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RESEARCH ARTICLE

Drought legacy effects on plant growth and plant–soil feedback are mediated by soil microbial communities independently of root exudates and root litter

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Abstract

1. Extreme droughts alter vegetation dynamics worldwide and the effects often persist after the drought ended. Indirect drought effects mediated by the soil microbial community can continue to affect plant growth during drought recovery and may impact plant–soil feedback (PSF), the effect a species has on its own growth via its rhizosphere microbiome. Changes in plant inputs to the soil, such as root exudates and litter, may drive these drought legacy effects through changes in soil bacterial and fungal communities.
2. In a three-stage greenhouse experiment, we assessed drought legacy effects on plant biomass and PSF of three common grassland species. In a first conditioning phase, soil was conditioned directly by plants under drought and ambient conditions. In a second conditioning phase, soil was conditioned by the addition of either conditioned soil inoculum or root exudates or root litter produced in the first phase by droughted or non-droughted plants. In the feedback phase, a new set of plants was grown in soil conditioned by the same species compared to soil conditioned by another species across all soil conditioning types and their biomass linked to soil microbial community data.
3. We found that only soil conditioning with plants, but not inoculum, exudates or litter, resulted in a consistent negative drought legacy effect on plant growth, which was linked to lower microbial biomass and shifts in bacterial and fungal community composition. We could identify a set of fungal and bacterial taxa which were differentially abundant in drought and well-watered soil and accurately predicted plant growth. PSF in plant-conditioned soil differed between species, but was only affected by drought in *Rumex acetosa*. This pattern was not reproduced through the addition of inoculum, root exudates or root litter.
4. *Synthesis.* Our results show that drought indirectly restricts plant growth, which is not mediated by root exudates or root litter, but through altering microbial biomass and community composition. These findings suggest that plant

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recovery from extreme drought is obstructed by persistent changes in soil microbial communities.

KEYWORDS

bacteria, climate change, drought legacy, fungi, plant–soil feedback, root exudates, root litter, soil microbes

1 | INTRODUCTION

The increasing frequency and intensity of extreme drought events under climate change results in altered plant community composition in ecosystems worldwide (Batbaatar et al., 2022; Liu et al., 2018). Grasslands cover around 40% of the global terrestrial surface (White et al., 2000) and are especially vulnerable to extreme drought events (Smith et al., 2024; Tilman & El Haddi, 1992). Drought impacts can persist after the drought has ended (Tilman & El Haddi, 1992) and alter long-term trajectories of vegetation dynamics (Müller & Bahn, 2022). These drought legacy effects on plant productivity and species composition have been linked to persistent changes in soil microbial communities (Fu, Chen, Jansa, et al., 2022; Schärer et al., 2023) and can even affect plants that never experienced drought themselves (de Long, Semchenko, et al., 2019; de Vries et al., 2012). To this date, the interplay between drought effects on plant and soil communities as well as feedbacks between the two, and the consequences for plant growth and community composition, are not understood in its whole complexity.

There are many examples across ecosystems where drought responses of plant communities are tightly intertwined with those of soil microbes, and their interactions are central in determining how long a drought legacy persists (Müller & Bahn, 2022). In grasslands, plant productivity usually declines under drought but recovers within 1 year (Mackie et al., 2019; Stampfli et al., 2018; Wu et al., 2018; Xie et al., 2020), often even exceeding pre-drought values after rewetting (Oram et al., 2023; Schärer et al., 2023). This overshoot effect has been linked to increased microbial nutrient mineralisation in the soil after the drought ended (Birch, 1958; Oram et al., 2023; Schärer et al., 2023). However, in a diversity experiment, it was shown that drought legacies that increase plant growth only occurred at high plant diversity (Xi, Chen, et al., 2022). Negative drought legacies at low plant diversity co-occurred with distinct bacterial and fungal communities and a stronger reduction of bacterial richness (Xi, Chen, et al., 2022). Drought-induced changes in plant community composition usually persist longer than those of productivity (De Boeck et al., 2018; Hoover et al., 2014; Stampfli & Zeiter, 2020) and can even permanently change the ecosystem state (Stampfli & Zeiter, 2004; Xu et al., 2017).

Plant–soil feedback (PSF; Bever et al., 1997), here defined as the net positive or negative effect of a plant-associated soil microbial community on plant fitness, presents one possible pathway through which extreme drought events can affect vegetation dynamics (van der Putten et al., 2016). Negative PSF is particularly

important for promoting species coexistence and stability in grasslands (Goossens et al., 2023; van der Putten et al., 2013). A commonly accepted hypothesis is that PSF becomes more positive under low resource availability and moderate stress, due to the higher dependence on mutualistic microbes and lower pathogen pressure (de Vries et al., 2023; Gundale & Kardol, 2021). Both fungal symbionts, in particular arbuscular mycorrhizal fungi (AMF), and plant growth-promoting rhizobacteria (PGPRB) are known to be specifically beneficial to their plant host under drought conditions (Jayne & Quigley, 2014; Kivlin et al., 2013; Rubin et al., 2017) and can facilitate plant community recovery from drought (Jia et al., 2020). Under extreme drought, however, these beneficial interactions may be disrupted and PSF more variable (de Vries et al., 2023; Fu, Chen, Rillig, et al., 2022). An increasing body of empirical studies has shown that PSF is generally responsive to drought (Crawford & Hawkes, 2020; Fry et al., 2018; Hassan et al., 2022; Martorell et al., 2021; Snyder & Harmon-Threatt, 2019), but also, that PSF drought responses are difficult to predict and vary between plant species, functional groups, growth strategies and experimental systems (Beals et al., 2020; de Vries et al., 2023). The few studies in which PSF drought responses were directly linked to soil microbial communities suggest a role for both fungi (Lozano et al., 2022; Xi, Crawford, & De Long, 2022) and bacteria (Fitzpatrick et al., 2018). Given the dynamic nature of PSF under environmental change, a mechanistic understanding of how plant and microbial drought responses interact in driving these drought-induced PSF shifts is essential if we want to predict drought legacies on vegetation patterns.

The nature and duration of microbial legacies in the soil is governed by plant drought responses, but also by direct physical–chemical impacts of drought and rewetting (Oram et al., 2025; Veach et al., 2020). During drought, the mortality of taxa with low drought tolerance results in a general loss of microbial biomass and a shift in community composition (Gliesch et al., 2024; Liu et al., 2022). Differences in drought response strategies (Malik & Bouskill, 2022; Metze et al., 2023) further shift species composition towards more slow-growing, fungal-dominated communities (Bapiri et al., 2010; de Vries et al., 2018; Fuchslueger et al., 2014; Liu et al., 2022; Preece et al., 2019), with a higher relative abundance of gram-positive bacteria (Bapiri et al., 2010; Fitzpatrick et al., 2018; Fuchslueger et al., 2014; Gliesch et al., 2024). Upon rewetting, a pulse of microbial activity results in the release of nutrients (Birch, 1958; Fierer & Schimel, 2003), which may be specifically advantageous for fast-growing, resource-acquisitive plant species (Williams & de Vries, 2020). Drought-resilient, opportunistic microbes with fast

growth rates can recolonise empty niche spaces, leading to further changes in the microbial community structure (Barnard et al., 2013; de Nijs et al., 2019; Evans & Wallenstein, 2014). These drought-induced shifts in soil microbial community structure, diversity and trait distribution can last between months up to longer than a year (de Vries et al., 2018; Fu, Chen, Jansa, et al., 2022; Oram et al., 2025; Wang & Allison, 2021) and carry the potential to affect PSF, plant community productivity and composition long after an extreme drought event.

Plants are a central element in shaping microbial community responses to drought (Bakker et al., 2018; Fitzpatrick et al., 2018; Gillespie et al., 2020; Gliesch et al., 2024; Preece et al., 2019). A reduction in root biomass (de Vries et al., 2016), species-dependent shifts in root traits (de Vries et al., 2016), changing patterns of root exudation (de Vries et al., 2019; Henry et al., 2007; Hou et al., 2025), and changes in the quality of litter inputs (Reinelt et al., 2023) can affect soil microorganisms. Root exudates differ between plant species based on their growth strategy and their root traits (Williams, Langridge, Straathof, Muhamadali, et al., 2021), play a central role in the assembly of the rhizosphere microbiome (Sasse et al., 2018; Zhalnina et al., 2018), shape microbial stress responses (Chen et al., 2022; Rolfe et al., 2019; Sharma et al., 2023; Vives-Peris et al., 2018) and impact PSF (Hu et al., 2018; Steinauer et al., 2023). Both quantitative (de Vries et al., 2019; Henry et al., 2007; Preece & Peñuelas, 2016; Reid & Mexal, 1977) and qualitative changes in the exudates of droughted plants have been documented across ecosystems, but with high variation across species (Canarini et al., 2016; Jiang et al., 2023; Ulrich et al., 2022) and limited capacity to recover (Gargallo-Garriga et al., 2018), indicating a persistent exudate-driven drought legacy. These shifts in root exudation can affect C transfer to different microbial groups during drought (Fuchslueger et al., 2014; Karlowicz et al., 2018) and trigger more soil respiration post-drought (Hou et al., 2025), which suggests that plants activate their rhizosphere microbial community to accelerate drought recovery (de Vries et al., 2019; Hou et al., 2025). Another pathway through which plant drought responses may affect microbial communities is through altered litter inputs. In grasslands, decaying root material is an important C source for soil microbes (Bardgett et al., 2014) and there is evidence that drought alters root C and N contents (de Vries et al., 2016; Lozano et al., 2020; Reinelt et al., 2023) and metabolite profiles (Gargallo-Garriga et al., 2014), which can positively or negatively affect their decomposition (García-Palacios et al., 2016; Kemp et al., 2003; Reinelt et al., 2023) and potentially alter feedbacks to plant growth (Veen et al., 2019). It still remains unclear which role these drought-induced changes in exudate and root litter quality play in shaping post-drought microbial communities and their feedbacks to plant growth.

