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 their soil fungi were analyzed. Under well-watered conditions, we grew 21 grassland species with those inocula from either 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, whereas 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.

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 & Dukes, 2012; de Vries *et al.*, 2016; Fitzpatrick *et al.*, 2018; Ochoa-Hueso *et al.*, 2018; Zhou *et al.*, 2018; Lozano *et al.*, 2020). 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 also can 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; Bennett & Klironomos, 2019; De Long *et al.*, 2019). However, the composition of soil microbial communities in particular, has been recognized as an important player (Schnitzer *et al.*, 2011; Bennett & Klironomos, 2019; De Long *et al.*, 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), because, for example, the accumulation of pathogens can suppress plant growth whereas the accumulation of mutualists can improve plant performance (van der Putten *et al.*, 2016; Bennett & Klironomos, 2019). Thus, plants that rely heavily on and invest in soil mutualists (Reich, 2014) would be highly affected as drought tends to negatively affect this fungal group (Ochoa-Hueso *et al.*, 2018; Lozano *et al.*, 2021). Likewise, an increase in saprotroph abundance and richness as a consequence of drought (Lozano *et al.*, 2021) could result in a positive feedback on plant growth (Van der Putten *et al.*, 2016), although effects also could be negative (Semchenko *et al.*, 2018).

The effect of drought on soil communities and subsequent feedback on plant species performance has been studied mainly

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 & Hawkes, 2020) and plant community structure (Meisner *et al.*, 2013b). 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 resulting from 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 resulting from 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 (Weemstra *et al.*, 2016; Kong *et al.*, 2017). Likewise, pathogens and saprotrophs probably 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 specific root surface area (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 also may respond to pathogen attack by an increase in root : shoot that cause a higher production of secondary metabolites for defense (Hartmann *et al.*, 2020). On the other hand, saprotroph abundance is positively correlated with root tissue density (RTD) and root diameter (Lozano *et al.*, 2021). Although having root systems with thicker roots represents a higher cost, it may be paid-off by the longer lifespan of such roots (Weemstra *et al.*, 2016; Kong *et al.*, 2017), which could benefit from carbon (C) mineralization driven by saprotrophs.

Changes in soil biota resulting from drought may affect root trait expression and thus plant performance. For instance, soil that previously was 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 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 PSF 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 PSF 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 the extent to which 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 PSF 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 PSF 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 also 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 (21 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 PSF. Based on that, we hypothesized a chain reaction where soil biota (here soil fungi) previously subjected to drought conditions indirectly affect PSF through effects on root traits. In order to test this, we collected soil from a previous experiment where 24 grassland species had grown under well-watered and drought conditions and whose soil fungal structure after being conditioned by those water conditions had been analyzed (Lozano *et al.*, 2021). Then, we prepared inoculum from those soils and established a new experiment where 21 grassland species (including graminoids, forbs and legumes), grew with inoculum from conspecific or heterospecific soils 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)

The soil conditioning phase was carried out in a previous experiment (Lozano *et al.*, 2021), in which sandy loam soil was conditioned (trained) with 24 different plant species growing under drought or nondrought (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)) whereas the other half were kept under nondrought conditions (70% WHC) for 2 months (see additional details in Lozano *et al.*, 2020). Soil free of roots was air-dried and stored for *c.* 18 months before using in the feedback phase. Although a decrease in microbial biomass C could be expected after that time (Cernohlavkova *et al.*, 2009), it 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 21 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 names 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 simulated 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, 20 seedlings of each of the 21 plant species were transplanted as single individuals back into sterile soil inoculated with (1) five conspecific soils previously subjected to drought, (2) five conspecific soils previously subjected to nondrought (watered) conditions, (3) five heterospecific soils previously subjected to drought and (4) five heterospecific soils previously subjected to nondrought (watered) conditions. Each replicate seedling was inoculated with independent soil replicates from the conditioning phase. Heterospecific soils were randomly assigned (Supporting Information Table S1). Our experimental design included 21 species × four soil inocula × 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 d later into 0.5-l 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 5 min 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 nondrought (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 2 months. All microcosms were watered twice per week with 70 ml of water to retain a water-holding capacity of *c.* 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°C : 18°C, day : night with relative humidity of *c.* 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 WinRhizo™ scanner-based system (v.2007; Regent Instruments Inc., Quebec, QC, Canada). These root measurements were used to calculate different root morphological traits: specific root surface area (SRSA; $cm^2\ mg^{-1}$), specific root length (SRL; $cm\ mg^{-1}$), root average diameter (RAD; mm) and root tissue density (RTD; root DW 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 PSF index following Armas *et al.* (2004) as:

$$\text{PSF trait} = \left(Y_{\text{conspecific}} - Y_{\text{heterospecific}}\right) / \left(Y_{\text{conspecific}} + Y_{\text{heterospecific}}\right)$$
$$\text{Eqn 1}$$

