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Drought legacy effects on root morphological traits and plant biomass via soil biota feedback

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SUMMARY

- Drought causes soil feedback effects on plant performance. However, how the linkages between conditioned soil biota and root traits contribute to explain plant-soil feedback (PSF) as a function of drought is unknown.
- We utilized soil inoculum from a conditioning experiment where grassland species grew under well-watered and drought conditions and whose soil fungi were analyzed. Under well-watered conditions, we grew 21 grassland species with those inocula from either

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conspecific or heterospecific soils. At harvest, plant biomass and root traits were measured.

- Negative PSF (higher biomass in heterospecific than in conspecific soils) was predominant, and favored in drought-conditioned soils. Previous drought affected the relationship between root traits and fungal groups. Specific root surface area (SRSA) was higher in heterospecific than in conspecific droughted soils and was linked to an increase in saprotroph richness. Overall, root diameter was higher in conspecific soils and was linked to mutualist and pathogen composition, while the decrease of root:shoot in heterospecific soils was linked to pathogenic fungi.
- Drought legacy affects biomass and root morphological traits via conditioned soil biota, even after the drought conditions have disappeared. This provides new insights into the role that soil biota have modulating PSF responses to drought.

KEY-WORDS: Aboveground-belowground interactions, Global change drivers, Plant-soil feedback, Plant-soil interactions, Root morphological traits, Soil inoculum, Soil fungal communities, Soil conditioned by drought.

INTRODUCTION

Climate change could modify precipitation patterns in ways that might affect plant-soil interactions (van der Putten et al., 2016; Pugnaire et al., 2019). Drought events are likely to become more frequent and intense (Dai, 2013, IPCC, 2018), which may directly affect plant growth, root traits, as well as the abundance and composition of soil biota (Hoeppner and Dukes, 2012, Lozano et al., 2020, de Vries et al., 2016, Zhou et al., 2018, Ochoa-Hueso et al., 2018, Fitzpatrick et al., 2018). Drought can alter different root traits, including diameter, tissue density, specific length or specific surface area (Lozano et al., 2020), and can change soil biota composition by increasing the relative abundance of saprotrophs and bacterial groups, decreasing the abundance and richness of mutualistic mycorrhizal fungi (Fitzpatrick et al., 2018, Ochoa-Hueso et al., 2018, Lozano et al., 2021), or by altering microbial networks (De Vries et al., 2018). These shifts in soil microbial communities can be linked with the adjustment of root traits to water scarcity (Lozano et al., 2021) and strongly depend on the plant species identity (Fitzpatrick et al., 2018, Lozano et al., 2020).

Drought can also indirectly affect plant performance through shifts in soil biota, which can feedback either on conspecific or heterospecific plant species (De Long et al., 2019). Plant-soil feedback (PSF) describes the relative growth of a plant with its own conspecific soil community compared with the heterospecific soil community conditioned by other plant species (Bever et al., 1997). It can range from negative to positive and is driven by different mechanisms linked with soil biota, chemical compounds or resource availability (Klironomos, 2002, Rodríguez-Echeverría et al., 2016, De Long et al., 2019, Bennett and Klironomos, 2019). However, the composition of soil microbial communities in particular, has been recognized as an important player (Schnitzer et al., 2011, De Long et al., 2019, Bennett and Klironomos, 2019). In fact, changes in abundance and composition of fungal communities strongly contribute to both negative and positive feedbacks on plant growth (Semchenko et al., 2018), as for example the accumulation of pathogens can suppress plant growth while the accumulation of mutualists can improve plant performance (Bennett and Klironomos, 2019, van der Putten et al., 2016). Thus, plants that heavily rely on and invest in soil mutualists (Reich, 2014), would be highly affected as drought tends to negatively affect this fungal group (Lozano et al., 2021, Ochoa-Hueso et al., 2018). Likewise, an increase in saprotroph abundance and richness due to drought (Lozano et al., 2021) could positively feedback on plant growth (Van der Putten et al., 2016), although effects could also be negative (Semchenko et al., 2018).

The effect of drought on soil communities and subsequent feedback on plant species performance has been mainly studied in terms of aboveground biomass. Research suggests that previous drought may have negative (Kaisermann et al., 2017) or neutral effects (Fry et al., 2018) on plant growth, and that these effects could vary depending on plant functional type (Hassan et al., 2021), with consequences for plant-plant interactions (Kaisermann et al., 2017; Crawford et al., 2020) and plant community structure (Meisner et al., 2013). However, scarce attention has been given to the feedback effect of drought as expressed via root traits, despite the fact that roots are in direct contact with the soil and their morphology is strongly influenced by drought (De Vries et al., 2016; Lozano et al., 2020). Indeed, the feedback effect of drought via a shift in root traits is practically overlooked within our current literature, with a single attempt to evaluate the feedback effect of drought as mediated by specific root length for two plant species, finding no effect (Fry et al., 2018).

Recent research suggests strong linkages between root trait adjustment to water scarcity and soil fungal communities, and proposes a chain reaction where changes in root traits due to drought modify fungal communities, with subsequent consequences for plant biomass (Lozano et al., 2021). It has been suggested that changes in the root : shoot ratio due to drought could cause shifts in fungal mutualist communities (Lozano et al., 2021) and that, depending on the plant species, roots can have increased diameter (Zhou et al., 2018, Lozano et al., 2020), likely to promote colonization by mutualistic soil biota (Kong et al., 2017, Weemstra et al., 2016). Similarly, pathogens and saprotrophs likely respond to drought-induced adjustment in root traits given their strong link to roots. On the one hand, fungal pathogen abundance is strongly linked to variation in SRSA and root : shoot (Lozano et al., 2021). This co-variation can occur because pathogens decrease root fineness by attacking preferentially first-order roots that are easier to infect (Emmett et al., 2014). Alternatively, plants may also respond to pathogen attack by an increase in root : shoot that cause a higher production of secondary metabolites for defense (Hartman et al., 2020). On the other hand, saprotroph abundance is positively correlated with root tissue density (RTD) and root diameter (Lozano et al., 2021). While having root systems with thicker roots represents a higher cost, it may be paid-off by the longer life span of such roots (Weemstra et al., 2016, Kong et al., 2017), which could benefit from carbon mineralization driven by saprotrophs.

Changes in soil biota due to drought may affect root trait expression and thus plant performance. For instance, soil that was previously conditioned by drought and that, as result of this treatment, contains lower richness and abundance of fungal mutualists (compared to a soil previously conditioned by well-watered conditions (Lozano et al., 2021)), would affect root traits of a next generation of plant individuals. Thus, these plants would have increased root diameter in order to promote colonization by the scarce fungal mutualists present in the drought-conditioned soil. This dynamic relationship between root trait expression and the local soil biota composition (e.g. changes in the relative abundance of fungal mutualists) can be explained by the fungal “collaboration” gradient, which may dominate the root economics space in plants (Bergmann et al., 2020). This fungal collaboration gradient varies across plant species from a “do-it-yourself” strategy to an “outsourcing” of functions strategy, and it illustrates variation in the investment in soil exploration by either the root itself or by its mycorrhizal fungal partners. Therefore, the exact feedback outcome of drought-conditioned

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soil biota would depend on the 'position' along the collaboration gradient a plant species can occupy, suggesting a key role of plant species identity in modulating plant-soil feedback responses to drought. For example, plants may increase root diameter to "outsource" arbuscular mycorrhizal fungi (AMF) either because it is their evolutionary strategy, or because the AMF abundance or richness is at such low level, that it merits investing in larger root diameters to favor establishment of mutualistic associations. Likewise, plants may increase SRSA either because they are located closer to the "doing-it-themselves" strategy or to establish relationships with the saprotrophic communities already present in order to increase C mineralization. This way, soil biota conditioned by drought would influence the root morphological trait expression affecting plant-soil feedback responses to drought.

Previous research has examined soil microbial communities at the end of the feedback phase (Kaisermann et al., 2017, Fry et al., 2018). That is, as a response to the previous drought together with the effect of the new plant species, and the soil water conditions used in the feedback phase. However, such a design cannot disentangle to what extent the conditioned soil communities could have driven feedback responses. That is, as an explanatory factor of the previous drought effects on plant performance in the feedback phase. Likewise, the relationship between soil communities and root traits explaining plant-soil feedback as a function of drought has not yet been elucidated, despite the strong relationship between root traits and soil biota (Lozano et al., 2021), and the fact that variation in plant-soil feedbacks could be predicted by root traits (Wilschut et al., 2019).