Here, we aimed to address the missing link between plant and soil microbial drought responses and the resulting consequences for plant community dynamics during drought recovery. Specifically, our objectives were to (A) assess legacy effects of severe drought on plant growth and PSF in grassland species and (B) assess the role of changes in root exudation and root litter inputs as well as changes

in microbial biomass and community composition in these legacy effects. We hypothesised that (1) drought legacy effects in the soil overall stimulate plant growth due to increased nutrient availability after rewetting; (2) drought legacy effects on PSF are species-specific as the microbial taxa driving PSF differ between species and may be differently affected by drought; (3) PSF responses to drought can be partially reproduced by the addition of exudates from droughted plants but less well by root litter addition, based on the more direct role of exudates in shaping rhizosphere microbial communities and their stronger drought response; and (4) bacterial communities are generally more affected by drought, but fungal responses are more relevant for plant growth and PSF responses to drought.

These hypotheses were tested in a three-stage greenhouse experiment, consisting of two soil conditioning phases followed by a feedback phase, using three common grassland species with contrasting drought responses. In conditioning phase I, all species were grown in live field soil with or without a severe drought treatment of 2 weeks. Conditioned soil, soil inoculum, root exudates and root litter were collected and added to the same field soil in conditioning phase II to isolate the contributions of different plant inputs to drought legacy effects on plant growth and PSF. In all soils, physical-chemical properties, microbial biomass, and bacterial and fungal community composition were assessed to determine legacy effects of drought and soil conditioning. In the feedback phase, new plants without drought history were planted in a full-factorial design in the conditioned soils from all species and their above- and below-ground biomass and PSF were quantified. We then linked these to the soil microbial properties at the end of conditioning phase II.

2 | MATERIALS AND METHODS

2.1 | Soil and plant origin

A sandy loam soil (SOM = $5.5 \pm 1.5\%$, pH = 7.75 ± 0.24 , soil moisture = 16.7%) which was excavated from a temperate grassland was sieved to 5 mm particle size before use in the experiment. Seeds of three common grassland species were sourced from a local company. We selected species that represent different growth strategies (slow vs. fast) and functional groups (grass vs. forb): the slow-growing grass *Anthoxanthum odoratum* (Ao), the fast-growing grass *Dactylis glomerata* (Dg) and the fast-growing forb *Rumex acetosa* (Ra). These species respond differently to drought in their biomass (de Vries et al., 2016), their PSF (Enderle et al., 2024), their root traits (Enderle et al., 2024; Sweeney et al., 2021) and their root exudation rate (Hou et al., 2025).

2.2 | Experimental setup

Drought legacy effects on plant growth and PSF were assessed in a three-stage greenhouse experiment (Figure 1). In conditioning

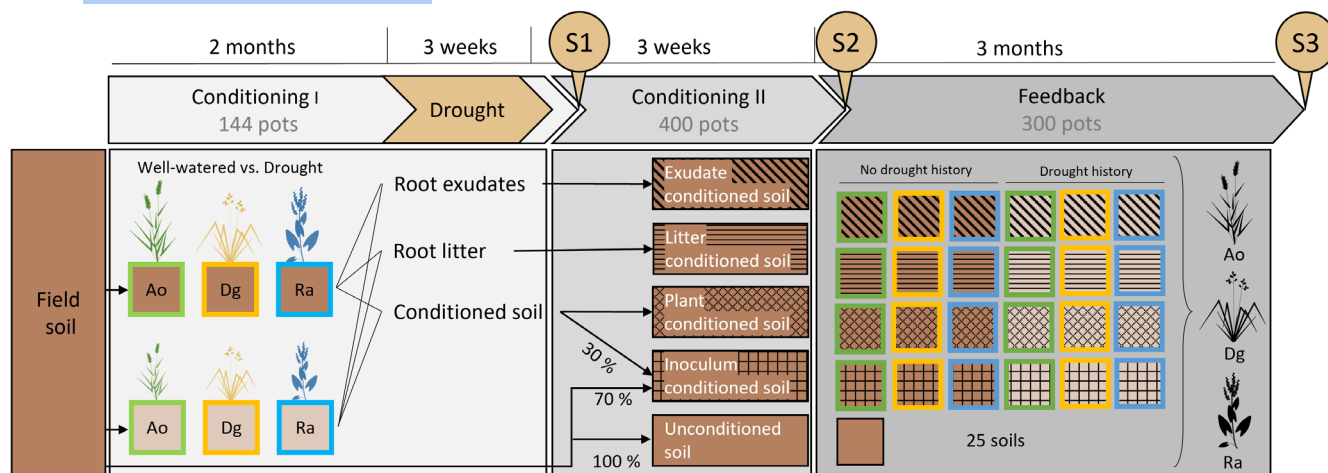


FIGURE 1 Experimental setup including a timeline of conditioning phases I and II and the feedback phase. S1 = sampling of plant biomass; S2 = sampling of soil; S3 = sampling of plant biomass. Ao = *Anthoxanthum odoratum*, Dg = *Dactylis glomerata*, Ra = *Rumex acetosa*.

phase I, part of the soil was conditioned by plants of all three species growing in the soil for 3 months, to assess the effects of all biological and physical-chemical impacts of these plants in the next phase. Root litter, root exudates and microbial inoculum (i.e. part of the fully conditioned soil) were collected at the harvest (Figure 1) for the second phase, to isolate those effects from those caused by growing the whole plant. In conditioning phase II, unconditioned background soil was conditioned by the addition of these root exudates, root litter or inoculum, either from droughted or well-watered plants. Plant-conditioned soil and unconditioned soil were included in conditioning II as negative and positive controls, respectively, including none or all of the conditioning and drought effects. In the feedback phase, new individuals of all species were grown in all soils in a full-factorial design. All experimental phases took place in an unheated greenhouse at an average temperature of 21°C and a day-night cycle of 16h to 8h.

2.3 | Conditioning phase I

In this experimental phase (March to July 2021), seeds of all three species were germinated in plug trays with the same soil used throughout the experiment for 3 weeks. Individual seedlings were transplanted into square pots (SOPARCO; 648 mL; 9 cm × 9 cm × 10 cm) with 630 g moist field soil and placed in saucers to avoid cross-contamination of microbial communities. All pots were watered at least every other day with rainwater, keeping them at a water-holding capacity (WHC) of 60% (18% gravimetric soil moisture). After 8 weeks of growth, half of the pots received a severe drought treatment at 20% WHC (7% gravimetric soil moisture) for 3 weeks based on similar experimental setups (de Vries et al., 2016, 2019; Hou et al., 2025), while the control group remained at 60% WHC. Dependent on the species, this level of moisture reduction resulted in moderate to extreme drought stress which was visible by wilting and senescence of leaves, but without

leading to seedling death. This variation of drought responses among different species at the same moisture level reflects plant community responses during a natural drought in which all plants experience the same conditions but differ in their tolerance. The drought treatment was followed by 1 week of recovery in a controlled growth chamber at 60% WHC to equalise environmental impacts before sampling and to ensure the roots are less sensitive to damage during root washing (Oburger & Jones, 2018; Vautrin et al., 2018; Williams, Langridge, Straathof, Fox, et al., 2021). After a total of 12 weeks of growth, plants were removed from the pots and soil was carefully shaken off the root system and stored at 4°C until conditioning phase II for use as plant-conditioned soil and to inoculate unconditioned soils. Plants were further used for exudate and litter collection.

2.4 | Exudate and litter collection

Root exudates were collected following the hybrid method described by (Williams, Langridge, Straathof, Fox, et al., 2021). After shaking off the soil, roots of intact plants were washed very carefully to cause as little injuries as possible in bowls with water and remaining debris was removed with tweezers. Plants were then transferred into 100 mL Schott bottles, darkened with aluminium foil, containing 100 mL hydroponic suspension (100 g dry, unconditioned soil/L) and positioned with parafilm. Bottles were continuously aerated (1 bubble/s) to ensure oxygen supply. The hydroponic suspension was changed once after 3 days. This growth period in hydroponics was implemented to ensure root recovery from possible damage during root washing, which has been shown to alter root exudation profiles (Williams, Langridge, Straathof, Fox, et al., 2021). After 1 week, the roots were washed thoroughly first in demineralised water and then in Milli-Q water; then, exudates were collected from intact plants in 100 mL autoclaved Milli-Q water on ice on a rotary shaker at 60 rpm for 2 h and vacuum-filtered through 2 µm filters (Whatman™, Little Chalfont, UK).