Yconspecific, trait value when the plant grew with conspecific soil inoculum; and Yheterospecific, 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 R/MISC package, and Student's *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 because they comprise evidence of the initial stage from which the feedback phase developed and because 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 (Eqn 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, whereas 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 PSF, we tested the effects of soil biota conditioned by different water regimes (drought and watered), and different plant species (21) on (1) shoot, root and total plant biomass, and on (2) root morphological traits. We performed linear models to test plant biomass and root morphological trait 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, whereas PSF 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 PSF, 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 R/RELAIMPO (Grömping, 2006). Subsequently, we performed a path analysis to test whether these fungal attributes directly affected PSF or did so indirectly through effects on root traits. For this analysis, the best predictors of PSF were selected based on the Akaike information criterion (AIC) by using the 'stepAIC' function from the R/MASS (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 χ^2, root mean square error of approximation (RMSEA), and comparative fit index (CFI). Adequate model fits are indicated by a χ^2 test ($P > 0.05$), high probability of a low RMSEA value ($P < 0.1$) (Pugesek *et al.*, 2003; Grace, 2006), and high CFI ($P > 0.95$, 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 ± 1 SE.

Results

Feedback effect on total plant biomass

Plant–soil feedback responses depended on whether the soil previously was 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). Likewise, with inoculum from droughted soils most species exhibited a negative PSF, whereas 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

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

	df	Shoot mass (g)	Root mass (g)	Total mass (g)	RAD	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 × 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. RAD, root average diameter; RTD, root tissue density; SRL, specific root length; SRSA, specific root surface area.
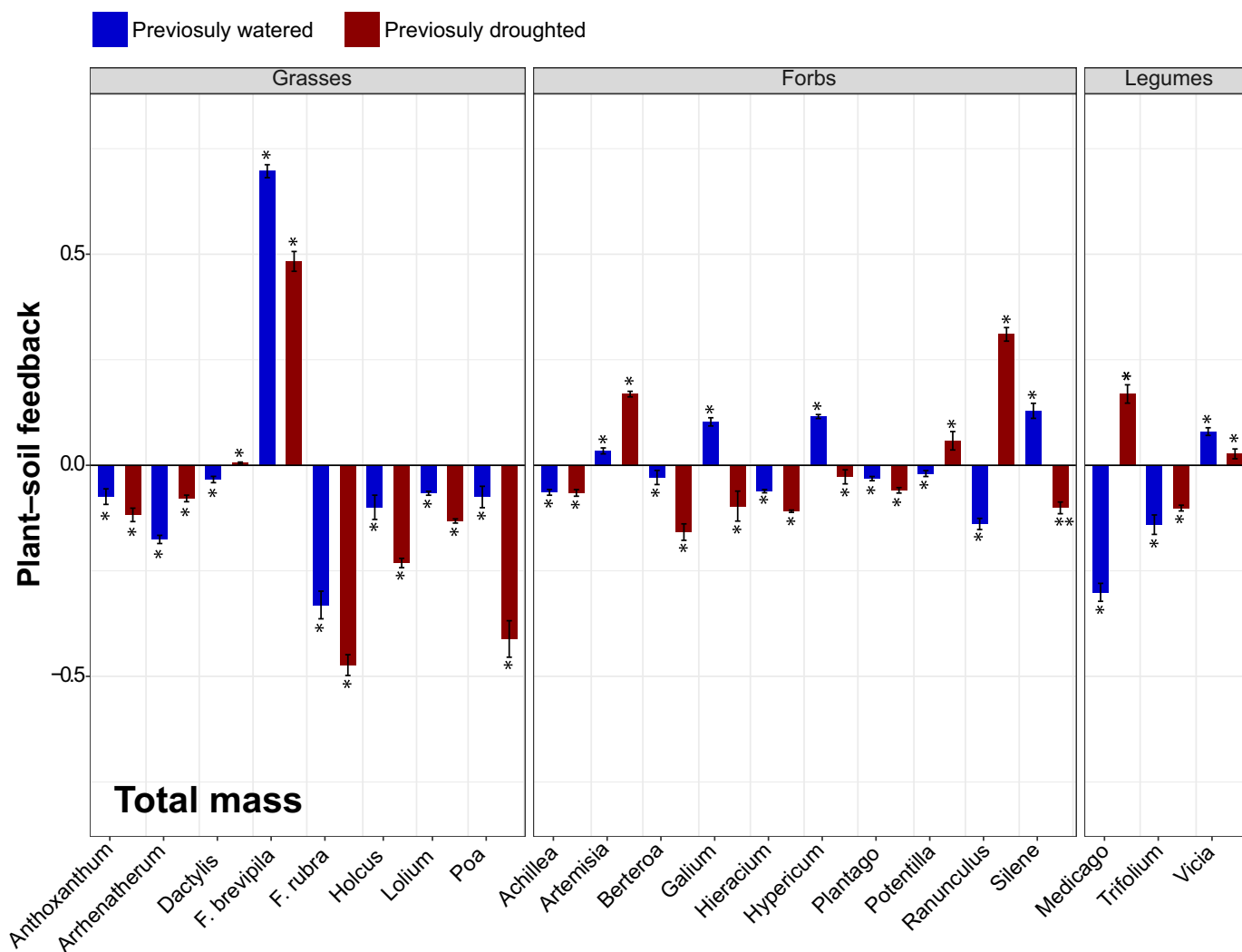