We hypothesized that the drought effects on soil biota directly influence the magnitude and direction of the feedback not only in terms of plant biomass, but via root traits expression. In other words, we aimed to study the legacy effect of drought on several root morphological traits associated with a variety of plant species (twenty-one grassland species), as this has not been studied yet. Likewise, we aimed to analyze for the first time the effect that soil communities conditioned by drought may have on plant-soil feedback. Based on that, we hypothesized a chain reaction where soil biota (here soil fungi) previously subjected to drought conditions indirectly affect plant-soil feedback through effects on root traits. In order to test this, we collected soil from a previous experiment where twenty-four grasslands species grew under well-watered and drought conditions and whose soil fungal structure after being conditioned by

those water conditions was analyzed (Lozano et al., 2021). Then, we prepared inoculum from those soils and established a new experiment where twenty-one grassland species (including graminoids, forbs and legumes), grew with inoculum from conspecific or heterospecific soil previously subjected to watered or drought conditions. Plant biomass and root morphological traits responses were measured at the end of this experiment.

Materials and Methods

Soil conditioning phase (previous experiment)

Soil conditioning phase was held in a previous experiment (Lozano et al., 2021). In that, sandy loam soil was conditioned (trained) with twenty-four different plant species growing under drought or non-drought (watered) conditions. Briefly, one individual seedling per species was planted into the center of each microcosm (10 replicates per plant species). Plants were well-watered for a month and then, half of the replicates were subjected to drought (30 % of water holding capacity (WHC)) while the other half were kept under non-drought conditions (70 % WHC) for two months (see additional details in Lozano et al., 2020). Soil free of roots was air-dried and stored for ~18 months before using in the feedback phase. While a decrease in microbial biomass C could be expected after that time (Cernohlavkova et al., 2009), such decrease does not represent a confounding factor or a bias in our experimental design as it would occur for both soils (i.e. those previously subjected to drought and those subjected to well-watered conditions).

Plant species selection (this study)

For the current experiment, we selected twenty-one plant species which included graminoids (*Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Festuca brevipila*, *Holcus lanatus*, *Poa angustifolia*, *Lolium perenne*, *Festuca rubra*, *Dactylis glomerata*), forbs (*Achillea millefolium*, *Artemisia ssp. Campestris*, *Berteroa incana*, *Galium verum*, *Hieracium pilosella*, *Hypericum perforatum*, *Plantago lanceolata*, *Potentilla argentea*, *Ranunculus acris*, *Silene vulgaris*) and legumes (*Trifolium repens*, *Vicia cracca*, *Medicago lupulina*). All of these common, frequent and co-occurring grassland species in Central Europe will be referred to by their generic name from here on (except for the two *Festuca* species to which we refer as *F. brevipila* and *F.*

rubra). Seeds of these plant species were obtained from commercial suppliers in the region (Rieger-Hofmann GmbH, Blaufelden, Germany).

Feedback phase (this study)

The feedback experiment was carried out under well-watered conditions. That is, it simulates the effects of a past drought event followed by wet conditions. Any effect on plant biomass and root traits would be driven exclusively by the conditioning of the soil inoculum (i.e., the legacy of drought or well-watered conditions). In May 2018, twenty seedlings of each of the twenty-one plant species were transplanted as single individuals back into sterile soil inoculated with (i) five conspecific soils previously subjected to drought, (ii) five conspecific soils previously subjected to non-drought (watered) conditions, (iii) five heterospecific soils previously subjected to drought and (iv) five heterospecific soils previously subjected to non-drought (watered) conditions. Each replicate seedling was inoculated with independent soil replicates from the conditioning phase. Heterospecific soils were randomly assigned (Table S1). Our experimental design included twenty-one species x four soil inocula x five replicates = 420 pots.

Seeds were surface-sterilized with 4 % sodium hypochlorite for 5 min and 75 % ethanol for 2 min, and thoroughly rinsed with sterile water. Then, seeds were germinated on sterile sand and transplanted 2 days later into 0.5-liter cones (6 cm diameter, 25 cm height) filled with 400 g of sterile sandy loamy soil from our field site (Lozano et al., 2020). Soil was autoclaved three times for 20 min at 120° C and then used as sterile substrate in microcosms. To prepare soil inocula, we followed recommendations by Van de Voorde et al. (2012), Rodríguez-Echeverría et al. (2013), Lozano et al. (2017). We took 200 g of live soil for each replicate of conspecifically or heterospecifically conditioned soil and stirred for five minutes in distilled, autoclaved water in a 1:2 (v:v) ratio. Then, soil was passed through a 0.5 mm sieve to remove soil particles, allowing fungal spores, hyphae, soil bacteria and microfauna to pass through (Van de Voorde et al., 2012). Sterile soil was watered with inoculum from conspecific or heterospecific soil previously subjected to drought (droughted soils) or to non-drought (watered soils) conditions, respectively (Table S1). This inoculum preparation procedure reduced any relative potential differential input of nutrients with inoculation (Rodríguez-Echeverría et al.,

2013), but could affect the fungal community, as some members could have been more sensitive to soil processing (e.g., stirring) or would have recolonized the soil better than others. However, as the inoculum preparation was the same in all pots (droughted and well-watered soils), we expect that those changes would have negligible effects on our experimental treatments. The feedback phase lasted two months. All microcosms were watered twice per week with 70 ml of water to keep water holding capacity ~ 60%, as this experiment was conducted under well-watered conditions. Plants were grown in a glasshouse chamber with a daylight period set at 12 h, 50 klx, and a temperature regime at 22/18 °C day/night with relative humidity of ~40 %. None of the plants died during the experiment. Microcosms were randomly distributed in the chamber and their position shifted three times to homogenize environmental conditions during the experiment.

Measurements

At harvest, roots were carefully removed from the soil and gently washed. Morphological traits in fine roots (i.e., < 2 mm in diameter which included mostly first to third order roots): length, surface area, volume and root average diameter were measured on a fresh sample using the WinRhizoTM scanner-based system (v.2007; Regent Instruments Inc., Quebec, Canada). These root measurements were used to calculate different root morphological traits: specific root surface area (SRSA; cm² mg⁻¹), specific root length (SRL; cm mg⁻¹), root average diameter (RAD; mm) and root tissue density (RTD; root dry weight per volume mg cm⁻³). Shoot and root mass were measured after drying samples at 70 °C for 48 h. Root : shoot was calculated.

Statistical analyses

Calculation of Plant Soil Feedback

Plant Soil Feedback was calculated by a bootstrap procedure for soils previously conditioned by well-watered (watered feedback) or drought conditions (drought feedback). For each plant species, we took a random plant replicate from the conspecific soil treatment and a second random plant replicate from the heterospecific soil treatment. Using these replicates, we calculated the Plant Soil Feedback (PSF) index following Armas et al. (2004) as:

$$\text{PSF}_{\text{trait}} = (Y_{\text{conspecific}} - Y_{\text{heterospecific}}) / (Y_{\text{conspecific}} + Y_{\text{heterospecific}}) \text{ (Eq. 1)}$$

Where $Y_{\text{conspecific}}$ is the trait value when the plant grew with conspecific soil inoculum, and $Y_{\text{heterospecific}}$ is the trait value when the plant grew with heterospecific soil inoculum. For each trait, we repeated the calculation of the (PSF) index 999 times by bootstrap sampling with replacement (Carvalho et al., 2010). The index was calculated for each conditioned soil treatment (i.e. watered, drought) and ranged from -1 to 1, with positive values indicating trait values greater with conspecific than with heterospecific soil inoculum and negative values indicating the opposite. Afterwards, we constructed 95 % confidence intervals by using the function “CI” from the “Rmisc” package, and t.test to determine whether the mean value of PSF index was different from zero.

Differences in fungal communities attributes between conspecific and heterospecific conditioned soils

Sequencing data from the fungal community structure of each conditioned soil (i.e., from the soil used for the inoculum preparation in the feedback phase), was extracted from Lozano et al. (2021). Other soil microbial groups present in the inocula which might play a role in the feedback phase (e.g., bacteria, protists) were not sequenced, but their combined effect (i.e., soil biota effect) can be observed in the feedback results. Briefly, fungal sequencing data were split into three functional groups: pathogens, saprotrophs and mutualists based on functional guild data associated with a given taxonomic level reported in the database FUNGuild (Nguyen et al., 2016) and other sources (see details in Lozano et al., 2021). Data on fungal community attributes (relative abundance, richness and composition) of the soil conditioned by drought and well-watered conditions were selected as evidence of the initial stage from which the feedback phase developed and, as they may help us understand the effect of previous drought on plant performance (biomass and root traits) via soil biota.