Shoots and roots were separated, dried at 70°C for 72h and weighed on a 4-decimal scale.

One third of the exudate solution was acidified with 0.25% HCl and directly measured for total organic carbon (TOC; enviro TOC, Elementar, Langensfeld, Germany) to determine the total root exudate carbon per plant (exudate C) and the specific root exudation rate (SER) as TOC per unit dry root biomass. The remaining two thirds of the exudate solution were freeze-dried and stored at -80°C until further processing. For reapplication in conditioning phase II, exudates were resuspended in 1mL sterile Milli-Q, pooled for all six individuals per species, treatment and replicate and diluted to a concentration of 75 µgC/mL to correct for the difference in exudate carbon between samples. Exudate extracts were stored in aliquots of 1 mL at -80°C until use.

Dried root samples were prepared for reapplication in conditioning phase II to mimic root litter deposition (Glass et al., 2023; Heredia-Acuña et al., 2023; Zhang et al., 2016). We collected live roots that were subsequently dried because the collection of naturally senesced roots was not feasible within the scope of this experiment (i.e. collection of root litter and exudates from the same individual). A representative quarter of the dried root system was collected from each plant and pooled across all six plants per species, treatment and replicate, cut into 0.5 cm long pieces and mixed thoroughly. Root samples were stored in paper bags at room temperature until use.

2.5 | Conditioning phase II

This experimental phase (October to November 2021) mimicked exudate and litter input throughout the control vs. drought period of 3 weeks that plant-conditioned soils experienced. Overall, 25 different soil mixtures, replicated four times, were prepared and each divided over four pots to be used in the feedback phase (Modiform; 228 mL; 7 cm × 7 cm × 8 cm; Figure 1). The plant-conditioned soil represented the full soil conditioned by live plants growing for 3 months. The inoculum-conditioned soil represented the conditioning effect on rhizosphere microbial communities while minimizing abiotic effects of drought and soil conditioning. Exudate- and litter-conditioned soil represented the effects of drought and soil conditioning on soil microbial communities mediated by these specific C input pathways. The same background soil used previously was used for the exudate-, litter- and inoculum-conditioned soils and the unconditioned control. We used alive background soil and not sterile soil in order to mimic as closely as possible what happens in the full plant-conditioned soil and to add the exudates and the root litter to the same microbial communities which were present in the beginning of conditioning phase I. For the exudate and control treatment, pots were filled directly with unconditioned background soil. For the exudate treatment, exudates (200 µL; 15 µgC) from all three species, from drought and control conditioning phase treatments, were applied every 3 days (seven times in total) by bringing a syringe about two-thirds deep into the soil and pulling it up while releasing its content to simulate exudation by real

roots. All other pots received the same treatment with Milli-Q water to control for the disturbance and the additional moisture. The syringe was cleaned from the outside with 70% EtOH and flushed inside with Milli-Q between treatments. For the litter treatment, 200 mg of root litter, again from each of the three species grown under drought and well-watered conditions, was mixed thoroughly into the unconditioned background soil before dividing them into pots (50 mg dry litter/pot). Plant-conditioned soil was pooled within each species, replicate and treatment. For the inoculum treatment, these pooled plant-conditioned soils were mixed with background soil in the ratio 3:7, based on dry weight. All pots contained the same amount of dry soil and were brought to the same moisture level (WHC 60%), which was maintained throughout the experiment. After 3 weeks, the soil of one pot of each treatment and replicate was collected, mixed, sieved to 2 mm and stored at 4°C for physical-chemical analyses or -20°C for microbial community analysis and the remaining three pots were used in the feedback phase.

2.6 | Feedback phase

The conditioned soil was used in the feedback phase (November 2021 to February 2022) to test how the conditioning treatments affected plant growth and PSF. Every species was grown in each soil conditioning treatment derived from droughted or from well-watered plants of all species in a full-factorial design (Figure 1). Before planting, all seeds were surface sterilised with 40 mL chlorine-based household bleach, diluted to <1% chlorine for 1 min in 50 mL tubes. Bleach was removed and seeds washed five times with 40 mL Milli-Q water to remove bleach residues. Multiple seeds, based on germination rates, were placed in each pot in 0.5 cm deep holes, covered with soil and watered with Milli-Q water for the first week to prevent contamination. Excess seedlings were gently removed with tweezers and pots were regularly weeded throughout the experiment. Plants were watered by sprinkling with rainwater for 3 s per pot, every 1–3 days dependent on the temperature. After 3 months of growth, above-ground biomass was cut at soil level, roots were washed, all debris removed with tweezers, above- and below-ground biomass was dried at 70°C for 72 h and weighed on a 4-decimal scale.

2.7 | Soil analyses

All soil analyses including the amplicon sequencing of microbial communities were carried out on all soils collected at the end of conditioning phase II. Gravimetric soil moisture was determined by the weight loss of 5 g fresh soil after drying at 105°C for 24 h on a 4-decimal scale. Soil organic matter content (SOM) was determined by loss on ignition at 550°C for 16 h, measured on a 5-decimal scale. Soil pH, dissolved organic carbon (DOC) and dissolved nutrients were measured in water extracts by shaking 15 g of fresh soil in 30 mL Milli-Q for 2 h at 150 rpm in a rotary shaker and vacuum-filtering

them through 2 µm filters (Whatman™, UK). DOC was analysed after acidifying extracts with 0.25% HCl in a TOC analyser (enviroTOC, Elementar). Dissolved nutrients were determined in an autoanalyzer (SAN⁺⁺ SYSTEM, SKZLAR) as total N, NO₂⁻, NO₃⁻, NH₄⁺ and PO₄⁻. Plant-available nitrogen (N; as NO₂⁻, NO₃⁻, NH₄⁺) and phosphorus (P; as PO₄⁻) were determined in 0.5 M KCl extracts, that were shaken for 1 h at 150 rpm and vacuum-filtered through 2 µm filters (Whatman™, UK) and diluted 1:9 on an autoanalyzer (SAN⁺⁺ SYSTEM, SKZLAR). Microbial biomass was determined through fumigation extraction with 0.05 M K₂SO₄. Around 5 g of fresh soil were either extracted immediately by shaking in 50 mL 0.05 M K₂SO₄ for 1 h at 150 rpm and vacuum filtration through 2 µm filters (Whatman™, UK) or placed in open glass bottles in a desiccator which contained a jar with chloroform at the bottom. Through three cycles of suction with a vacuum pump a chloroform atmosphere was created to release elements of all living organisms. Once at the end of the day the chloroform sphere was reestablished. After 24 h, nutrients and C were extracted as described before. Both unfumigated and fumigated extracts were analysed for total N (SAN⁺⁺ SYSTEM, SKZLAR) and TOC in acidified extracts (0.25% HCl; enviroTOC, Elementar). Microbial biomass C and N were corrected with a correction factor of 0.45 and 0.54, respectively, based on the soil type (Brookes et al., 1985; Vance et al., 1987).

2.8 | Amplicon sequencing of soil microbial communities

DNA was extracted from 0.5 g frozen soil with the DNeasy PowerSoil extraction kit (QIAGEN GmbH, Hilden, Germany) following the protocol and stored at -20°C. For bacteria and archaea, the 16S ribosomal DNA region (16S) and for fungi the internal transcribed spacer 1 (ITS1) region were amplified by polymerase chain reactions (PCR) using primers with adapters for the library preparation (16S: 515F, 806R; ITS: ITS1-F, ITS2; primer sequences and PCR conditions see Table S1). Successful amplification of PCR products was controlled by agarose gel electrophoresis, and yield quantified with the Qubit fluorometer (Thermo Fisher Scientific, Waltham, USA), following the respective protocol. PCR products were purified with the AMPure XP Kit (Beckman Coulter, Indianapolis, USA). Library preparation, amplicon sequencing and read pre-processing was done by the Earlham Institute (Norwich, UK). Prior to sequencing, adaptor dimers were removed in an additional cleaning step (0.65X bead, AMPureXP, Beckman Coulter, Indianapolis, USA) in both 16S and ITS libraries. Sequencing was performed in a MiSeqv2 flow cell on the Illumina MiSeq sequencer under addition of 20% Phix spike for quality control, yielding demultiplexed paired-end reads with a length of 250 bp.

