium*, bacterial communities in the root endosphere and rhizosphere shifted detectably along the drought intensity gradient, reflecting how hydration governs processes such as substrate diffusion, enzymatic activity and microbial connectivity within the soil matrix (Bouskill et al., 2013; Carson et al., 2010; Manzoni et al., 2016). As soils dry, reduced nutrient mobility and osmotic stress selectively disadvantage moisture-sensitive taxa, leading to gradual changes in relative abundance rather than wholesale turnover (Schimel, 2018). In contrast, fungal composition changed only weakly with moisture, consistent with several fungal traits that buffer fungi against drying: thicker cell walls and osmolyte production that enhance desiccation tolerance, the ability to exploit complex substrates less affected by diffusion limitations and the spatial continuity of hyphal networks that maintain access to heterogeneous microsites (De Vries et al., 2018; Hannula & Veen, 2025; Ochoa-Hueso et al., 2018; Treseder & Lennon, 2015). These combined features contribute to the greater compositional stability of fungal communities under drought.

Across taxonomic, functional and indicator patterns, a consistent picture emerged in which microbial responses to moisture were highly selective and strongly filtered by compartment and species identity rather than producing broad community shifts. The dominance of compartment-level structuring, especially the distinct

profiles of the rhizosphere versus root endosphere, is well documented in plant microbiome systems and reflects sharp gradients in oxygen, substrate types and host-derived compounds that shape microbial niches (Hereira-Pacheco et al., 2023; Santos-Medellín et al., 2017). Indicator analyses reinforced this pattern: most indicator ASVs were linked to a single compartment or plant species, and overlap among moisture-, climate- and host-associated sets was minimal. Such restricted overlap suggests that drought effects operate through fine-scale ecological filtering, altering subsets of taxa whose traits match local microsite conditions, rather than inducing wholesale community turnover (Allison & Martiny, 2008). Functional predictions were similarly stable across treatments, consistent with functional redundancy in diverse microbiomes where taxonomic shifts do not necessarily translate into altered metabolic potential (Louca et al., 2018). Such redundancy means that different microbial taxa can perform overlapping functions, allowing the community to maintain key processes under changing moisture and climatic conditions and thereby contributing to resilience in plant performance. Together, these patterns indicate that moisture imposed targeted, compartment- and host-specific adjustments on microbial communities, while the broader taxonomic and functional architecture of plant-microbe interactions remained robust across the drought gradient.

4.3 | Effects of warming and elevated CO₂ on plant traits and microbial communities

Climatic conditions, manipulated as the combined increase in temperature and CO₂, influenced plant performance in both species, yet these changes did not conform to the expectation that future climate would buffer drought impacts (H3). Instead, biomass declined under the future climate, particularly in *Lolium*, indicating that the warming component likely intensified evaporative demand and amplified water limitation, overriding any potential CO₂-driven gains in water-use efficiency (De Kauwe et al., 2013; Dusenage et al., 2019). The modest increase in SLA under future conditions suggests a shift towards more acquisitive leaf traits, but this adjustment was insufficient to offset the combined climatic stress. These outcomes align with evidence that warming often exacerbates drought impacts and constrains productivity in grasslands, even under elevated CO₂ (Denney et al., 2024; Zelikova et al., 2014). *Festuca*'s relative insensitivity to climate reflects its stress-tolerant strategy, whereas the stronger reductions in *Lolium* biomass highlight the vulnerability of acquisitive competitors to warming-driven moisture depletion.

Climatic conditions also interacted with moisture to shape microbial responses, but these effects were selective and varied across compartments. In line with H3, bacteria showed the clearest climate × drought interactions: under the current climate, bacterial richness decreased with increasing drought intensity in both the root endosphere and rhizosphere, whereas these relationships became non-significant under the future climate. This attenuation mirrors findings from controlled warming and CO₂ experiments