Then, we calculated for each plant species, whether the abundance and richness of three fungal guilds (pathogens, saprotrophs and mutualists), differed between conditioned conspecific and heterospecific soils. To do this comparison, we calculated the PSF index (Eq. 1) for richness and abundance following the same bootstrapping procedure explained above. Positive values of the index indicated that conditioned conspecific soils had higher abundance

or richness in a fungal guild than conditioned heterospecific soils, while negative values indicated the opposite.

Feedback analysis: Effects of previous drought on plant biomass and root traits

In order to test whether previous drought influenced plant-soil feedback, we tested the effects of soil biota conditioned by different water regimes (drought and watered), and different plant species (twenty-one) on (i) shoot, root and total plant biomass, and on (ii) root morphological traits. We performed linear models to test plant biomass and root morphological traits response to previous drought. Soil with inoculum from previous watered or drought conditions (previous drought), plant species and their interactions were considered as fixed factors, while plant-soil feedback indices in terms of plant biomass, and root traits, were the response variables. Root mass and diameter were log-transformed to validate normality and variance homogeneity assumptions.

Soil fungi and root traits as predictors of plant-soil feedback

In order to determine the fungal attributes that best explain plant-soil feedback, we assessed the relative importance (%) of each fungal attribute (richness, abundance and composition of pathogens, saprotrophs and mutualists) to watered or droughted feedback for conspecific or heterospecific soils. Plant-soil feedback was evaluated in terms of total plant biomass. We did this by using the metric 'pmvd' from the "RELAIMPO" R package (Grömping, 2006).

Subsequently, we performed a path analysis to test whether these fungal attributes directly affected plant-soil feedback or did so indirectly through effects on root traits. For this analysis, the best predictors of plant-soil feedback were selected based on the Akaike information criterion (AIC) by using the 'stepAIC' function from the "MASS" R package (Venables & Ripley, 2002), from attributes of pathogens, saprotrophs, mutualists and for root traits. The selected predictors were retained for use in the path analysis. PCoAs axes that represent fungal composition were extracted from Lozano et al. (2021). The most parsimonious model was selected by comparing the AIC. We evaluated the fit of our final models using a minimum set of parameters, including X^2 , root mean square error of approximation (RMSEA), and comparative fit index (CFI). Adequate model fits are indicated by a X^2 test ($P > 0.05$), high probability of a low RMSEA value (< 0.1) (Pugesek et al., 2003; Grace, 2006), and high CFI ($>$

0.95, Byrne, 1994). Analyses were conducted using R v.3.5.3 (R Core Team, 2019). Results shown throughout the text and figures are mean values \pm 1 SE.

RESULTS

Feedback effect on total plant biomass

Plant-soil feedback responses depended on whether the soil was previously subjected to drought or well-watered conditions, the plant species and the trait analyzed (Table 1). With inoculum from watered soils, most plant species experienced a negative PSF (higher total plant biomass with heterospecific than with conspecific soil biota, Fig. 1, Table S2), although some species (*F. brevipila*, *Artemisia*, *Galium*, *Hypericum*, *Silene*, *Vicia*) displayed a positive PSF (better growth with conspecific than with heterospecific soil biota). Similarly, with inoculum from droughted soils most species exhibited a negative PSF while few had a positive PSF (e.g., *F. brevipila*, *Ranunculus*, *Artemisia*, *Medicago*). Some species (i.e., *Galium*, *Silene* and *Hypericum*) switched from positive PSF with inoculum from watered soils to negative PSF with inoculum from droughted soils. Other species showed the opposite pattern switching from negative to positive PSF (e.g., *Ranunculus*, *Medicago*, Table S2). In addition, without changing the direction of the effect, previous drought altered the magnitude of the PSF effect. That is, for some species (e.g., *Anthoxanthum*, *Lolium*, *Poa*, *Achillea*), the negative PSF with inoculum from watered soils was exacerbated with inoculum from droughted soils, while for other species (e.g., *Arrhenatherum*, *F. rubra*, *Artemisia*) it was less negative. Overall, shoot and root masses exhibited a similar response to previous drought among plant species (Fig. S1a,b). Changes in root : shoot ratio were also evident. For instance, with inoculum from watered soils, *F. rubra*, *Holcus*, *Berteroa*, *Galium*, *Hypericum*, *Potentilla*, *Ranunculus* and *Silene* had a higher root : shoot in conspecific than in heterospecific soils (or lower in heterospecific soils), while with inoculum from droughted soils, *F. rubra*, *Achillea*, *Galium*, *Hypericum*, *Potentilla*, *Ranunculus*, *Silene* and *Medicago* showed this pattern (Fig. S1c).

Feedback effects as mediated by root morphological traits

Specific root surface area (SRSA): With inoculum from watered soils most species had higher SRSA with conspecific than with heterospecific soil biota, while with inoculum from

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droughted soils plants exhibited the opposite pattern. *Achillea*, *Artemisia*, *Ranunculus*, *Trifolium* and *Lolium* had higher SRSA with conspecific than with heterospecific inoculum from watered soils, but higher SRSA with heterospecific than with conspecific inoculum from droughted soils, while species as *F.rubra*, *Hieracium* and *Vicia* showed the opposite pattern. *Holcus*, *Berteroia* and *Plantago* showed higher SRSA with conspecific than with heterospecific soil biota, a response neutralized by drought (Fig 2a, Table S2).

Specific root length (SRL): Similar to SRSA, with inoculum from watered soils, most species had higher SRL with conspecific than with heterospecific soil biota, while with inoculum from droughted soils a higher SRL with heterospecific than with conspecific soil was more frequent (Fig 2b, Table S2). In addition, some species switched from higher SRSA with conspecific than with heterospecific inoculum from watered soils to higher SRSA with heterospecific than with conspecific inoculum from droughted soils (i.e., *Holcus*, *Achillea*, *Artemisia*, *Ranunculus*, *Trifolium*), while other species switched from higher SRL with heterospecific than with conspecific soils to an opposite pattern (i.e., *F.rubra*, *Hieracium* and *Vicia*). In addition, *Dactylis* and *Lolium* had higher SRL with conspecific than with heterospecific soil biota, a response that was neutralized by drought (Table S2).

Root diameter: With inoculum from watered soils, ten out of twenty-one species (e.g., *Dactylis*, *F.brevipila*, *F.rubra*, *Silene*, *Medicago*) had a higher diameter with heterospecific than with conspecific soil biota, while the others exhibited the opposite pattern (Fig. 2c, Table S2). Several species (i.e., *Dactylis*, *F.brevipila*, *Artemisia*, *Potentilla*, *Silene*, *Medicago*) switched from higher diameter with heterospecific than with conspecific inoculum from watered soils to the opposite pattern with inoculum from droughted soils (Table S2); while few species switched to a higher diameter with heterospecific than with conspecific inoculum from droughted soils (i.e., *Lolium*, *Plantago*, *Vicia*). Finally, *Hieracium* had a higher diameter with conspecific than with heterospecific soil biota, which was canceled out by drought (Fig. 2c, Table S2).

Root tissue density (RTD): With inoculum from watered soils, most species had a higher RTD with heterospecific than with conspecific soil biota, but had the opposite pattern with inoculum from droughted soils (Fig. 2d, Table S2). Specifically, some species (i.e., *Anthoxanthum*, *Lolium*, *Achillea*, *Artemisia*, *Plantago*, *Ranunculus*) switched from higher RTD

with heterospecific than with conspecific inoculum from watered soils to the opposite pattern with inoculum from droughted soils, while other species (i.e., *F.rubra*, *Potentilla*, *Silene* and *Vicia*) switched towards higher RTD with heterospecific than with conspecific inoculum from droughted soils.