Demultiplexed raw reads were processed at the University of Amsterdam using the Dadasnake pipeline (Weißbecker et al., 2020). Primers were removed with *cutadapt*. All 16S sequences were pooled during the filtering with DADA2 with the following settings: truncation at 170 bp forward and 130 bp reverse, a quality filter at 13, a maximum

estimated error of 0.2 and length-filtering with a minimum of 245 bp and a maximum of 275 bp. Chimeras were removed. Bacterial and archaeal sequences were taxonomically classified based on the SILVA database (Quast et al., 2013). For ITS reads, no truncation length was applied, the minimal quality was set to 15, the maximum estimated error to 3 and the minimum length to 40 bp. For taxonomic classification, we used the Unite database (Abarenkov et al., 2024). Read quality and depth were assessed through QC plots, error estimation plots and rarefaction curves.

2.9 | Calculation of plant–soil feedback

To estimate how beneficial a plant species' own home soil is for its own growth, we calculated a PSF index based on total plant dry biomass at the end of the feedback phase as follows (Brinkman et al., 2010; Petermann et al., 2008):

$$PSF_A = \ln \left(\frac{\text{biomass } A_a}{\text{biomass } A_b} \right).$$

Here, A stands for the focal species; *a* for its own conditioned soil (home soil) and *b* for soil conditioned by another species B (away soil). Feedback was calculated for each species pair, soil conditioning type and drought treatment separately according to the replicate blocks of the experimental design. Values above zero indicate that the focal species grows better in its own conditioned soil compared to soil conditioned by another species, whereas negative values indicate less biomass in own soil.

2.10 | Statistical analysis

All statistical analyses and data visualisations were executed in R version 4.3.1 (R Core Team, 2024) using RStudio version 2022.07.2 (Posit Team, 2024). If not stated otherwise, treatment effects on soil properties, root exudation, plant biomass and PSF were assessed in linear mixed effects models using the package *lme4* (Bates et al., 2015) with experimental block as a random effect. All models were tested for normal distribution of their residuals and homogeneity of variances and if necessary transformed. Treatment effects and their interactions were tested in a type III analysis of variance (ANOVA) with Kenward–Roger approximation to correct for small sample size. Significant main effects or interactions were further investigated with TUKEY post hoc tests.

Plant total, shoot and root biomass, total exudate C and SER at the end of conditioning I were tested in models with species, drought treatment and their interaction. All soil properties at the end of conditioning II were tested in models with soil conditioning, drought treatment and conditioning species. Total dry biomass at the end of the feedback phase was assessed by species, drought treatment, soil conditioning and conditioning species and their interactions. To be able to include unconditioned soil in the comparison of soil conditioning types, we built a second model only assessing

soil conditioning and species effects on biomass. PSF was tested with species, drought and soil conditioning as main effects and with conditioning species as an additional random effect.

Correlations between plant biomass and soil properties as well as of PSF in soils with different types of soil conditioning were assessed with Pearson correlation coefficients and the respective correlation tests.

For statistical analysis and visualisation of the amplicon sequencing data, we used the *phyloseq* package (McMurdie & Holmes, 2013). To limit the impact of artefacts from sequencing errors, amplicon sequence variants (ASVs) which were not assigned at the domain level and reads which occurred in less than 2% of the samples or less than three times in total were removed. To determine measures of alpha diversity, the data was rarefied to the sample with the lowest read depth. For all other analyses, composition-sensitive methods were chosen whenever possible (Gloor et al., 2017). Beta diversity was assessed using principal component analysis (PCA) and distance-based redundancy analysis (dbRDA) on centred log-ratio (CLR) transformed data using the *vegan* package (Oksanen et al., 2024). Differences in microbial community composition between treatments were determined through permutational analysis of variances (PERMANOVA) on CLR-transformed data.

To identify differentially abundant (DA) taxa between drought and ambient treatments in plant-conditioned soil, we used three different common DA tools, which performed well in different comparative studies and are suitable for compositional data: DESeq2, ALDEx2 and ANCOM-II (Calgareo et al., 2020; Nearing et al., 2022; Quinn et al., 2018; Weiss et al., 2017). DESeq2 was executed with the *DESeq2* package (Love et al., 2014) on filtered reads with a local fit type, size factor type was set to 'poscounts' to account for data sparsity and DA tested with a Wald test with an alpha of 0.05. ALDEx2 was performed with the *ALDEx2* package (Fernandes et al., 2014), with an interquartile log-ratio (IQLR) transformation, 999 Monte Carlo samples and a Wilcoxon test with an alpha of 0.05, corrected for type I errors with the Benjamini–Hochberg procedure. ANCOM-II was executed using the *ANCOMBC* package (Lin & Das Peddada, 2023; Mandal et al., 2015) without prior detection of structural zeros to avoid overestimating the impact of rare taxa. ANCOM-II performs an additive log-ratio transformation to account for compositional data. We used a significance threshold of 0.8 with an alpha of 0.05, adjusted with the Benjamini–Hochberg procedure. Taxa which were identified by all three methods could be classified as differentially abundant with high confidence.

To select those taxa whose abundance pattern best explains plant biomass production in the feedback phase, we then used the *coda4microbiome* package (Calle et al., 2023) on the subset of taxa which were identified by at least one of the DA methods. In this way, we select taxa which are both differentially abundant under drought and well-watered conditions and influence plant biomass production. Based on pairwise log-ratios, this approach selects a generalised linear model (GLM) with those taxa contributing the most to explaining variation in biomass through penalised regression. To adjust zero counts for the log transformation, the value one

was added to all data points with the function *impute_zeros*. We selected an elastic net penalty parameter of $\alpha=0.9$ and λ within one standard error (λ_{1se}) to reduce the risk of overfitting the model (Susin et al., 2020) and added plant species and experimental block as covariates.

To link PSF to differences in soil microbial community composition, we first performed an indicator species analysis with the *indicspecies* package (Severns & Sykes, 2020) on plant-conditioned soil. To identify taxa which are either specifically associated with each of the three species or absent in their soil, we selected indicator taxa for individual species and combinations of two species. The *multipatt* function was executed with 999 permutations based on point biserial correlations ($func = 'r.g'$), controlling for uneven groups. We selected all taxa under a cut-off of $p=0.1$ associated with one or two plant species, once when found in home soil and once when found in away soil, as an input for the regression analysis with *coda4microbiome*, which was executed as explained above for biomass. Here, only experimental block was added as a covariate, because we are specifically interested in differences in PSF between species.

3 | RESULTS

3.1 | Direct effects of drought on plant growth and root exudation

At the end of conditioning phase I, plant biomass was reduced under drought in all species (Figure S1). *R. acetosa* was most severely affected, which was mostly driven by below-ground differences in biomass loss (Figure S1). Above-ground biomass reduction under drought did not differ between species (Figure S1). Both SER and exudate C differed between the species, with *A. odoratum* having the highest and *R. acetosa* the lowest root exudate C per plant and per unit root biomass (Figure S2; ANOVA, main effect species, Exudate C: $F_{2,133} = 11.9, p < 0.0001$, SER: $F_{2,133} = 23.2, p < 0.0001$). In drought-exposed plants, total exudate C was unchanged (Figure S2B; ANOVA, main effect drought, $F_{1,133} = 1.5, p = 0.22$), which was the result of reduced root biomass being compensated by an increase in SER across species (Figure S2A; ANOVA, main effect drought, $F_{1,133} = 6.9, p = 0.001$).

3.2 | Effects of drought and soil conditioning on soil biological and physical–chemical properties

Soil conditioning and drought variously affected soil physical–chemical and biological properties (Tables S2 and S3; Figure 2). Soil moisture at the end of conditioning II was no longer affected by the drought treatment (Table S3; ANOVA, main effect drought, $F_{1,69} = 0.4, p = 0.55$), but was slightly increased in plant- and inoculum-conditioned soils (Table S3; ANOVA, main effect soil conditioning, $F_{3,69} = 4.7, p = 0.005$). Plant-available nutrients (N and P) were generally increased by drought across all conditioning treatments (Table S3; ANOVA, main