Fig. 1 Plant–soil feedback (PSF) of 21 species growing in sterile soil watered with inoculum from soil previously subjected to watered (blue) or drought (red) conditions. PSF in terms of total plant mass. Mean values and its confidence intervals are shown. Positive values indicate better performance in conspecific than in heterospecific soils. Negative values indicate the opposite. Student's *t*-test values showed strong (*, *P* < 0.01) and moderate (**, *P* < 0.05) evidence that the mean values were different from zero.

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, whereas 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 also were 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), whereas 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 With inoculum from watered soils most species had higher SRSA with conspecific than with heterospecific soil biota, whereas with inoculum from 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, whereas species such as *F. rubra*, *Hieracium* and *Vicia* showed the opposite pattern. *Holcus*, *Berteroa* and *Plantago* showed higher SRSA with conspecific than with heterospecific soil biota, a response neutralized by drought (Fig. 2a; Table S2).

Specific root length Similar to SRSA, with inoculum from watered soils, most species had higher SRL with conspecific than

with heterospecific soil biota, whereas 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*), whereas 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 average diameter With inoculum from watered soils, 10 of 21 species (e.g. *Dactylis*, *F. brevipila*, *F. rubra*, *Silene*, *Medicago*) had a higher RAD with heterospecific than with conspecific soil biota, whereas the others exhibited the opposite pattern (Fig. 2c; Table S2). Several species (i.e. *Dactylis*, *F. brevipila*, *Artemisia*, *Potentilla*, *Silene*, *Medicago*) switched from higher RAD with heterospecific than with conspecific inoculum from
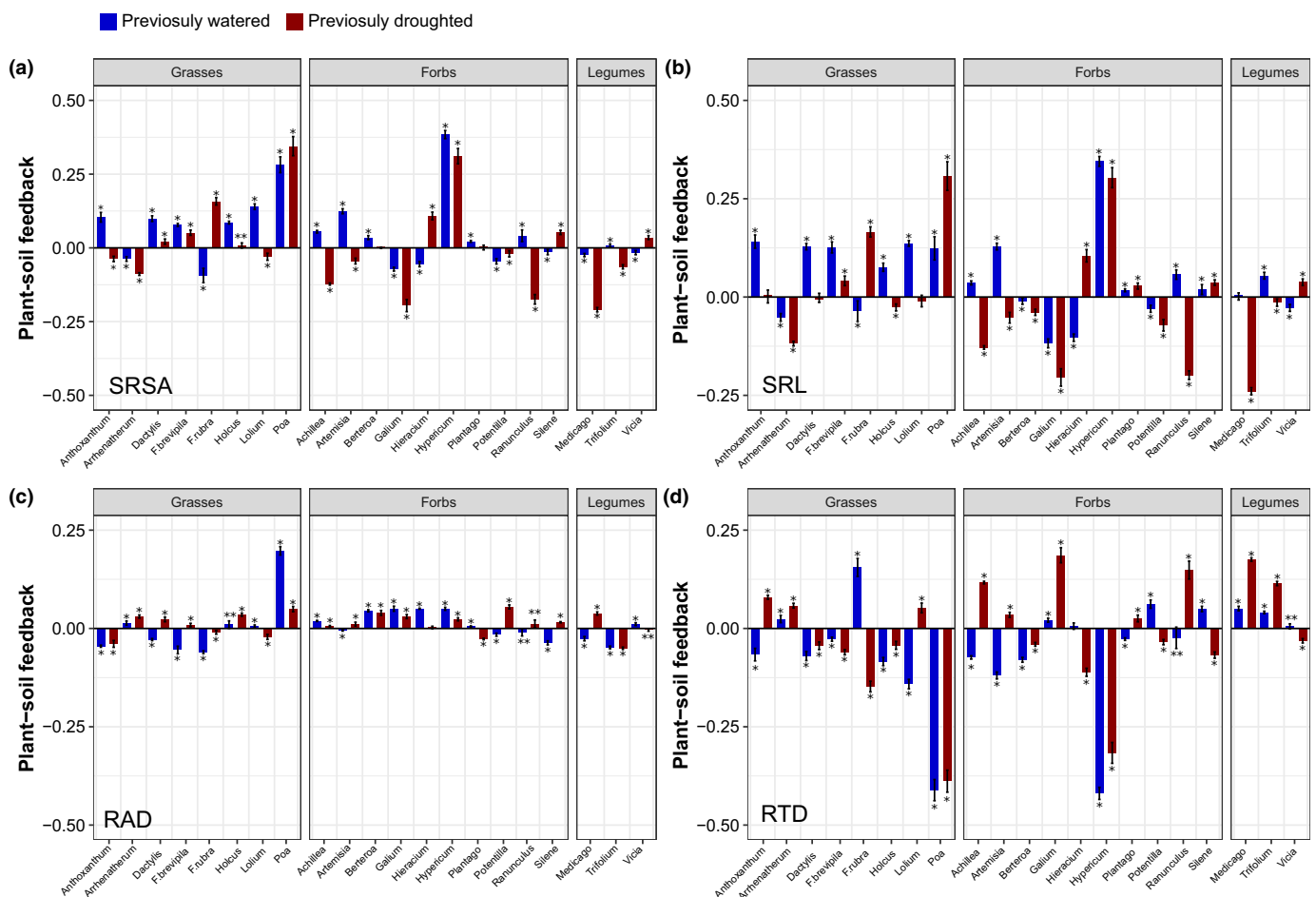