Differences in fungal community attributes between conspecific and heterospecific conditioned soil

Conditioned soil used to extract the inoculum with which the plant species were treated (i.e., from previously watered or droughted soils), showed differences in richness and abundance of fungal communities according to the origin of the soil (conspecific or heterospecific, Fig. S2). For instance, pathogen abundance was higher in conspecific than in heterospecific soils of *Arrhenatherum*, *F. rubra*, *Holcus*, *Poa*, *Achillea*, *Artemisia*, *Hypericum*, *Silene*, *Medicago* and *Vicia*, previously subjected to well-watered conditions, while it was higher in conspecific than heterospecific soils of *Arrhenatherum*, *Dactylis*, *Poa*, *Galium*, *Hieracium*, *Hypericum*, *Plantago*, *Ranunculus*, *Silene* and *Trifolium*, previously subjected to drought conditions. Soil biota also differed in richness and abundance of saprotrophs and mutualists for each plant species (Fig. S2).

Relative importance of conditioned fungal communities to plant-soil feedback

Overall, feedback with a drought-conditioned soil was more strongly negative than with a well-watered conditioned soil (Fig. 3a). The attributes of each fungal group that best explained feedback with a well-watered legacy for conspecific soils were: pathogen composition (25.8 %), saprotroph abundance (6.8 %) and mutualist composition (27 %) (Fig. 3b), while for heterospecific soils were: pathogen abundance (5.2 %), saprotroph richness (50.1 %) and mutualist composition (9.7 %) (Fig. 3c). On the other hand, the attributes of each fungal group that best explained feedback with a drought legacy for conspecific soils were: pathogen composition (0.004 %), saprotroph richness (0.49 %) and mutualist composition (0.14 %) (Fig. 3d). The low contribution of these fungal attributes suggests that additional factors may also play a key role determining PSF. For heterospecific soils the fungal attributes that best explained feedback were pathogen, saprotroph and mutualist composition (17.3 %, 22.9 % and 13.9 %, respectively, Fig. 3e).

Fungal communities and root traits as predictors of plant-soil feedback

The magnitude of watered feedback in conspecific soils was best predicted by the composition of mutualists from the conditioning phase ($\beta = 0.17$, $p=0.11$, Fig. 4) as well as for root : shoot ($\beta = -0.41$, $p < 0.01$) and root diameter ($\beta = -0.18$, $p=0.03$) while in heterospecific soils, watered feedback was best predicted by saprotroph richness ($\beta = -0.16$, $p=0.07$), root : shoot ($\beta = 0.35$, $p < 0.01$) and specific root surface area ($\beta = 0.14$, $p=0.12$). On the other hand, the magnitude of droughted feedback in conspecific soils was best predicted by saprotroph richness ($\beta = -0.29$, $p=0.01$) and specific root surface area ($\beta = -0.29$, $p=0.01$), while in heterospecific soils it was only predicted by root : shoot ($\beta = 0.23$, $p=0.01$).

DISCUSSION

Our results showed that previous drought exacerbated the negative plant-soil feedback and affects that outcome via root morphological traits. Specific root surface area (SRSA) was higher with heterospecific than with conspecific soils, while root tissue density (RTD) showed the opposite pattern. Likewise, we found that the different groups of soil biota conditioned by drought correlated with different root traits. For instance, saprotroph fungal richness was strongly correlated with SRSA, mutualistic fungal composition with root diameter, while pathogen composition was correlated with root : shoot and root diameter. These linkages between soil biota and root traits help explain the negative plant-soil feedback as a legacy of drought.

Previous drought exacerbates negative PSF

Our results showed that a negative PSF was predominant among the 21 plant species in our study (i.e. better performance with heterospecific than with conspecific soils), which is in agreement with previous work, e.g. Kaiserman et al. (2017) and Hassan et al. (2021). In addition, we showed that previous drought exacerbates the negative PSF. That is, for 72 % of the plant species (15 out of 21), the magnitude of the negative PSF was higher with soils previously subjected to drought compared to well-watered conditions.

Plant species likely benefit from growing with other species' soil biota (negative PSF) because pathogens are more specialized than plant growth-promoting soil biota (Cortois et al., 2016). In

fact, previous studies have observed that pathogens may outcompete mutualists for infection sites or photosynthates (Graham, 2001, Sikes et al., 2014, Bennett and Klironomos, 2019). We observed for instance, that all graminoids (except *F. brevipila*) and most forbs had a negative PSF, in agreement with Cortois et al. (2016), likely because of less net negative effects (in graminoids) and more net positive soil biota effects (in forbs) of heterospecific soil biota. Previous drought could affect microbial abundance and richness: increasing these attributes in saprotrophs and decreasing them in mutualists (Lozano et al., 2021). Likewise, drought may change fungal and bacterial composition (Fitzpatrick et al., 2018, Lozano et al., 2021), which together with its destabilizing effect on microbial networks (De Vries et al., 2018) may help explain the exacerbated negative PSF after drought. Finally, negative PSF may occur through resource depletion (Bennett et al., 2017, van der Putten et al., 2016), which was avoided in this experiment as the inoculum preparation prevented a potential differential input of nutrients via inoculation (Rodríguez-Echeverría et al., 2013, Lozano et al., 2017), and as this experiment was maintained under well-watered conditions.

Previous drought effects on soil biota alter root morphological traits

Our results showed for the first time that the legacy effect of drought shaping soil microbial communities affects root morphological traits expression, even after the drought conditions have disappeared.

From the root economic spectrum perspective, traits positively associated with nutrient uptake capacity, like high SRSA or SRL, should correlate negatively with root tissue investment (RTD) (Reich, 2014, Wright et al., 2004). Our results agree with that perspective as SRSA and SRL were positively correlated among each other and inversely correlated with RTD, with inoculum from either droughted or watered soils. However, the direction of the correlation changed due to the legacy effect of drought. We observed that soil microbial communities shaped by previously watered and drought conditions had a contrasting effect on root morphological trait expression.

With inoculum from watered soils, ~13 out of 21 species had a higher SRSA and SRL (root fineness) and a lower RTD with conspecific than with heterospecific soil biota. A higher SRSA and SRL has been discussed as part of a strategy to improve soil moisture acquisition

with a low plant investment (Debinski et al., 2010, Comas et al., 2013). Our results suggest that these root morphological responses can be driven by the soil biota shaped by previous watering conditions (drought or watered). Fine non-woody roots are thought to decompose faster (Smith et al., 2014) and to interact intensively with saprotrophs, a fungal group that increases with drought (Lozano et al., 2021), as root exudates and litter production are an important C source for saprotrophs. These fungi are a primary agent of litter decomposition by releasing easily degradable carbohydrates, unlocking nutrients held in the soil organic matter (Kuzyakov et al., 2000). Thus, an increase in fine roots with conspecific soil biota can be related with the home field advantage hypotheses, which suggest higher decomposition in a plant's own environments than elsewhere (Gholz et al., 2000, Austin et al., 2014). In that sense, plants and soil biota could adapt to each other in the same local environment (Rúa et al., 2016) as it has been observed in different ecosystems (Lozano et al., 2019). Although this phenomenon may occur, the hypothesis that faster root decomposition occurs in conspecific soils as a legacy of drought needs to be tested.

In contrast to watered soils, plants with inoculum from droughted soils exhibited a higher RTD but a lower SRL and SRSA with conspecific than with heterospecific soil biota. High root tissue density (RTD) is associated with slower plant growth rates (Tjoelker et al., 2005), and depending on plant species identity, this response is typical of drought environments (de Vries et al., 2016, Lozano et al., 2020). RTD has been linked with drought resistance (Fort et al., 2013, Tjoelker et al., 2005, Wahl and Ryser, 2000), root longevity (Eissenstat et al., 2000), and with changes in root diameter mainly associated with water transport capacity (Fort et al., 2013). However, in this experiment the plant species were not subjected to drought but simply to a soil community modified by previous drought, suggesting that the known increase in root tissue density under drought conditions would also be related to the legacy effect of on soil biota in addition to the well-established adjustment to water scarcity (De Vries et al., 2016, Lozano et al., 2020, Weemstra et al., 2016, Fort et al., 2013). Drought can decline mutualist abundance and richness (Lozano et al., 2021, Ochoa-Hueso et al., 2018), reason why plants may have higher root diameter/ tissue density if they have a greater dependence on mycorrhizal fungi. We found a stronger effect of soil biota on root tissue density than on root diameter (as the magnitude of the effect on root diameter was around 0.1, while for RTD it was around 0.25). This suggests that the stele-cortex ratio, more than the diameter itself can be a

key root trait for understanding the responses of plant species to drought conditions, which is supported by the fact that stele-cortex ratios play an important role when establishing mycorrhizal associations (Valverde-Barrantes and Blackwood, 2016, Kong et al., 2017).