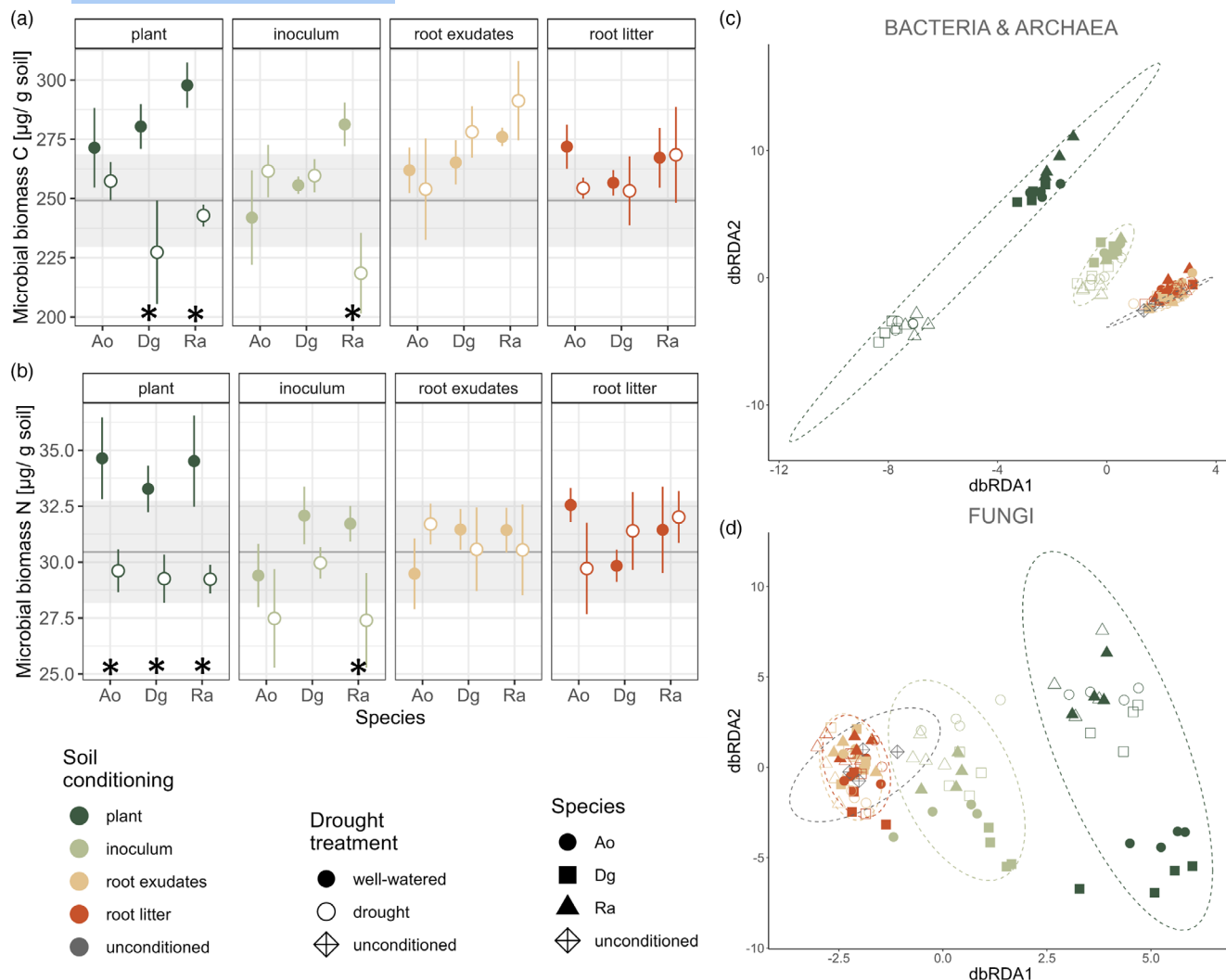


FIGURE 2 Microbial biomass and community composition across soil treatments at the end of conditioning phase II. (a) Microbial biomass C and (b) microbial biomass N based on chloroform fumigation extraction. Points and bars represent group means and standard errors, respectively. Grey horizontal lines and shaded areas represent the mean and standard error of the unconditioned soil. Asterisks show significant differences between well-watered and droughted treatments (pairwise Tukey, $p < 0.05$). (c, d) First two axes of a distance-based redundancy analysis (dbRDA) of CLR-transformed amplicon sequencing reads from (c) bacteria and archaea (16s) and (d) fungi (ITS) based on variation explained by soil conditioning, drought and species. Soil conditioning is indicated by colour, conditioning species by shape. Full symbols represent the well-watered treatment and open symbols the drought treatment; unconditioned background soil is shown with a grey, framed plus. *Ao* = *Anthoxanthum odoratum*, *Dg* = *Dactylis glomerata*, *Ra* = *Rumex acetosa*.

effect drought, $F_{1,69} = 10.6$, $p = 0.002$ and $F_{1,69} = 9.4$, $p = 0.003$). For plant-available N, this was the result of an increase in extractable nitrate (Table S3; ANOVA, main effect drought, $F_{1,69} = 11.5$, $p = 0.001$), but not ammonium, and this was accompanied by an increase in total dissolved N (Table S3; ANOVA, main effect drought, $F_{1,69} = 10.9$, $p = 0.002$). For plant-available P, the difference between drought and well-watered conditions was strongest in plant- and inoculum-conditioned soil (Table S3; ANOVA, interaction drought: soil conditioning, $F_{3,69} = 2.4$, $p = 0.08$).

Microbial properties were most affected by drought in plant-conditioned soil (Figure 2). Both microbial biomass C and N increased through plant conditioning under well-watered conditions compared to unconditioned soil, but not under drought (Figure 2a,b; ANOVA, interaction conditioning: drought, $F_{3,69} = 3.9$, $p = 0.01$ and $F_{3,69} = 5.2$,

$p = 0.003$). This difference between drought and well-watered conditions in microbial C was species-specific, with a stronger reduction under drought in *D. glomerata* and *R. acetosa*, whereas it was consistent across species for microbial N (Figure 2a, ANOVA, interaction conditioning:drought:species, $F_{6,69} = 2.6$, $p = 0.02$). A similar but weaker pattern was observed in soil conditioned by inoculum (Figure 2a,b). Soil conditioning with root exudates and root litter had limited effects on microbial N, but exudate addition, especially from *R. acetosa*, increased microbial C independent of drought (Figure 2a).

To assess the effects of soil conditioning, species and drought on microbial community composition, we performed a dbRDA (Figure 2c,d), together with a PERMANOVA (Table 1). The explanatory variables explained overall 28% of variation in bacterial and 30% of variation in fungal community composition. For bacteria and

TABLE 1 PERMANOVA results for bacterial and fungal communities at the end of conditioning phase II based on the experimental block, soil conditioning type, drought treatment and conditioning species and their interactions.

Term	Bacteria and archaea					Fungi				
	df	SS	R ²	F	p	df	SS	R ²	F	p
Block	3	36,587	0.037	1.226	0.0001	3	16,499	0.036	1.184	0.0002
Soil conditioning (C)	3	63,420	0.063	2.126	0.0001	3	27,994	0.061	2.009	0.0001
Drought (D)	1	15,107	0.015	1.519	0.0002	1	5340	0.012	1.150	0.0184
Species (S)	2	20,467	0.020	1.029	0.1876	2	9940	0.022	1.070	0.0585
C:D	3	10,192	0.040	1.347	0.0001	3	15,362	0.033	1.102	0.0060
C:S	6	60,313	0.060	1.011	0.2915	6	27,977	0.061	1.004	0.4049
D:S	2	20,121	0.020	1.012	0.3042	2	9152	0.020	0.985	0.6057
C:D:S	6	60,013	0.060	1.006	0.3470	6	28,262	0.061	1.014	0.2670
Residual	69	686,140	0.684			69	320,498	0.695		
Total	95	1,002,359	1.000			95	461,025	1.000		

Abbreviations: df, degrees of freedom; SS, sum of squares.

Note: Significant terms at $p < 0.05$ are highlighted in bold.

archaea, dbRDA1 and dbRDA2 explained 18% and 12% of all variation of the constrained analysis and for fungi 13% and 5%, respectively. The type of soil conditioning had the largest influence on the community composition of bacteria and fungi (Table 1). Both bacterial and fungal communities in plant-conditioned soil were most distant from those in unconditioned soil, with inoculated soil clustered between these two. Exudate- and litter-conditioned communities were not different from unconditioned soil (Figure 2c,d). The effect of drought on soil microbial communities depended on the type of conditioning (Figure 2c,d; Table 1). Drought clearly separated soil microbial communities in plant-conditioned soil, and this effect was stronger for bacteria than fungi (Figure 2c,d; Table 1). Drought effects on community composition were weaker in inoculum-conditioned soil and absent in exudate- and litter-conditioned soils (Figure 2c,d). Plant species marginally affected fungal communities but not bacterial communities (Table 1).

3.3 | Drought legacy effects on plant biomass production and PSF

The three species displayed a similar pattern of biomass production in the feedback phase across the different soil types (Figure 3a). All species produced the least biomass in soil conditioned by plants or inoculum and the most biomass in unconditioned soil or soil amended with root litter (Figure 3a; Table S4; ANOVA, main effect soil conditioning, $F_{3,212}=42.9$, $p < 0.0001$). Root exudate and litter addition did not change plant growth compared to unconditioned soil (Figure 3a). Effects of drought in the conditioning phase on plant biomass in the feedback phase depended on the type of soil conditioning: In all species, we observed a negative drought legacy effect on plant growth in plant-conditioned soil, but in none of the other conditioning types (Figure 3a; ANOVA, interaction soil conditioning: drought, $F_{3,212}=15.0$, $p < 0.0001$). Only *D. glomerata* plants produced more biomass in soil

with exudates from droughted plants compared to exudates from well-watered plants (Figure 3a), which was most pronounced with exudates from *A. odoratum* (Figure S2). Overall, plant growth in the feedback phase depended on the combination of the species growing and the conditioning species (Figure S3; ANOVA, interaction species: species soil, $F_{4,212}=4.0$, $p=0.003$). All species grew the least in their own conditioned soil and best in soil conditioning by a species from another functional group (Figure S3).