Fig. 2 Plant–soil feedback (PSF) of 21 species growing in sterile soil watered with inoculum from soil previously subjected to watered (blue) or drought (red) conditions. PSF responses as reflected in (a) specific root surface area (SRSA); (b) specific root length (SRL); (c) root diameter (RAD) and (d) 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. Student's *t*-test values showed strong (*, $P < 0.01$) and moderate (**, $P < 0.05$) evidence that the mean values were different from zero.

watered soils to the opposite pattern with inoculum from droughted soils (Table S2), whereas few species switched to a higher RAD with heterospecific than with conspecific inoculum from droughted soils (i.e. *Lolium*, *Plantago*, *Vicia*). Finally, *Hieracium* had a higher RAD with conspecific than with heterospecific soil biota, which was cancelled out by drought (Fig. 2c; Table S2).

Root tissue density 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, whereas 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, whereas 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). On the one hand, 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), whereas for heterospecific soils they 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 also may 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

On the one hand, 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 RAD ($\beta = -0.18$, $P = 0.03$). However, 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 SRSA ($\beta = 0.14$, $P = 0.12$). 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 SRSA ($\beta = -0.29$, $P = 0.01$), whereas 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 PSF and affects that outcome via root morphological traits. Specific root surface area was higher with heterospecific than with conspecific soils, whereas 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 and mutualistic fungal composition with root diameter, whereas pathogen composition was correlated with root : shoot and RAD. These linkages between soil biota and root traits help explain the negative PSF 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, such as Kaisermann *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 of 21), the magnitude of the negative PSF was higher with soils previously subjected to drought compared to well-watered conditions.