Soil fungi conditioned by drought and their linkages with root traits contribute to explaining plant-soil feedback

We found that soil fungi previously conditioned by drought contribute to explaining the negative PSF. This is a novel finding as previous research about drought feedback effects on plant performance have analyzed the soil microbial communities as a response variable rather than as an explanatory variable. Soil fungi (saprotrophs, mutualists and pathogens) shaped by drought, differed in their relative abundance and richness among plant species. However, we also observed general patterns of soil biota shaped by drought influencing root morphological trait expression with likely consequences for plant biomass. For instance, overall, changes in saprotroph richness due to drought play a key role promoting a negative PSF, via effects on root traits such as specific root surface area (SRSA).

A decrease in saprotroph richness in heterospecific soils (or an increase in conspecific soils), may help explain the exacerbated negative PSF with inoculum from droughted soils. In these soils, saprotroph richness was linked with root fineness (SRSA), a relationship that did not occur with inoculum from watered soils. Drought may induce shifts in soil fungal communities that in turn can be linked to root traits (Lozano et al., 2021). Specifically, a diverse community of saprotrophs may be linked to plant species with thin roots (Semchenko et al. 2018, Lozano et al., 2021), a pattern that we observed with conspecific soils. Plant species may develop thinner roots as a strategy to face drought (Lozano et al., 2020). In addition, our results suggest that the legacy effect of drought on soil biota, specifically, on saprotroph richness, may affect root traits (i.e., SRSA), a relationship that contributed to the negative PSF.

It has been argued that saprotrophs can contribute to a positive PSF due to their role in decomposition processes (Van der Putten et al., 2016). However, we found the opposite pattern: saprotroph richness was correlated with negative PSF. A similar pattern has been observed by Semchenko et al. (2018) related to specialist saprotrophs, a situation that highly depends on saprotrophs interactions with other fungal groups. That is, the net outcome for

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plant growth depends on antagonistic and synergistic interactions among saprotrophs, pathogens and mutualists (Van der Putten et al 2016). Thus, mutualists may have had a protective effect on plant tissues and, simultaneously, pathogens may have enhanced the abundance of dead tissue available to specialist saprotrophs (Semchenko et al., 2018), favoring decomposition processes in heterospecific soils (negative PSF).

Likewise, our path analyses showed a positive relationship between root : shoot and PSF, which implies that a decrease in root : shoot with heterospecific soil biota, may promote a negative plant-soil feedback. Although plant species may increase root mass as a possible strategy to increase resource availability for heterospecific soil biota via altered root turnover and/ or root exudation (Eisenhauer et al., 2017), our results showed that in soils previously subjected to drought this is not the case, as plants decrease root: shoot and invest in other root traits such as SRSA to increase plant-soil feedback with heterospecific soil biota. Root : shoot was linked with pathogen abundance in well-watered soils. A root : shoot decrease may reduce the probability of pathogenic infection explaining the higher plant biomass in heterospecific soils compared to conspecific soils (negative PSF). In addition, pathogen composition was linked with root diameter in conspecific soils. It is known that pathogen colonization is related to root diameter and, especially, to the hierarchical branching order of fine roots (Emmett et al., 2014; King et al., 2021). Apart from their differences in structure and function (e.g., active cortex and uptake/resource functions), high-order roots may be preferentially protected from pathogens because the entire downstream branches depend upon them (Wells and Eissenstat, 2003), while first-order roots are most likely to encounter pathogen propagules (Emmett et al., 2014). Thus, an increase in root diameter in conspecific soils, would be associated with a greater pathogenic infection of first order roots (finest roots). Likewise, root diameter was also correlated with mutualist composition. Pathogens, which can colonize the roots faster than mutualists, could have affected the establishment of mycorrhizal associations in conspecific soils, which added to pathogenic effects on first order roots, contributing to the negative PSF.

Other changes in soil fungal community composition due to previous drought also contribute to explaining the negative PSF. Our path analyses showed that mutualist composition appears to be a key fungal attribute determining plant-soil feedback. Mutualistic fungi are known to

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promote drought resistance (Hartman et al., 2020) and to support positive PSF (Van der Putten et al., 2016), however, they can, on occasion, reduce plant growth of conspecific plants over heterospecific ones (Lekberg et al., 2014, Bever, 1999), causing a negative PSF.

Mutualists can drive negative PSF, especially in young plants, as the initial carbon drained to AM fungi can be costly for plants with little photosynthetic capacity (Schroeder et al., 2004, Jifon et al., 2002), or because young plants can be poorly colonized by mutualists (in comparison with pathogenic fungi, mutualists may need more time to develop their fungal structures to establish associations with conspecific plants).

Growth depressions (negative PSF) may also arise from P deficiency which is thought to occur when AM fungi, while transferring phosphate to the root, impair or eliminate direct, root-mediated phosphate uptake (Lekberg et al., 2014). This phenomenon has been observed in plants poorly colonized by AM (Smith and Smith 2012) as young plants can be, so that the transfer of phosphate from the fungus to the plant is insufficient to make up for the loss of phosphate uptake via the direct, root-mediated pathway, which in the end may promote a negative PSF. However, as growth depression is a transient phenomenon, it is very likely that a positive association between plants and AM fungi would be observed in more mature plants.

The legacy effect of drought can be influenced by the fluctuations in water availability itself (i.e., drying / rewetting). That is, we would expect the largest shift in soil microbial community composition in the shift from drought (conditioning phase) to well-watered conditions (feedback phase), rather than from well-watered (conditioning phase) to well-watered conditions (feedback phase). However, grassland soils are frequently exposed to drying/rewetting events, so most of the soil biota present would be microbes tolerant to these fluctuations (Van der Putten et al., 2016). Research shows contrasting results on this matter. For example, some studies reported that during a drying/rewetting fluctuation fungal richness and abundance slightly decrease (Meisner et al., 2018), while other studies found negligible effects on fungal community composition during a similar fluctuation (Barnard et al., 2013). Although previous drought might decrease microbial growth, respiration rates or biomass, these attributes could start to increase immediately upon rewetting, reaching the rate of a well-watered soil after a week for 1-year dried soil (Meisner et al., 2013b, Lundquist et al., 1999, Scheu and Parkinson, 1994), but see Gordon et al (2008) for a contrary view. Even so, the low fungal biomass or respiration during that first week after rewetting, may have had negative

consequences on the net plant performance which would explain the stronger negative feedback observed under droughted soils, and the minor contribution of droughted soil biota to the negative feedback.

We found that saprotroph richness, and mutualistic and pathogen composition were the key fungal attributes promoting negative PSF. However, other soil biota such as symbiotic soil bacteria and nematodes may also play a role modulating PSF (Pugnaire et al., 2019; Van de Voorde et al., 2012). Future research in field and controlled conditions that explicitly measure the complexity of the whole soil biota are needed to fully understand the legacy effect of drought on plant-soil feedbacks. In addition, it is still uncertain how long the legacy effects of drought on root traits and on PSF may last. Therefore, short-term experiments, such as ours (in which the effect of the soil biota is emphasized), as well as those of longer duration that allow a better development of, for instance, mutualist associations, are necessary in order to better understand plant-soil feedbacks.

Our results showed strong linkages between fungal communities and root traits modulating plant-soil feedback. For instance, we found strong linkages between saprotrophs and root fineness (SRSA), mutualists and root diameter, and between pathogens and root : shoot/root diameter. This research provides new insights into the role that soil fungi play modulating plant-soil feedback response to drought, via effects on root traits.

AUTHOR CONTRIBUTIONS

YML conceived the ideas and designed methodology with input from CAA-T and MCR; YML, CAA-T and JMO established and maintained the experiment in the greenhouse; JMO collected the data with the help of YML; YML analyzed the data and wrote the first draft. All authors contributed to the draft and gave final approval for publication. We have no conflict of interest to declare.

DATA AVAILABILITY

Data that support the findings of this study are available from the corresponding author upon request.

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Supporting information

Table S1. Experimental design

Table S2. Summary of plant-soil feedback responses

Figure S1. Plant-soil feedback as reflected by shoot mass, root mass and root : shoot.