Only in plant-conditioned soil we could identify a clear pattern of PSF, which was not replicated in any of the other soil conditioning types (Figure 3b; Figure S4). PSF in plant-conditioned soil was generally negative and differed between species (Figure 3b; Table S4). *A. odoratum* experienced neutral PSF, *D. glomerata* negative PSF and *R. acetosa* the most negative PSF (Figure 3b). A similar, but weaker pattern of PSF could be observed in inoculum-treated soil, but the negative PSF of *D. glomerata* was neutralised (Figure 3b). PSF in exudate-treated soils seemed to be reversed compared to plant-conditioned soils, with *A. odoratum* experiencing the most negative PSF and *R. acetosa* the most positive PSF, whereas the addition of root litter did not induce any PSF (Figure 3b). Drought only had a strong effect on PSF in plant-conditioned soil, where it intensified the negative PSF of *R. acetosa*. In inoculum- and exudate-conditioned soil, we observed a trend of PSF being neutralised under drought (Figure 3b).

3.4 | The role of soil microbes in mediating drought legacy effects on plant growth

To explain the observed negative legacy effect of drought on plant growth, we correlated plant biomass at the end of the feedback phase with soil properties at the end of conditioning phase II (Figure S5). As these effects were only observed in plant-conditioned soil, all following results only refer to this part of the dataset. Total plant

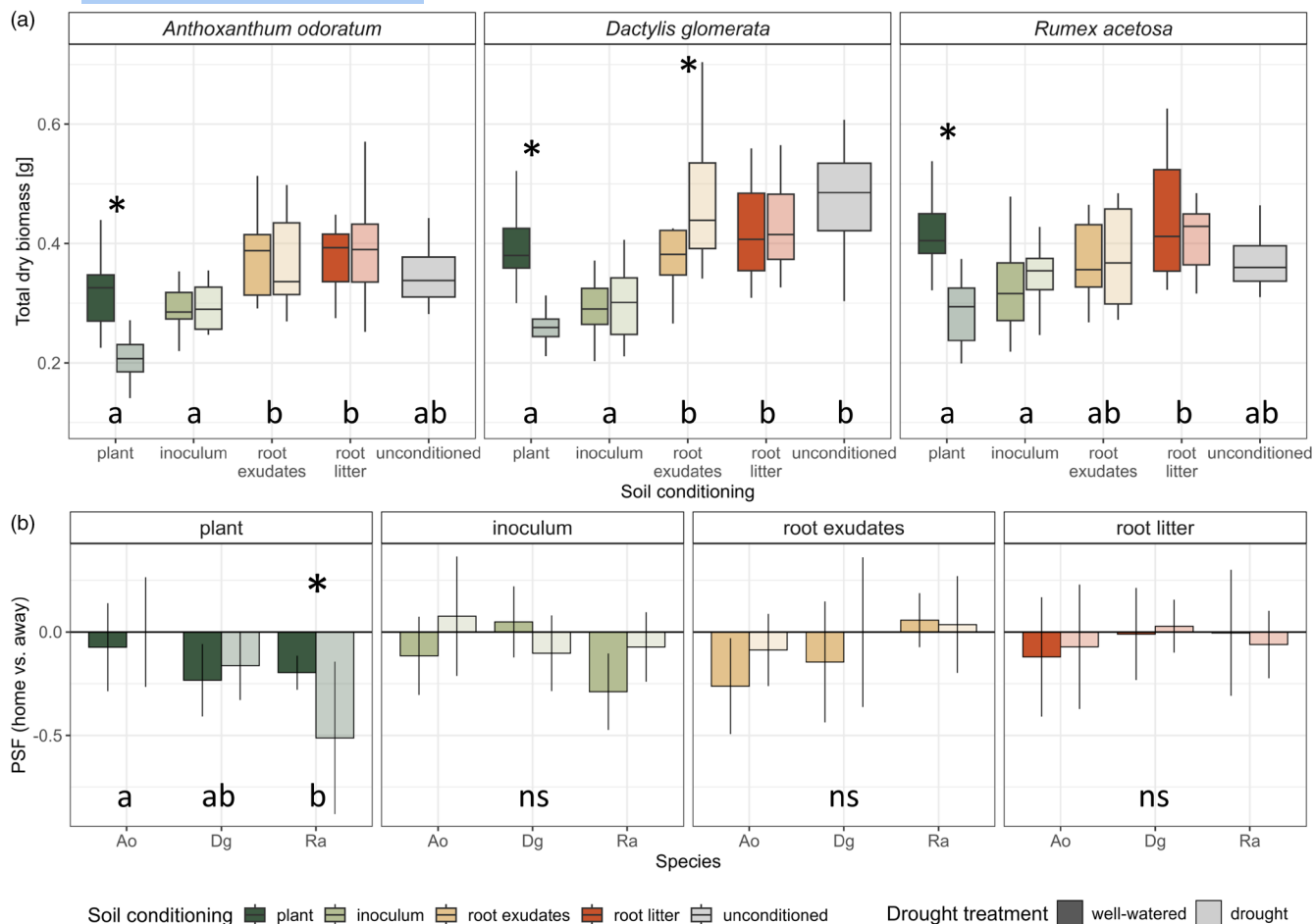


FIGURE 3 Plant biomass and PSF at the end of the feedback phase. (a) Box plots of total dry biomass. Different letters represent differences between soil conditioning treatments, and asterisks represent differences between well-watered and drought conditions within a conditioning treatment (TUKEY, $p < 0.05$). $n = 300$. (b) PSF index. Different letters represent differences between species, and asterisks represent differences between well-watered and drought conditions within a species (TUKEY, $p < 0.05$). $n = 192$. Ao = *Anthoxanthum odoratum*, Dg = *Dactylis glomerata*, Ra = *Rumex acetosa*.

biomass was positively correlated with microbial biomass N and C (Figure S5A,B) and negatively correlated with plant available N and P (Figure S5C,D).

To link the negative drought legacy effect on plant growth to soil microbial community composition, we first identified differentially abundant microbial taxa in droughted and well-watered soils using three common methods of differential abundance analysis (Figure 4). For bacteria and archaea, out of a total of 20,236 taxa present in plant-conditioned soil, 803 taxa (3.97%) were identified by at least one of the methods, of which 91 taxa (0.45%) were identified by all three methods as differentially abundant in droughted compared to well-watered soil (Figure 4a; Table S5). For fungi, out of a total of 2814 taxa, 27 taxa (0.96%) were identified by at least one of the methods, of which two taxa (0.07%) were identified by all three methods (Figure 4b). In both cases, DESeq2 was the most liberal and ALDEx2 the most conservative selection method. The two bacterial taxa which were identified with the most confidence belong to the genera *Pseudomonas* and *Marmoricola* and were both more abundant in soil that had experienced a drought compared to well-watered

conditions, whereas the majority of identified taxa was less abundant in soil with a drought legacy (Figure 4c). Of the only two identified fungal taxa, one increased and one decreased in abundance in response to drought and they could both not be classified further taxonomically (Figure 4c).

We then used the subset of taxa that were identified as differentially abundant in drought compared to well-watered soil by at least one DA method (803 bacterial and 27 fungal taxa) to link their abundance with plant biomass in the feedback phase using a GLM-based approach for compositional data (Figure 5). Plant biomass in the feedback phase could best be predicted by a selection of 13 bacterial or six fungal taxa, resulting in an apparent R^2 of 0.53 for both models (Figure 5). The bacterial taxon contributing by far the most negatively to the model belonged to the order *Chitinophagales*, followed by a bacterium from the order *Sphingobacteriales* and a taxon from the family *Obscuribacteraceae* (Figure 5c). The most positively contributing bacterial taxa were from the genus *Thaurea*, the family *Microscillaceae* and the order *Polyangia* (Figure 5c). For fungi, two taxa were positively and four taxa negatively associated with plant