showing that future climate scenarios can alter microbial moisture sensitivity by modifying carbon allocation, substrate diffusion and soil drying rates (Meisner et al., 2018; Metze et al., 2023). Although warming can intensify soil desiccation through higher evaporation and transpiration (Radolinski et al., 2025; Reinthaler et al., 2021), the combined warming and elevated CO₂ in our study did not amplify moisture effects on richness; instead, it weakened bacteria's responsiveness to water availability. Climate also influenced community composition, especially for endosphere bacteria and rhizosphere fungi, but these shifts remained modest and did not correspond with changes in predicted functional pathways or fungal trophic modes, suggesting strong functional redundancy and compartment-driven filtering (Louca et al., 2018; Toju et al., 2018). Interactions between climate and moisture were largely absent for leaf-associated bacteria and for all fungal richness metrics, consistent with studies reporting limited higher

order interactions among warming, CO₂ and drought in microbial communities (Canarini et al., 2024; Eisenhauer et al., 2012; In 'T Zandt et al., 2025; Kardol et al., 2010). Overall, warming and elevated CO₂ altered the sensitivity of microbial communities to moisture rather than restructuring their diversity or functional capacity.

4.4 | Integrated plant–microbe responses to drought and climatic conditions

Our structural equation models show that *Festuca* and *Lolium* rely on distinct pathways linking soil moisture, climate, plant traits and microbial communities. In *Festuca*, moisture effects propagated through multiple microbial components in both the root endosphere and rhizosphere, indicating that bacterial richness and compositional