Figure S2. Soil fungal structure from the conditioned phase

Figure 1. Plant-soil feedback (PSF) of twenty-one species growing in sterile soil watered with inoculum from soil previously subjected to watered (blue) or drought (red) conditions. PSF in terms of total plant mass. Mean values and its confidence intervals are shown. Positive values indicate better performance in conspecific than in heterospecific soils. Negative values indicate the opposite. T-test values showed strong (*, $p < 0.01$) and moderate (+, $p < 0.05$) evidence that the mean values were different from zero.

Figure 2. Plant-soil feedback (PSF) of twenty-one species growing in sterile soil watered with inoculum from soil previously subjected to watered (blue) or drought (red) conditions. PSF

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responses as reflected in (A) specific root surface area, SRSA; (B) specific root length; (C) root diameter, RAD and (D) root tissue density, RTD. Mean values and its confidence intervals are shown. Positive numbers indicate higher values of the trait in conspecific than in heterospecific soils. Negative numbers indicate higher values of the trait in heterospecific than in conspecific soils. T-test values showed strong (*, $p < 0.01$) and moderate (+, $p < 0.05$) evidence that the mean values were different from zero.

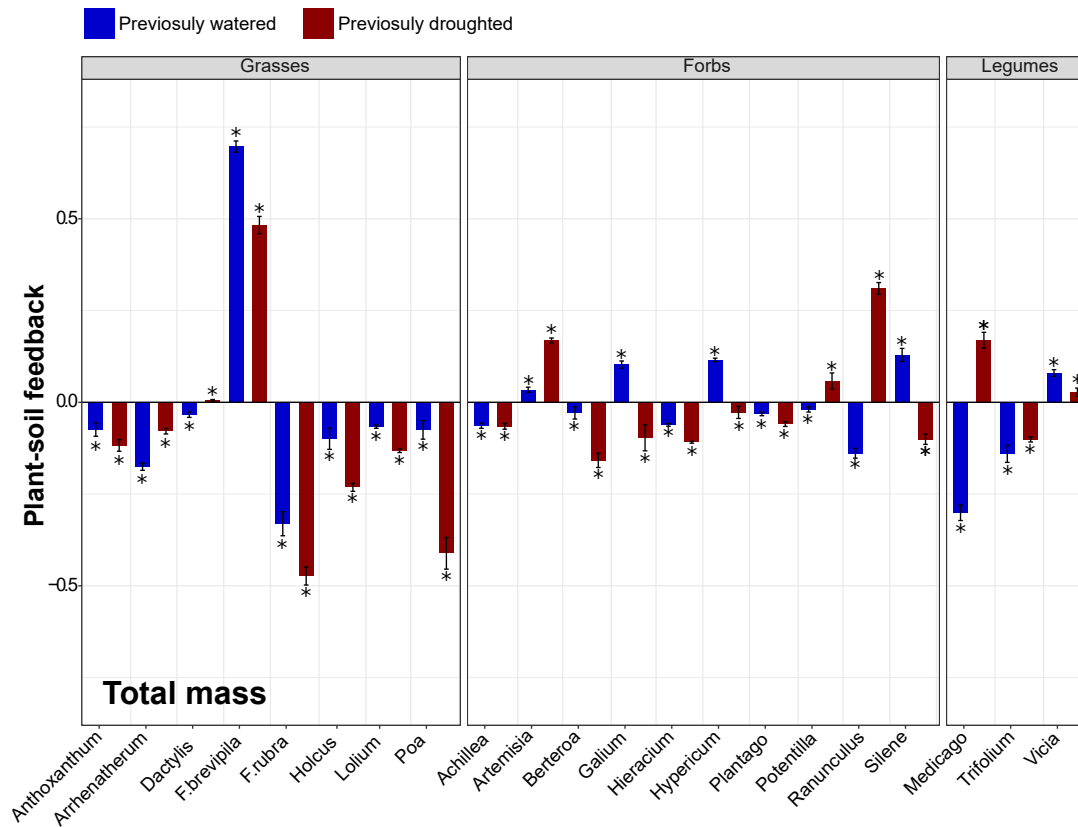
Figure 3. Mean values of (A) plant-soil feedback response to previous drought conditions. Confidence intervals are quite small that can not be appreciated in the panel. Negative values in panel (A) indicate higher plant biomass in heterospecific than in conspecific soils. T-test evidence a strong difference from zero (*, $p < 0.01$). Four additional panels explain the relative importance of the fungal attributes to plant-soil feedback in (B,D) conspecific and (C,E) heterospecific soils previously subjected to watered or drought conditions. Composition of conditioned soil fungi corresponds to the two principal coordinate axes (data from Lozano et al., 2021). The metric for assessing relative importance of regressors in the linear model was 'pmvd'.

Figure 4. Path analyses of the relationships between soil fungal communities, root traits, soil properties and (A) watered feedback (plant biomass in the feedback phase with inoculum from soil previously subjected to well-watered conditions) or (B) droughted feedback (plant biomass in the feedback phase with inoculum from soil previously subjected to drought conditions). The coefficient adjacent to each arrow is the strength of the effect of each standardized path and its evidence (P value). The width of the arrows is proportional to the magnitude of the path coefficients. Full arrows indicate positive relationships and dotted arrows negative relationships. Single-headed arrows indicate a hypothesized causal influence of one variable upon another. Linkages with fungal composition (grey boxes) do not imply positive correlations but a relationship.

Table 1 Results of linear models for plant biomass and root morphological trait responses to plant-soil feedback.

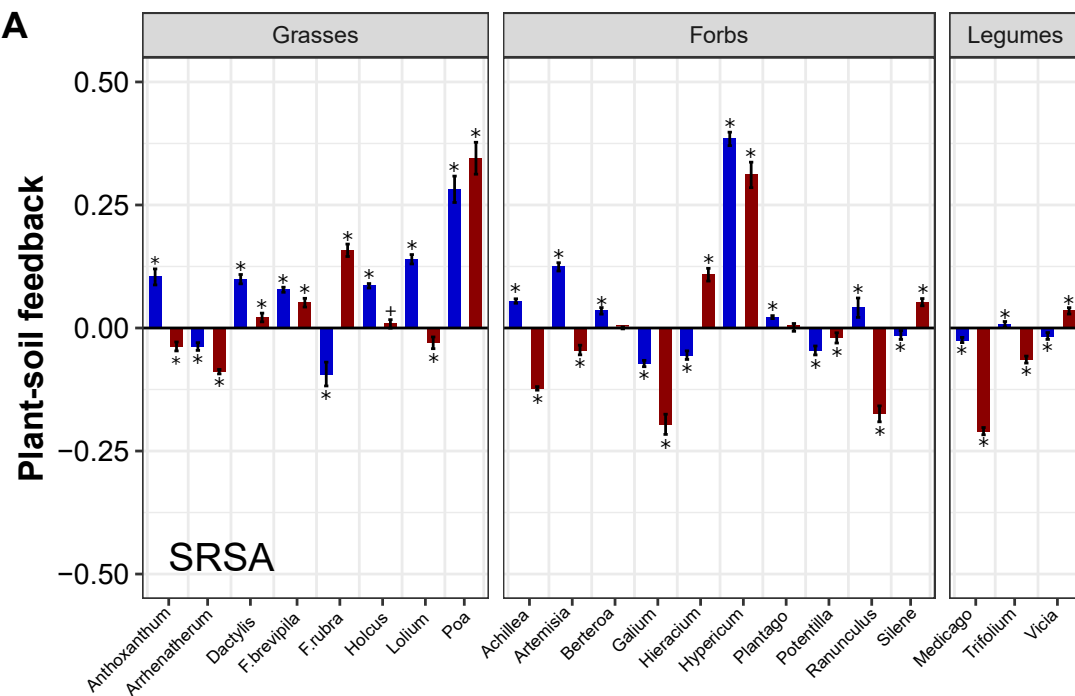
	df	Shoot mass	Root mass	Total mass	Diameter	RTD	SRL	SRSA
Previous drought (Pd)	1	89.706 (<0.001)	85 (<0.001)	89.7 (<0.01)	309 (<0.001)	1738.12 (<0.001)	2099.7(<0.001)	2408.77 (<0.001)
Plant species (Ps)	20	5855.88 (<0.001)	466 (<0.001)	5855.8 (<0.01)	533 (<0.001)	881.53 (<0.001)	500.80 (<0.001)	559.88 (<0.001)
Pd x Ps	20	324.711 (<0.001)	337 (<0.001)	324.7 (<0.001)	232 (<0.001)	304.96 (<0.001)	252.46 (<0.001)	267.10 (<0.001)

F values and p-values (in parentheses) are shown. Previous drought refers to whether the soil from which the inoculum was obtained was subjected to drought or well-watered conditions in the conditioning phase. RDA (root tissue density), SRL (specific root length), SRSA (specific root surface area).