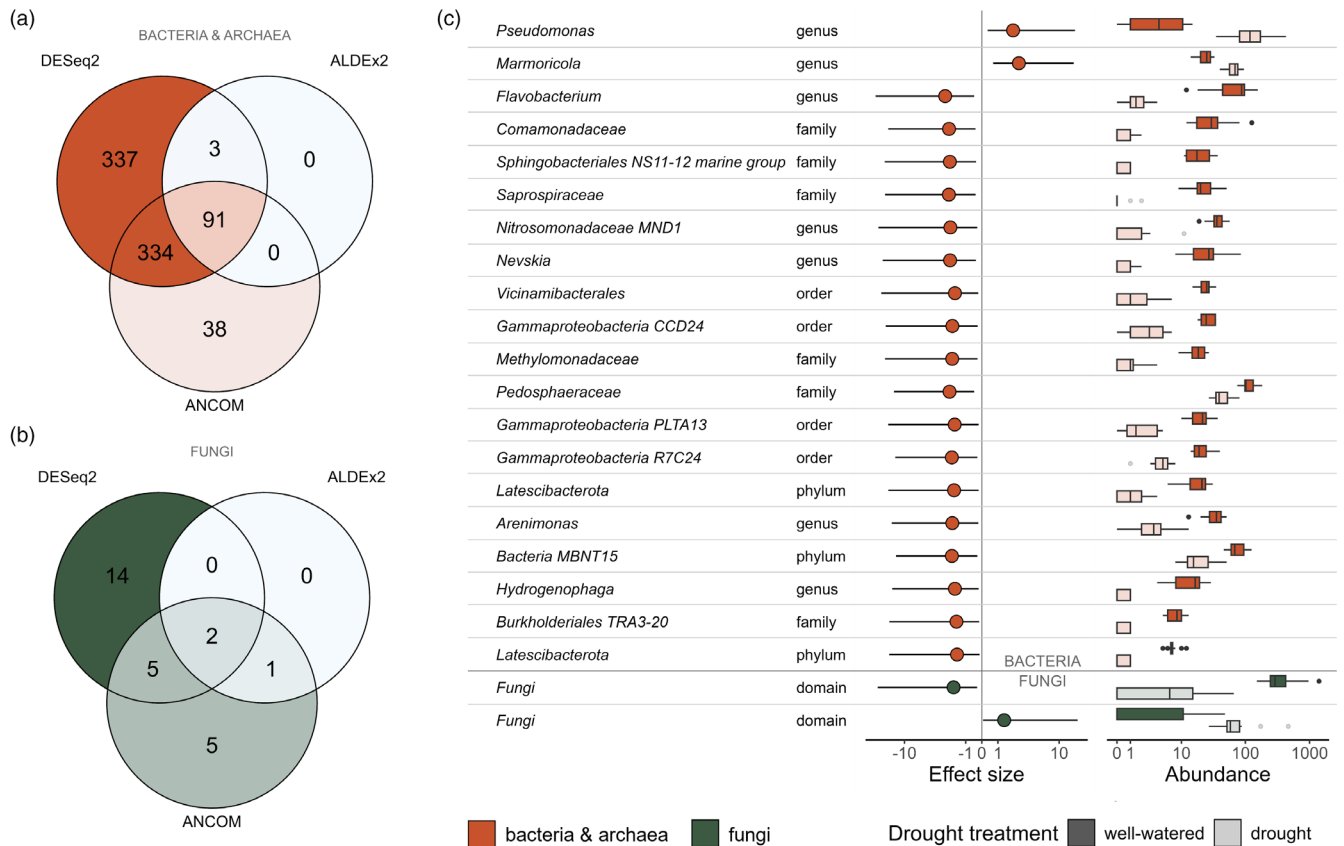


FIGURE 4 Differentially abundant bacteria and fungi between droughted and well-watered plant-conditioned soil. Number of taxa identified as differentially abundant by one or more of the methods DESeq2, ALDEx2 and ANCOM for (a) bacteria and archaea (red) and (b) fungi (green). (c) Taxonomic classification on the highest identifiable rank, effect size and abundance of the 20 taxa with the lowest p -value for bacteria and the two taxa for fungi identified by all three methods. Effect sizes, confidence intervals and p -values are based on ALDEx2. Abundance is shown in absolute read counts per sample.

growth, of which only one taxon could be identified further than domain level and belonged to the order *Helotiales* (Figure 5d).

3.5 | The role of soil microbes in mediating PSF

To link PSF to microbial community composition, we first identified indicator taxa whose abundance was linked to soil conditioning by one or two of the species by indicator species analysis. At a cut-off of $\alpha=0.1$, 817 bacterial taxa were identified, of which 251 were associated with *R. acetosa*, 209 with *A. odoratum*, 208 with *D. glomerata* and 149 taxa with two of the species (Figure 6a). For fungi, a total of 154 taxa were identified as indicator species, of which 64 were associated with *A. odoratum*, 43 with *D. glomerata*, 28 with *R. acetosa*, and 19 taxa with two of the species (Figure 6b). All identified taxa were used as candidate taxa in home and away soil to predict PSF with a balance between positively and negatively associated taxa, similar to the analysis for plant biomass described in the previous section. PSF was best predicted by a selection of 13 bacterial taxa or two fungal taxa, resulting in an R^2 of 0.68 or 0.44, respectively (Figure 6). In both cases, the majority of predictor taxa came from home soil. The two most negatively contributing bacterial taxa

belong to the family *Myxococcaceae* and the order *Vicinamibacteriales*, and the three most positively contributing taxa belong to the genus *Bdellovibrio*, the family *Vicinamibacteraceae* and the order *Polyangiales* (Figure 6e). Only two taxa from away soil contributed to a small extent to the bacterial model explaining PSF, belonging to the genera *Aboriccoccus* and *Haliangium* (Figure 6e). In the fungal model, a taxon from the phylum *Blastocladiomycota* contributed negatively and the species *Scedosporium dehoogii* contributed positively to determine PSF (Figure 6e).

4 | DISCUSSION

Our research demonstrated consistent negative drought legacy effects on plant growth, contrary to our first hypothesis and species-specific drought effects on PSF, confirming hypothesis two. Drought legacy effects on plant growth and PSF seemed to be mediated by direct interactions between plant and soil communities rather than via changes in root exudate or litter quality, which diverges from our third hypothesis. The differences in plant biomass between soils with and without drought history were explained by a few bacterial and fungal taxa that were differentially abundant in droughted and well-watered

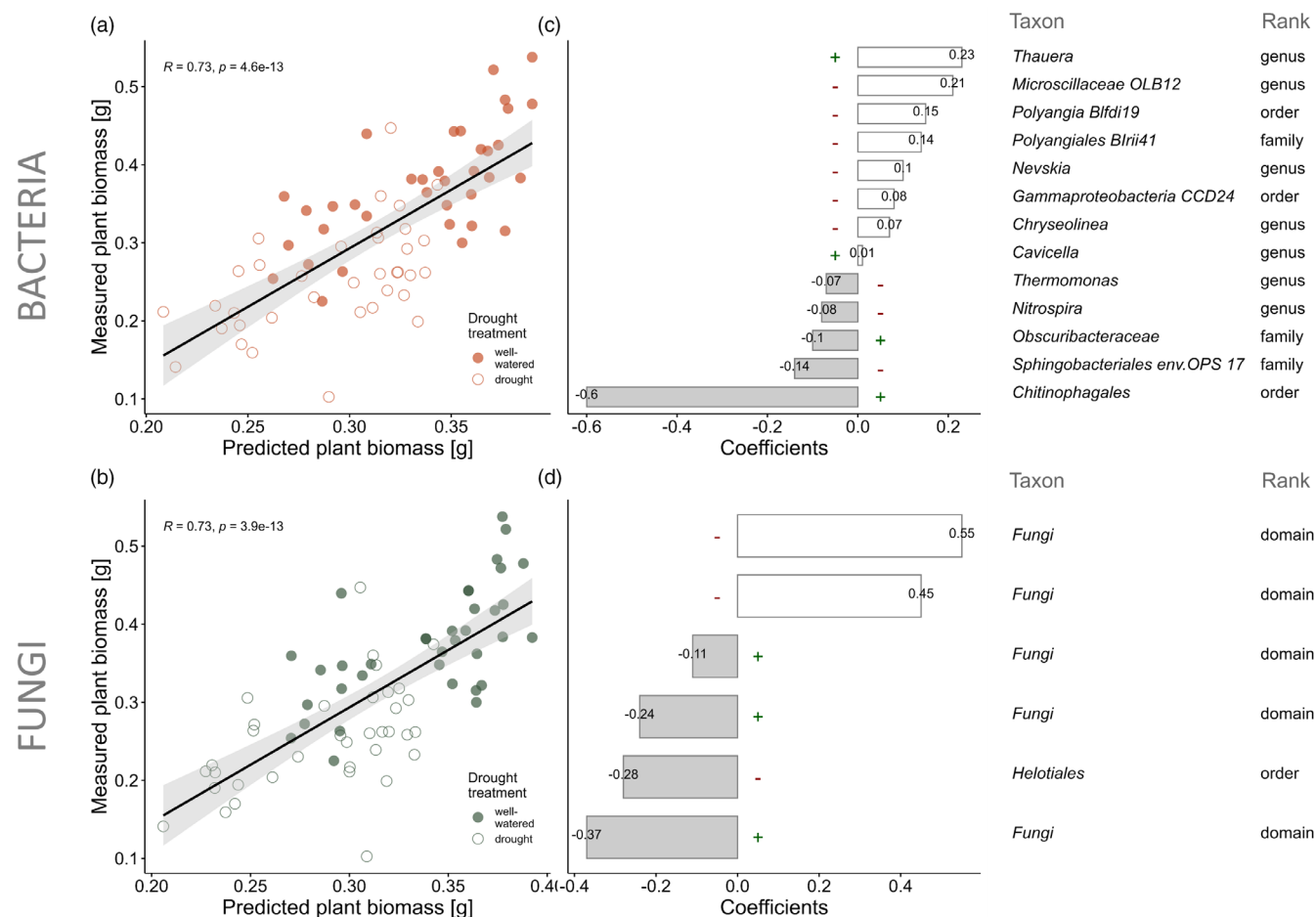


FIGURE 5 Prediction of plant biomass by soil microbes. Signature taxa for plant biomass in plant-conditioned soil and biomass prediction based on their abundance. Predicted and measured values for plant biomass based on (a) bacterial and (b) fungal amplicon sequence variants (ASVs). Microbial signature of (c) bacterial and (d) fungal ASVs associated with plant biomass in the feedback phase, their model coefficients and their highest classified taxonomic rank. Taxa which increased in abundance under drought are marked with a green plus, taxa which decreased with a red minus.

soils. While bacteria were more responsive to drought, both bacteria and fungi were of comparable importance for plant drought responses, partially confirming our fourth hypothesis.