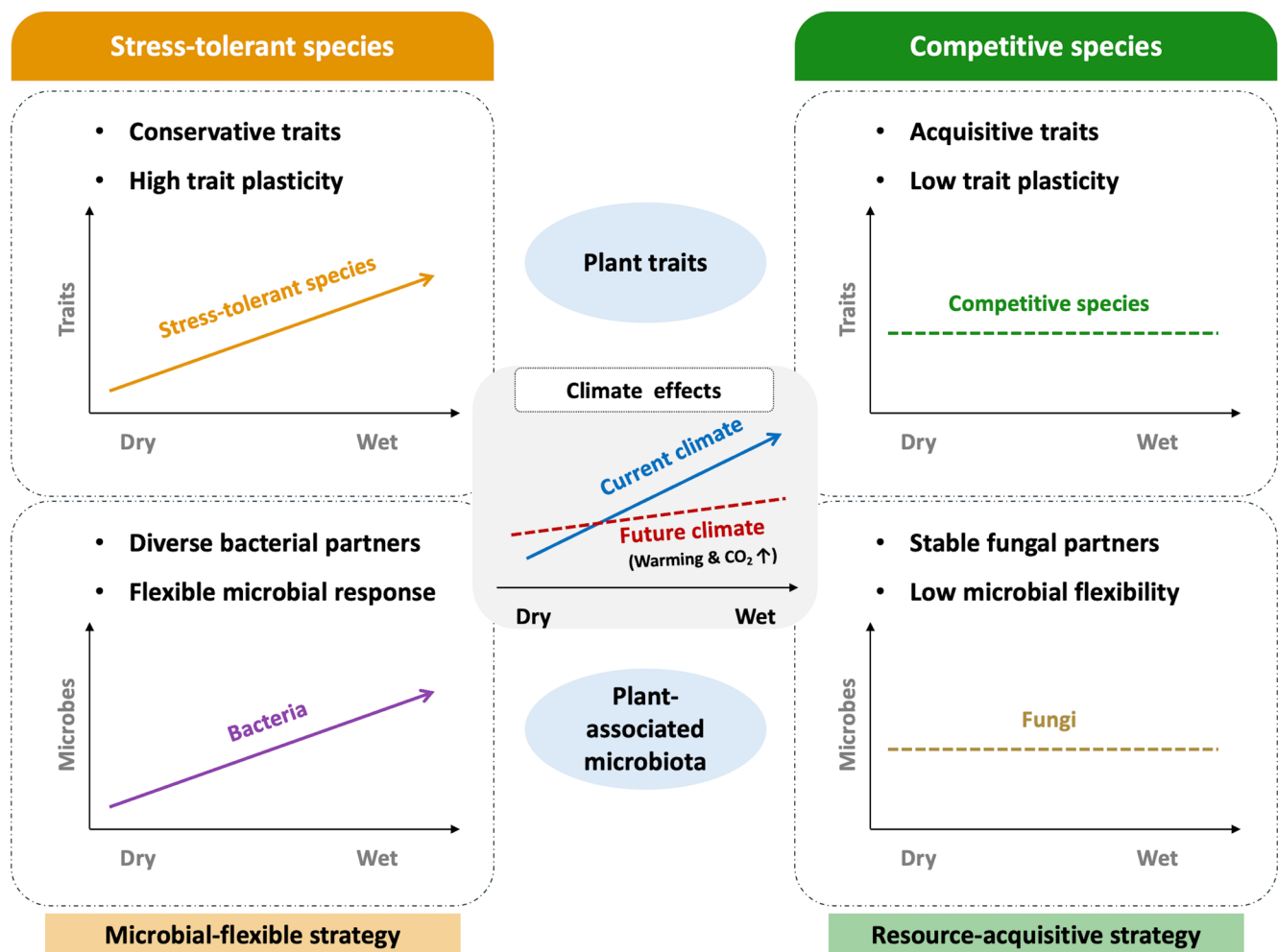


FIGURE 7 Conceptual model illustrating contrasting strategies through which stress-tolerant and competitive grassland species respond to drought under current versus future climatic conditions. Stress-tolerant plant species tend to combine conservative traits with greater trait plasticity and associations with taxonomically diverse, moisture-responsive microbial communities—forming a microbial-flexible strategy, in which associated microbial communities (particularly bacteria) respond strongly along the moisture gradient and support coordinated adjustment to drought. In contrast, competitive plant species rely more on acquisitive traits with lower trait plasticity and relatively stable microbial associations, including fungal partners, reflecting a resource-acquisitive strategy in which drought sensitivity arises primarily from direct physiological responses and competitive interactions. Warming and elevated CO₂ may modify moisture availability or trait expression to some extent but generally exert weaker effects than drought. This framework highlights how contrasting plant–microbe interaction strategies shape grassland responses to environmental stress.

shifts contribute to its drought-response strategy even when not directly linked to biomass. This aligns with the idea that stress-tolerant species integrate flexible microbial partners capable of rapidly responding to resource fluctuations (Compant et al., 2010; Naylor & Coleman-Derr, 2018). Several moisture $\times$ climate interactions further suggest that *Festuca*'s microbial networks remain responsive under warming and elevated CO₂, enabling coordinated adjustment of both plant traits and microbiomes.

In contrast, *Lolium* exhibited a streamlined response structure in which biomass was driven mainly by direct effects of moisture, climate and competition, with little contribution from microbial attributes. This pattern reflects the strategy of acquisitive grasses, which generally rely on rapid physiological responses rather than microbiome restructuring (Bever et al., 2012; Pérez-Ramos et al., 2019), and is consistent with evidence that productive grasses often maintain relatively stable, low-plasticity microbial associations under stress (Morella et al., 2020; Sheik et al., 2011).

Although competitive and stress-tolerant plant species are often broadly associated with bacterial- versus fungal-dominated soil microbial communities, respectively (Bergmann et al., 2020; De Vries et al., 2012), these frameworks primarily describe patterns of microbial dominance. The contrasting pathways identified by our structural equation models emphasize differences in microbial responsiveness and stability within drought-response networks. In this system, stress-tolerant *Festuca* was associated with more moisture-responsive and taxonomically diverse bacterial communities, whereas competitive *Lolium* maintained relatively stable fungal associations, highlighting a context-dependent axis of plant-microbe coordination under drought gradients.

Across both species, correlations between plant traits (SLA, LDMC) and microbial attributes were weak, suggesting that drought responses were not strongly mediated through coordinated trait-microbiome adjustments. This decoupling mirrors findings that plant functional traits and microbial communities often operate on different temporal and spatial scales—traits reflect whole-plant strategies, whereas microbiomes track microsite conditions, exudation chemistry and root turnover dynamics (Egidi et al., 2025; Trivedi et al., 2022). Moreover, the strongest microbial responses occurred below-ground, whereas SLA and LDMC represent above-ground leaf economics, a trait axis known to correlate weakly with root traits and below-ground microbial dynamics in grasslands (Freschet et al., 2018; Laughlin et al., 2021). Because climate manipulations were applied at the plot level, climate effects should be interpreted cautiously; nonetheless, the consistency of plant and microbial patterns across analyses supports the robustness of the identified pathways. Together, these results indicate that plant-microbe coordination under drought and future climate arises not from tight trait-microbiome coupling but from species-specific response pathways: *Festuca* mobilizing flexible microbial partners to adjust to environmental variation and *Lolium* relying primarily on direct environmental sensitivity and competitive interactions.