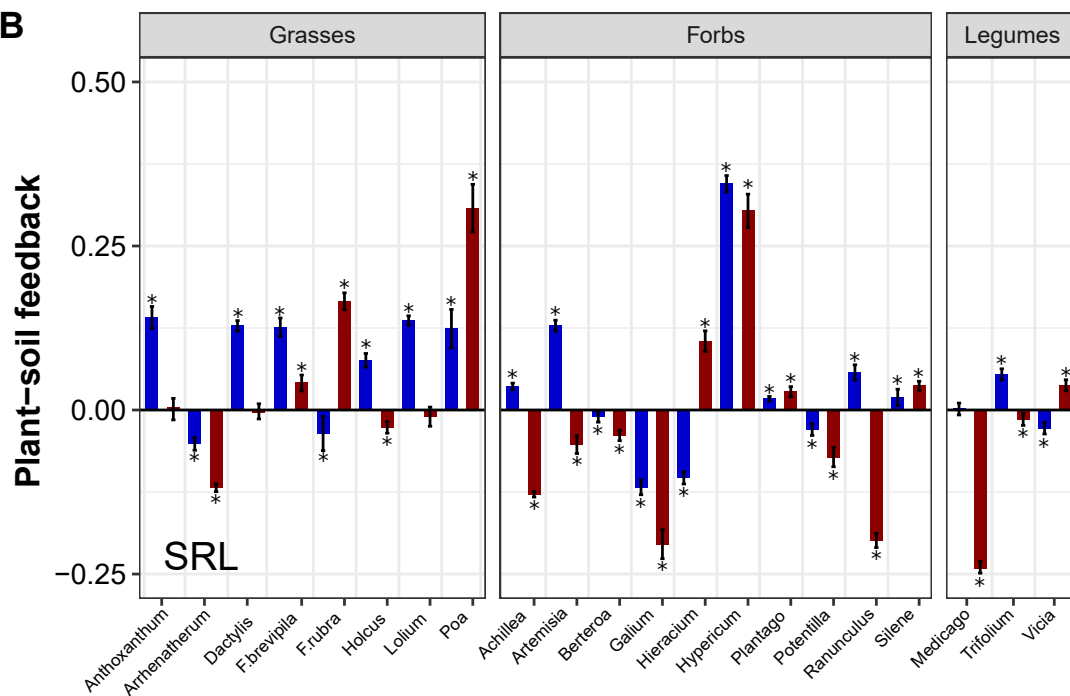


■ Previously watered ■ Previously droughted

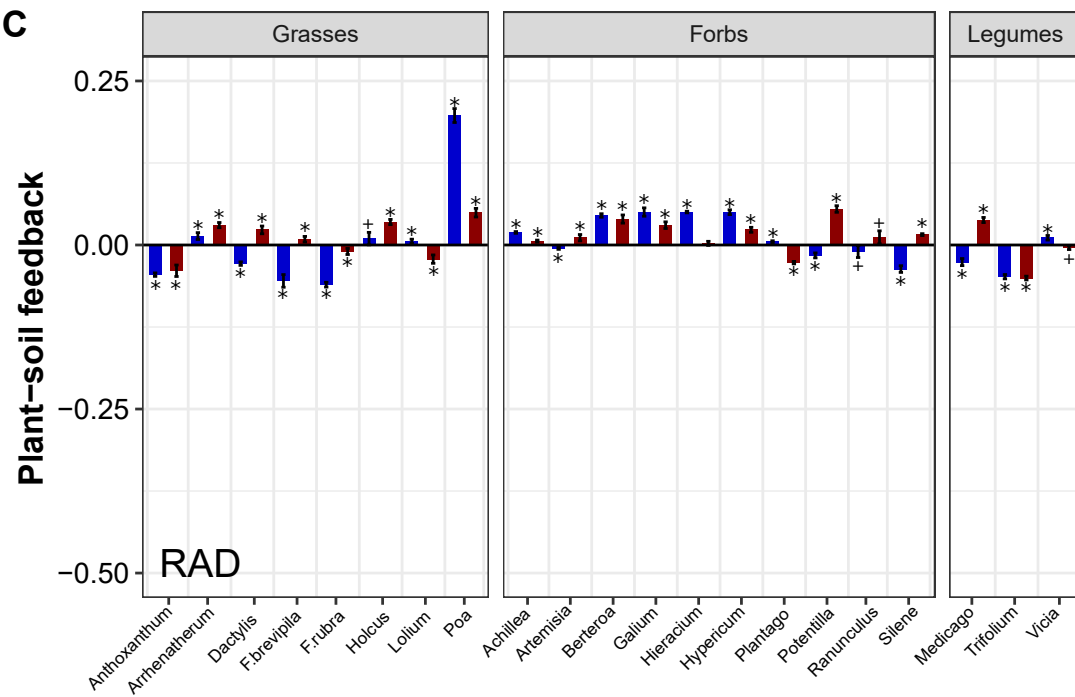
A



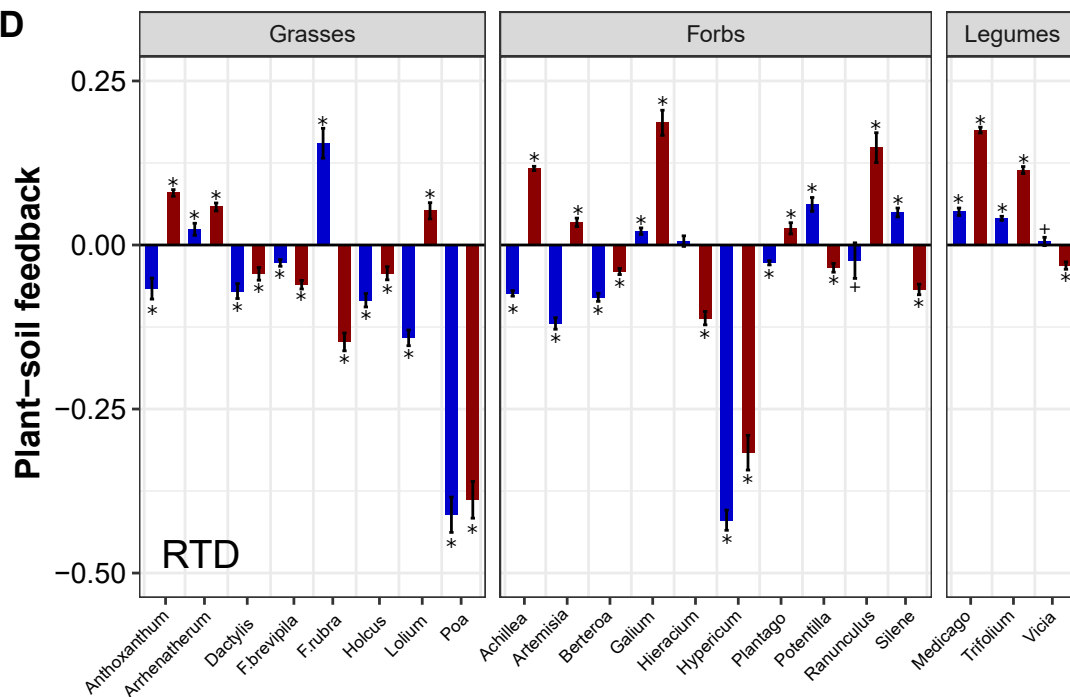
B

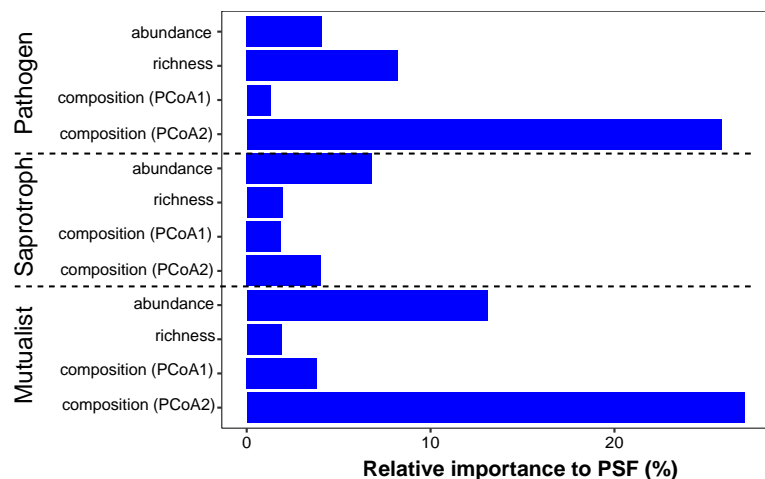
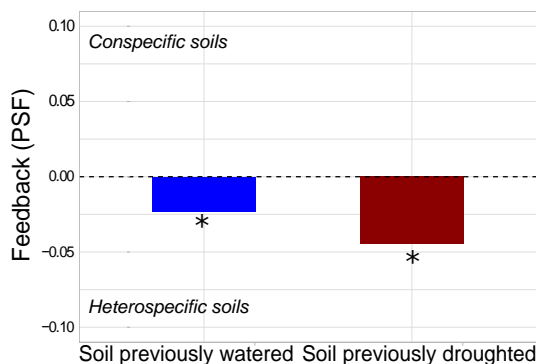
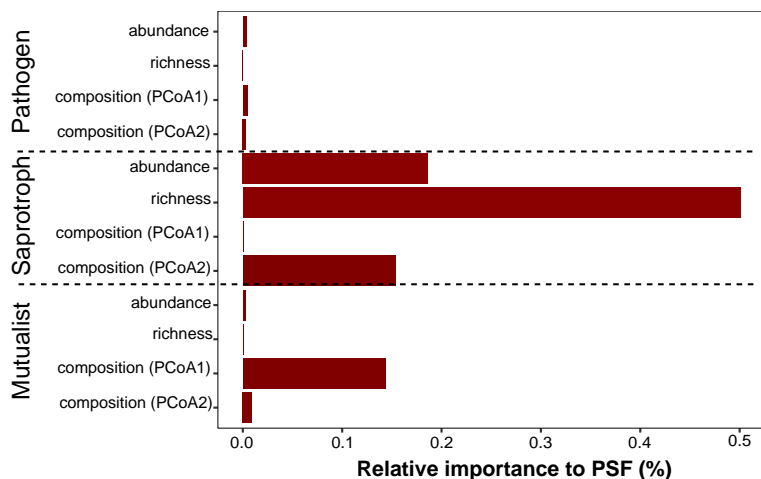
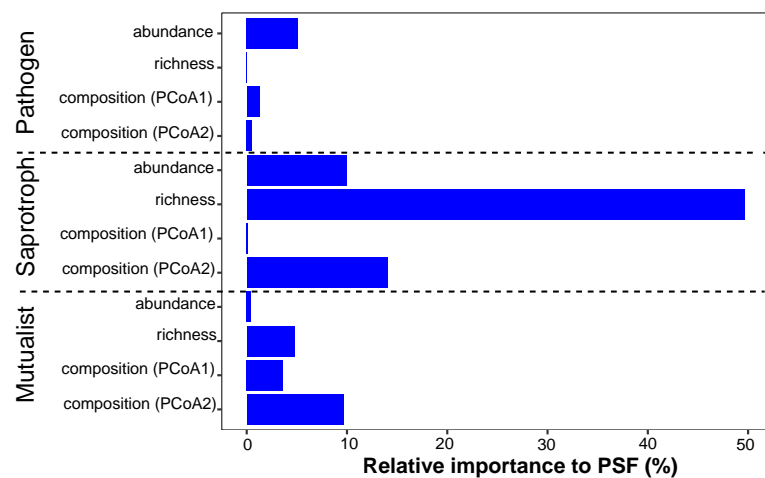
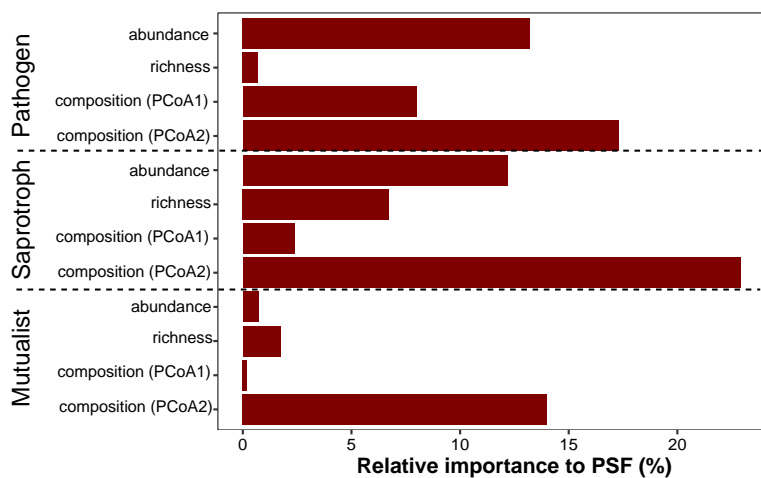


C