4.1 | The role of soil microbial communities in mediating plant drought responses

Drought legacy effects in the plant-conditioned soil consistently restricted the growth of plants in the feedback phase, which is in stark contrast to the nutrient-driven drought legacy effects that are usually observed after rewetting (Oram et al., 2023; Schärer et al., 2023). In line with these studies, we found that plant-available sources of N and P were consistently higher in droughted soils. However, this increase in nutrient availability did not lead to a boost in plant growth, but instead was negatively correlated with plant biomass in the feedback phase. Moreover, while microbial biomass was consistently reduced by drought (Gliesch et al., 2024; Hueso et al., 2012; Preece et al., 2019), in particular

in the plant- and inoculum-conditioned soil, microbial biomass was positively correlated with plant growth in the feedback phase. This reduction in microbial biomass together with a shift in both bacterial and fungal community composition in plant-conditioned soil was linked to the negative drought legacy effect on plant growth across all species. As often found before (Barnard et al., 2013; Bouasria et al., 2012; de Vries et al., 2018), bacterial communities were more affected by drought than fungal communities, and more bacterial taxa were differentially abundant in droughted, compared to well-watered soil (0.45% of all bacterial taxa vs. 0.07% of all fungal taxa). A selection of a few fungal or bacterial taxa was sufficient to explain a large amount of variation in plant biomass in the feedback phase, suggesting a role for both bacteria and fungi in the negative plant growth response to drought. The strongest bacterial predictor of plant biomass belongs to the order of *Chitinophagales* (*Bacteroidota*), a group that showed resistance to salt stress (Vera-Gargallo et al., 2023), was more abundant in our study under drought and was negatively linked to plant growth, possibly directly restricting plant growth in previously droughted soil. Prior research

across increasing levels of stress and recovery thereof in future research.

4.3 | The role of soil microbial communities in mediating PSF

As drought effects on PSF were limited, we linked PSF to soil microbial communities independent of their watering treatment and identified microbial indicator taxa which were associated with each plant species. *R. acetosa* had the highest number of bacterial indicator taxa, whereas *A. odoratum* had the highest number of associated fungal taxa. In contrast, previous work indicates a higher abundance of fungi in soils cultivated by forbs compared to grasses (Bezemer et al., 2006; Hannula et al., 2019). From this pool of indicator taxa, we identified 13 bacterial and two fungal taxa, which collectively explained a large part of the variation in PSF, suggesting that few key taxa may determine the direction and strength of PSF. The bacterial predictors were more effective in explaining variation in PSF, most of which were associated with grasses, and more so the fast-growing grass *D. glomerata*. Interestingly, most of the predictors were associated only with one of the three species, suggesting that PSF may be driven more by specialists than by generalists (Semchenko et al., 2022). Most microbial predictors matched the general pattern of PSF we observed among the three species. For instance, PSF was more negative with higher abundances of a taxon belonging to the family of *Myxococcaceae* (*Myxococcota*) in home soil, an indicator taxon of *D. glomerata*. In line with this finding, *D. glomerata* experienced moderate negative PSF—under well-watered conditions the most negative among the three species. A taxon from the bacterivorous genus *Bdellovibrio* (Williams & Chen, 2020) was linked to positive PSF. By feeding on harmful bacteria, such as the plant pathogen *Pseudomonas phaseolicola* (Stolp & Petzold, 1962), this bacterium may benefit the associated plant. We found that members of the *Actinobacteria* and *Acidobacteria* were both positively and negatively associated with PSF. These are groups that play important roles in the soil ecosystem, are responsive to drought (Barnard et al., 2013) and provide a variety of services to plants such as disease suppression, growth promotion and improved nutrition (Kalam et al., 2020; Palaniyandi et al., 2013).

4.4 | The role of root exudates and root litter in mediating plant–soil interactions in response to drought

In contrast to our third hypothesis, we could not identify a role of root exudates or root litter in mediating plant growth and PSF after drought. This is surprising, as both root exudates and root litter play an important role in mediating below-ground plant–microbial interactions, in addition to direct interaction pathways such as mycorrhizal associations (Rolfe et al., 2019; Semchenko et al., 2022;

Tedersoo et al., 2020; Veen et al., 2019). We expected their combined effect to explain at least a part of the observed PSF in plant-conditioned soil. The lack of effect of root litter addition could be due to our use of freshly collected, oven-dried root material, which may differ from naturally senesced roots. The addition of root exudates had generally very little impact on both microbial communities and plant growth, with the exception that *D. glomerata* generally grew better with exudates from droughted plants than well-watered plants. This could be the consequence of stimulated microbial activity caused by increased C transfer to soil microbes through exudates from droughted plants (de Vries et al., 2019; Hou et al., 2025). Fast-growing plant species in particular could profit from the resulting increase in nutrient availability, as shown in a mesocosm experiment conducted by (de Vries et al., 2018), where plant communities shifted towards higher abundances of *D. glomerata* during drought recovery. PSF in plant-conditioned soil did not correlate with PSF in inoculum, root exudate or litter-conditioned soils, questioning the role of these components in regulating feedbacks between plants and soil microbes. However, this could also be the consequence of priority effects by existing microbial communities in the live background soil (Debray et al., 2022). This approach has the advantage of providing a broad range of microbes as a basis for selection processes, but it may also prevent the assembly of new communities by soil conditioning (Brinkman et al., 2010). The pattern of PSF in exudate-conditioned soil even seemed opposite of that of plant-conditioned soil, implying that there must be other mechanisms in place that control PSF. It has to be noted, that the approach of extracting root exudates and litter, storing them and reapplying them to soil cannot perfectly simulate the temporal and spatial patterns of root exudation or litter inputs by a real plant. Additionally, the recovery period after the drought has ended and the subsequent growth in hydroponics could have masked immediate changes in root exudation right after the drought ended. Our approach could have thus not been sensitive enough to detect exudate- or litter-induced drought effects on PSF.

5 | CONCLUSIONS

In conclusion, our work revealed strong connections between plant and microbial drought responses in grassland species, which may result in altered vegetation dynamics after an extreme drought event. We demonstrated that microbial legacies of drought persist in the soil and continue to have negative impacts on plant growth, and that these effects overpower the effects of higher nutrient availability after drought. Our research suggests that both soil fungi and bacteria have a role in shaping negative drought legacies on plant growth, as well as PSF in droughted and non-droughted soils, which was largely independent of root exudates or root litter. They suggest that the role of root exudates and litter inputs in shaping PSF is limited, and that drought legacy effects on plant growth and PSF are driven by direct root–microbe interactions.

AUTHOR CONTRIBUTIONS

Eileen Enderle and Franciska T. de Vries conceived the ideas for the manuscript and designed the research methodology; Eileen Enderle, Leonardo Hinojosa, Victor Lombard and Fangbin Hou collected the data; Eileen Enderle led the data analysis with assistance from Victor Lombard and guidance from Franciska T. de Vries; Eileen Enderle led the writing of the manuscript together with Franciska T. de Vries. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

Franciska de Vries is an Associate Editor of the *Journal of Ecology*, but took no part in the peer review and decision-making processes for this paper.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70160>.

DATA AVAILABILITY STATEMENT

All corresponding data including a dynamic document with the code for the data analysis and a static PDF containing the analysis output are publicly available on Figshare under <https://doi.org/10.21942/uva.28356221> (Enderle et al., 2025). Raw sequence data have been deposited in the Sequence Read Archive (SRA) of NCBI within the project PRJNA746101.

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SUPPORTING INFORMATION

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

Data S1. Data analysis.

Figure S1. Direct drought effects on plant biomass.

Figure S2. Total exudate C and exudation rate at the end of conditioning phase I.

Figure S3. Plant biomass at the end of the feedback phase across all soils.

Figure S4. Correlation of PSF in plant-conditioned soil with other conditioning types.

Figure S5. Correlation between plant biomass in the feedback phase and soil parameters in plant-conditioned soil.

Table S1. Primer sequences and PCR cycle.

Table S2. Soil properties at the end of conditioning phase II.

Table S3. ANOVA outputs for soil properties.

Table S4. ANOVA outputs for plant biomass and PSF.

Table S5. Full table of differentially abundant taxa.

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