These species-specific pathways are summarized in a conceptual model (Figure 7), which illustrates how drought and climatic

conditions interact with contrasting plant ecological strategies to generate two distinct response frameworks: a microbially flexible strategy, where stress-tolerant plants draw on diverse and moisture-responsive bacterial partners, and a resource-acquisitive strategy, where competitive plants rely primarily on direct physiological and competitive responses with relatively stable fungal associations. The model provides a general mechanism for understanding how plant-microbe coordination shapes grassland responses to environmental change. Overall, these findings reveal that drought and climatic conditions reshape grassland functioning not through a single plant-microbiome response, but through species-specific strategies that determine whether plants depend on microbial flexibility or direct physiological adjustment under stress.

5 | CONCLUSION

Our findings show that drought and the combined warming and elevated CO₂ treatment shape grassland functioning through species-specific coordination between plant traits and microbial communities. *Festuca* mobilized moisture-responsive bacterial assemblages, whereas *Lolium* relied primarily on direct physiological and competitive responses, producing distinct pathways of drought and climate change sensitivity. Although moisture strongly influenced bacterial richness and composition, microbial functional profiles remained comparatively stable, reflecting strong compartment filtering and redundancy. These results highlight that plant-microbe interactions do not follow a uniform strategy across grassland species; instead, contrasting ecological strategies determine whether plants draw on microbial flexibility or direct resource-use shifts under environmental stress. Recognizing this diversity in plant-microbiome responses will be essential for predicting grassland dynamics under increasing drought and atmospheric change.

AUTHOR CONTRIBUTIONS

Gang Yang conducted laboratory work and microbial analyses, led the writing of the original draft and contributed to formal analysis, methodology development and data visualization. Lisa Capponi was primarily responsible for maintaining the field experiment and contributed to manuscript review. Michael Bahn and Andreas Schaumberger provided project administration and resources and contributed to reviewing and editing the manuscript. Zuzana Münzbergová conceived the study, contributed to methodology and supervision, provided resources and participated in manuscript review and editing. All authors contributed critically to the drafts and approved the final version for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70251>.

DATA AVAILABILITY STATEMENT

Raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1402917 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1402917>). All processed data and analysis R scripts are available on GitHub (https://github.com/al-YangG/Drought_Plant-Microbes_Two-Grasses) and archived via Zenodo with the DOI: <https://doi.org/10.5281/zenodo.18232656> (Yang, 2026).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Method S1. DNA extraction, PCR amplification and sequencing.

Method S2. Bioinformatics and sequence processing.

Method S3. Statistical analyses and structural equation models.

Figure S1. Experimental design and plot layout. (a) Layout of four experimental plots under future (diamonds) and current (circles) climate scenarios. Each plot contained 12 mesocosms and planted with either a stress-tolerant (orange) or competitive (green) plant community. Six watering regimes were applied per plot to establish a drought gradient. (b) Photograph (left) and schematic diagram (right) of a single plot, showing mesocosm arrangement, central smart field lysimeter and CO₂ fumigation ring (diameter of 2.2m). Watering regimes were randomly assigned to mesocosms within each plot to reduce potential bias from small-scale heterogeneity.

Figure S2. Relationship between watering treatments and soil moisture at peak drought. Gravimetric water content (GWC) of all mesocosms plotted against watering amount (ml per event). Points represent individual mesocosms and are coloured by plant species. The solid black line shows the overall linear regression with 95% confidence interval (grey shading). Species-specific regressions are shown as a solid line for *Festuca* (significant, orange) and a dashed line for *Lolium* (non-significant, green). Reported *p*-values correspond to the significance of each linear model. This figure demonstrates that

the imposed watering treatments generated a clear soil-moisture gradient across mesocosms and that the two plant species differed in the strength of their responses.

Figure S3. Rarefaction curves of bacterial and fungal communities across plant compartments. Curves show the expected number of observed amplicon sequence variants (ASVs) as a function of sequencing depth. Thin grey lines represent individual samples, and thick red lines indicate median curves for each data set. Blue dashed vertical lines mark the rarefaction thresholds applied in downstream analyses: 3848 reads for bacterial rhizosphere, 4463 for bacterial root endosphere, 10 for fungal leaf endosphere, 854 for fungal root endosphere and 1024 for fungal rhizosphere.