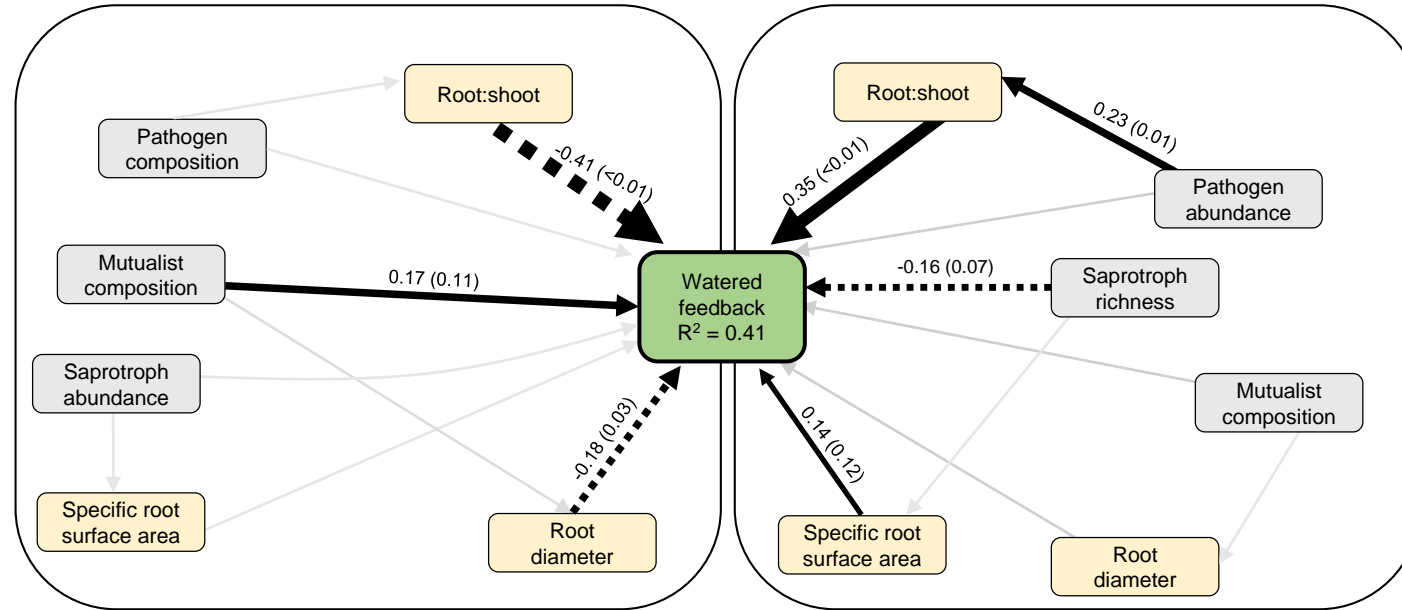


D



B Soil biota from **conspecific** well-watered soils**D** Soil biota from **conspecific** droughted soils**C** Soil biota from **heterospecific** well-watered soils**E** Soil biota from **heterospecific** droughted soils

A

Conspecific soil $R^2 = 0.24$ Heterospecific soil $R^2 = 0.17$ 

B

Conspecific soil $R^2 = 0.18$ Heterospecific soil $R^2 = 0.12$ 