Figure S4. Venn diagrams showing the number of shared and exclusive bacterial (left panels) and fungal (right panels) ASVs across (a, b) compartments (leaf: leaf endosphere, root: root endosphere and soil: rhizosphere), (c, d) climatic conditions (current vs. future) and (e, f) plant species (*Festuca* vs. *Lolium*).

Figure S5. Pairwise correlations among moisture, plant traits and microbial metrics for *Festuca* (left) and *Lolium* (right). The heat map shows Pearson correlation coefficients (r) between all pairs of variables. Only statistically significant correlations ($p < 0.05$) are coloured, with positive correlations shown in shades of red and negative correlations in shades of blue, and colour intensity proportional to the strength of the correlation. Each tile is annotated with the corresponding r -value.

Figure S6. Plant performance responses to the moisture gradient across climate conditions. (a) Moisture–trait relationships for species biomass, biomass proportion, specific leaf area (SLA) and leaf dry matter content (LDMC), with all data pooled across plant species and climatic conditions. Grey points represent observed trait values, and blue lines show fitted linear regressions with 95% confidence ribbons. Slope estimates (all non-significant, 'ns') describe the overall moisture effect for each trait. Shaded backgrounds distinguish severe drought (0–400 mL), moderate drought (400–1000 mL) and well-watered conditions (1000–1600 mL). (b) Moisture–trait relationships separated by climatic conditions (current vs. future). Points and lines are coloured by climate (current = blue; future = red). Dashed lines indicate non-significant moisture effects, and solid lines indicate significant effects ($p < 0.05$). Slope estimates printed in each panel correspond to the regression line of the same colour and quantify the moisture response of each species; significance is indicated by '*' ($p < 0.05$) and 'ns' ($p \geq 0.05$). Shaded backgrounds follow panel (a).

Figure S7. Plant performance differences between climatic conditions and plant species. (a) Effects of climatic conditions (current vs. future) on species biomass, biomass proportion, specific leaf area (SLA) and leaf dry matter content (LDMC). Violin plots show the distribution of individual samples, black points denote medians and vertical bars denote interquartile ranges. Compact letter displays above violins indicate statistically distinct groups based on linear models (biomass, biomass proportion) and linear mixed-effects models (SLA, LDMC) with pairwise comparisons; different letters represent significantly different means at $p < 0.05$. (b) Combined effects of climate and

plant species (*Festuca* and *Lolium*) on plant performance traits. Violin plots are separated by species identity and climatic conditions. Black points and vertical bars represent medians and interquartile ranges. Compact letter displays indicate statistically distinct groups within each trait ($p < 0.05$).

Figure S8. Microbial richness responses to the moisture gradient under different climate conditions. (a) Relationships between bacterial and fungal ASV richness and the moisture gradient for leaf, root and rhizosphere compartments. Points represent individual samples. Linear model fits are shown as blue lines; solid lines indicate significant moisture effects ($p < 0.05$) and dashed lines indicate non-significant effects. Slope estimates printed in each panel correspond to the regression line of the same colour and quantify the moisture response of each species; significance is indicated by '*' ($p < 0.05$), '**' ($p < 0.01$) and 'ns' ($p \geq 0.05$). Shaded backgrounds denote, from left to right, severe drought (0–400 mL), moderate drought (400–1000 mL) and well-watered conditions (1000–1600 mL). (b) Bacterial richness–moisture relationships shown separately for current and future climatic conditions. Points and regression lines are coloured by climatic conditions (current = blue; future = red). Line styles and shaded backgrounds follow those in (a). (c) Fungal richness–moisture relationships shown separately for current and future climate conditions. Points, regression lines and line styles follow the conventions in (b).

Figure S9. Microbial richness across climate conditions and plant species. (a) Bacterial and fungal ASV richness in leaf, root and rhizosphere compartments under current and future climatic conditions. Violin plots show the distribution of individual samples; black points denote medians and vertical bars denote interquartile ranges. Compact letter displays above violins indicate statistically distinct groups based on linear mixed-effects models with pairwise comparisons; different letters represent significantly different means at $p < 0.05$. (b) Bacterial and fungal ASV richness shown jointly for climatic conditions and plant species (*Festuca* and *Lolium*). Violin plots are grouped by species identity and climatic conditions. Black points represent medians and vertical bars represent interquartile ranges. Compact letter displays indicate statistically distinct groups within each compartment of bacteria or fungi ($p < 0.05$).

Figure S10. Principal component analysis (PCA) of bacterial and fungal community composition in the leaf endosphere, root endosphere and rhizosphere. Each point represents one sample and is coloured by climatic conditions (Current = blue, Future = red), with point size indicating the applied moisture level. Convex hulls enclose samples of the same plant species. Black arrows indicate the direction of the moisture gradient based on fitted environmental vectors (envfit). Percentages on axes denote the proportion of variance explained by the first two principal components. Numbers in parentheses in panel titles represent the number of ASVs included in each data set.

Figure S11. Relative abundances of the top five bacterial and fungal taxa across environmental and host factors. Stacked bar plots show the mean relative abundance (%) of (a) bacterial phyla, (b) fungal phyla, (c) bacterial orders and (d) fungal orders, grouped by compartment (leaf endosphere, root endosphere, rhizosphere), moisture level

(M1–M6, from driest to wettest), plant species (*Festuca* and *Lolium*) and climatic conditions (current and future). Bars within each facet are normalized to 100%. 'Other' includes all remaining taxa outside the top five most abundant groups at each taxonomic rank.

Figure S12. Predicted bacterial functional profiles (KEGG Level 3) across plant species, moisture levels and compartments. (a) Relative abundance (%) of predicted pathways grouped by plant species (*Festuca*, *Lolium*). (b) Relative abundance (%) of predicted pathways across moisture levels (M1–M6; from driest to wettest). (c) Relative abundance (%) of predicted pathways across compartments (leaf endosphere, root endosphere, rhizosphere). Bars represent the mean relative abundance of pathways assigned to KEGG Level 3 categories, colour-coded by their parent Level 2 functional class. Only the top 15 most abundant pathways are displayed in each panel for readability.

Figure S13. Fungal trophic modes across environmental and host factors. (a) Mean relative abundance (%) of fungal trophic modes grouped by compartment (leaf endosphere, root endosphere, rhizosphere), moisture level (M1–M6, from driest to wettest), plant species (*Festuca* and *Lolium*) and climatic conditions (current and future). Bars within each facet are normalized to 100%, with colours indicating different trophic modes inferred from FUNGuild. 'Unassigned' denotes ASVs without functional annotations or with low-confidence assignments. (b) Effects of compartment on the relative abundance of each trophic mode. Bars represent mean $\pm$ SE across mesocosms. Different letters indicate significant differences among compartments based on beta regression (Tukey-adjusted $p < 0.05$).

Figure S14. Summary of moisture-responsive ASVs (identified by *DESeq2*) and group-specific indicator ASVs (identified by *indicspecies*) across environmental and host factors. (a) Number of moisture-responsive bacterial and fungal ASVs detected by *DESeq2* across compartments (leaf endosphere, root endosphere, rhizosphere), plant species (*Festuca*, *Lolium*) and climatic conditions (current vs. future). Bars show positive (upregulated) and negative (downregulated) responses to increasing moisture, with the exact numbers printed inside each bar. (b) Number of ASVs associated with each compartment (leaf endosphere, root endosphere, rhizosphere) and classified according to whether they were identified as compartment indicators (*indicspecies*), as moisture-responsive ASVs (*DESeq2*) or both. (c) Number of ASVs associated with plant species (*Festuca* or *Lolium*) and classified according to whether they were identified as species indicators (*indicspecies*), as moisture-responsive ASVs (*DESeq2*) or both. (d) Number of ASVs associated with climatic conditions (current or future) and classified according to whether they were identified as climate indicators (*indicspecies*), as moisture-responsive ASVs (*DESeq2*) or both. For (b–d), results are shown separately for bacteria and fungi. Blue dots represent ASVs identified only by *indicspecies*, purple dots represent ASVs identified only by *DESeq2* as moisture-responsive and red dots represent ASVs identified as being moisture responsive and simultaneously as indicators of compartment (b), species (c) or climate (d). Numeric labels indicate the number of ASVs in each category.

Figure S15. Shared and unique moisture-responsive ASVs between *Festuca* and *Lolium*. (a) Venn diagrams showing the number of moisture-responsive ASVs unique to *Festuca* or *Lolium* and those shared between the two plant species, for bacteria (left) and fungi (right). Across both domains, two bacterial ASVs and one fungal ASV were shared between species. (b) $\log_2$ moisture-response of shared ASVs, showing fold-change in abundance along the moisture gradient in *Festuca* (x-axis) and *Lolium* (y-axis). Each point represents an ASV identified as moisture-responsive using differential abundance analysis in the *DESeq2* package. The solid diagonal line represents a 1:1 relationship between the responses of the two host species. (c) Taxonomic summaries for the three shared ASVs highlighted in (b), including domain, phylum, class, family and trophic mode.

Figure S16. Indicator ASVs across compartments, plant species and climatic conditions and their taxonomic composition. (a) Number of significant bacterial and fungal indicator ASVs identified for each compartment (leaf endosphere, root endosphere, rhizosphere), plant species (*Festuca*, *Lolium*) and climatic condition (current, future). Values above bars indicate total indicator counts within each category. (b–d) Phylum-level composition of bacterial (top) and fungal (bottom) indicator ASVs across (b) compartments, (c) plant species and (d) climatic conditions. Bubble size represents the number of indicator ASVs within each phylum, and bubble colour indicates the mean IndVal score. IndVal values were obtained from the indicator species analysis in the *indicspecies* package, where higher values denote stronger associations with the respective group. Phylum labels show the number of indicator ASVs relative to the total ASVs detected in that phylum in the full data set. Because compartments yielded far more indicator ASVs and a broader taxonomic spread than plant species or climatic species, only the most represented phyla are shown in (b) (bacteria: top 10; fungi: top 5) to maintain clarity. (c) and (d) display all phyla detected for each factor.

Table S1. Target percentages of field capacity used to structure the watering gradient, corresponding watering amounts and allocation of watering treatments to the future and current climate plots illustrated in Figure S1.

Table S2. Summary of read depths across six datasets. For each dataset, the total number of samples is given in parentheses. Values are reported as minimum, median, mean and maximum sequencing depths (reads per sample).

Table S3. Sample retention at different rarefaction thresholds. For each dataset, the total number of samples is given in parentheses. Rows indicate the number of samples retained and the percentage retained at thresholds from 1000 to 15,000 reads.

Table S4. Results of linear mixed-effects models testing the effects of moisture (M), climatic conditions (C), plant species (P) and their interactions on bacterial and fungal diversity across plant compartments (leaf endosphere, root endosphere and rhizosphere; sample size $n = 96$ for all datasets except fungal leaf endosphere, where $n = 95$). Diversity was quantified using (a) the Shannon and (b) the inverse Simpson indices. Values show χ^2 -statistics with associated p -values. Significant treatment effects are in bold ($p < 0.05$), marginally significant effects are in *italics* ($0.05 \leq p < 0.1$).

Table S5. Results of linear models for species biomass (sample size, $n=48$), beta regression for biomass proportion ($n=48$) and linear mixed-effects models for specific leaf area (SLA; $n=96$) and leaf dry matter content (LDMC; $n=96$). Values show F - or χ^2 -statistics with associated p -values for the effects of moisture (M), climatic conditions (C), plant species (P) and their interactions. Significant treatment effects are in bold ($p < 0.05$); marginally significant effects are in *italics* ($0.05 \leq p < 0.1$).

Table S6. Results of linear mixed-effects models testing the effects of moisture (M), climatic conditions (C), plant species (P) and their interactions on bacterial and fungal richness across plant compartments (leaf endosphere, root endosphere and rhizosphere; sample size $n=96$ for all datasets except fungal leaf endosphere, where $n=95$). Values show χ^2 -statistics with associated p -values. Significant treatment effects are in bold ($p < 0.05$); marginally significant effects are in *italics* ($0.05 \leq p < 0.1$).

Table S7. Results of redundancy analyses (RDA) testing the effects of moisture (M), climatic conditions (C), plant species (P) and their interactions on bacterial and fungal community composition in different plant compartments. Values show F -statistics and associated p -values for each term. Significant treatment effects are in bold ($p < 0.05$); marginally significant effects are in *italics* ($0.05 \leq p < 0.1$). Sample sizes (n) are indicated for each compartment.

Table S8. Results of linear models for species biomass (sample size, $n=48$), beta regression for biomass proportion ($n=48$) and linear mixed-effects models for specific leaf area (SLA; $n=96$) and leaf dry matter content (LDMC; $n=96$). Values show F - or χ^2 -statistics with associated p -values for the effects of soil gravimetric water content (GWC), climatic conditions (C), plant species (P) and their interactions. Significant treatment effects are in bold ($p < 0.05$); marginally significant effects are in *italics* ($0.05 \leq p < 0.1$).

Table S9. Results of linear mixed-effects models testing the effects of soil gravimetric water content (GWC), climatic conditions (C), plant species (P) and their interactions on bacterial and fungal richness across plant compartments (leaf endosphere, root endosphere and rhizosphere; sample size $n=96$ for all datasets except fungal leaf endosphere, where $n=95$). Values show χ^2 -statistics with associated p -values. Significant treatment effects are in bold ($p < 0.05$); marginally significant effects are in *italics* ($0.05 \leq p < 0.1$).

Table S10. Results of redundancy analyses (RDA) testing the effects of soil gravimetric water content (GWC), climatic conditions (C), plant species (P) and their interactions on bacterial and fungal community composition in different plant compartments. Values show F -statistics and associated p -values for each term. Significant treatment effects are in bold ($p < 0.05$); marginally significant effects are in *italics* ($0.05 \leq p < 0.1$). Sample sizes (n) are indicated for each compartment.

Table S11. Variables considered in structural equation models (SEMs). Summary of environmental, plant, microbial and biotic context variables evaluated in the SEMs linking moisture, climate, plant traits, microbial communities and species biomass.

Table S12. Final structural equation model (SEM) structures and model fit statistics for *Festuca* and *Lolium*. Component equations and covariances included in the final SEMs linking moisture, climate, plant traits, microbial community attributes and species biomass.

Table S13. Summary of beta regression results testing the effects of experimental factors on the relative abundances of the five most abundant bacterial and fungal taxa at the phylum and order levels. Shown are Wald χ^2 statistics and associated p -values for the fixed effects of compartment (Cp), moisture (M), climatic conditions (C), plant species (P) and their interactions. Panels indicate (a) bacterial phyla, (b) fungal phyla, (c) bacterial orders and (d) fungal orders. Significant effects are shown in bold ($p < 0.05$).

Table S14. Summary of beta regression results testing the effects of experimental factors on the relative abundances of fungal trophic modes. Shown are Wald χ^2 statistics and associated p -values for the fixed effects of compartment (Cp), moisture (M), climatic conditions (C), plant species (P) and their interactions. Significant effects are shown in bold ($p < 0.05$).

Table S17. Summary of path coefficients and covariances in structural equation models (SEMs) for *Festuca* and *Lolium*. Standardized path coefficients (Std.Estimate) are shown for all relationships included in the final SEMs. Covariances (~~) represent residual correlations between microbial or trait variables after accounting for modelled predictors. Degrees of freedom (df) and associated p -values are provided for each path. Significant effects ($p < 0.05$) are highlighted in bold.

Table S18. Marginal and conditional R^2 values for component models within structural equation models (SEMs) for *Festuca* and *Lolium*. Marginal R^2 represents variance explained by fixed effects (moisture, climate and their interaction), while conditional R^2 represents variance explained by both fixed and random effects (mesocosm identity). 'NA' indicates models without random effects.

Table S15. Moisture-responsive bacterial ASVs detected in *Festuca* and *Lolium* using DESeq2. Summary of ASVs whose abundances changed significantly along the experimental moisture gradient in *Festuca* and *Lolium*.

Table S16. ASVs jointly identified as indicators by *indicspecies* and DESeq2 across compartments, plant species and climatic conditions. Summary of bacterial and fungal ASVs that were detected as significant indicator taxa by both *indicspecies* and DESeq2 for each experimental grouping (compartment, plant species and climatic condition).

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