Plant species probably 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 & 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), probably 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
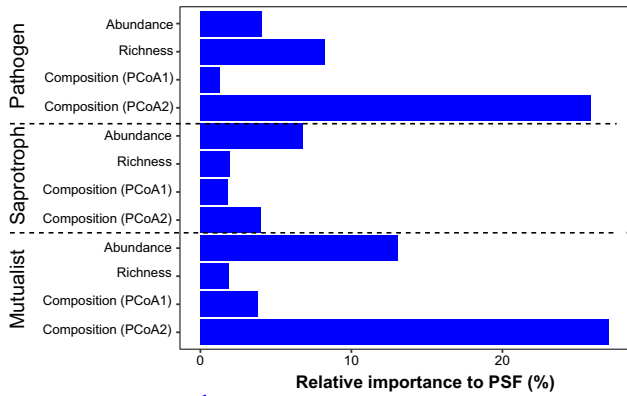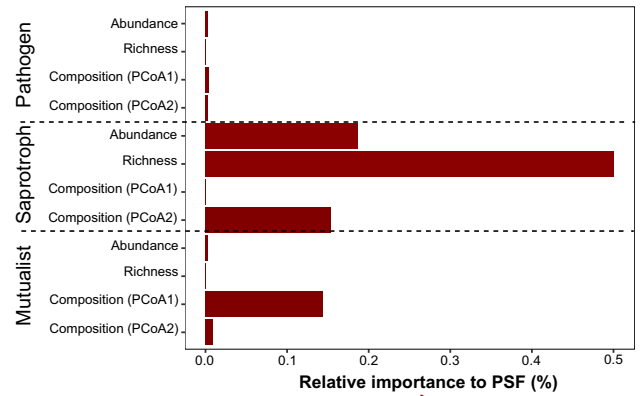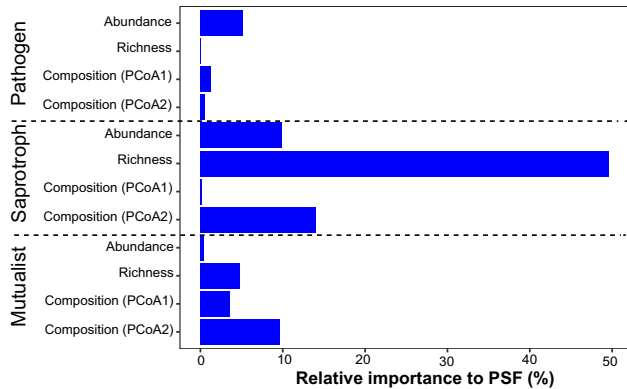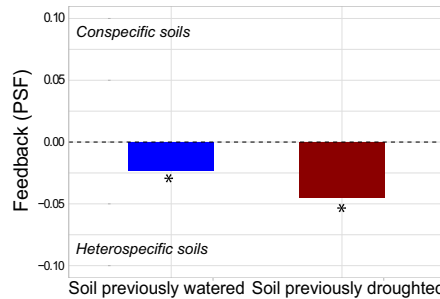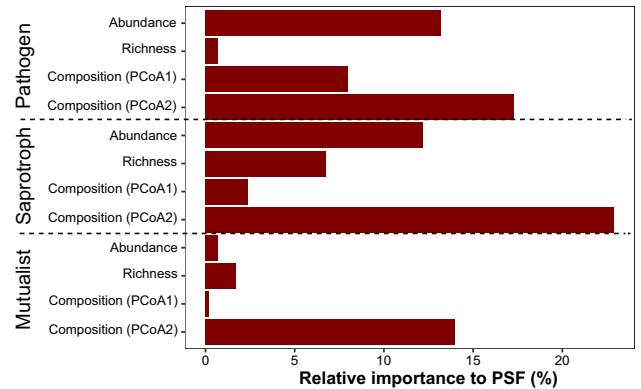
Fig. 3 Mean values of (a) plant–soil feedback (PSF) response to previous drought conditions. Confidence intervals are quite small that cannot be appreciated in the panel. Negative values in panel (a) indicate higher plant biomass in heterospecific than in conspecific soils. Student's t-test revealed a strong difference from zero (*, $P < 0.01$). Four additional panels explain the relative importance of the fungal attributes to PSF 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'.

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 (van der Putten *et al.*, 2016; Bennett *et al.*, 2017), 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 trait expression, even after the drought conditions have disappeared.
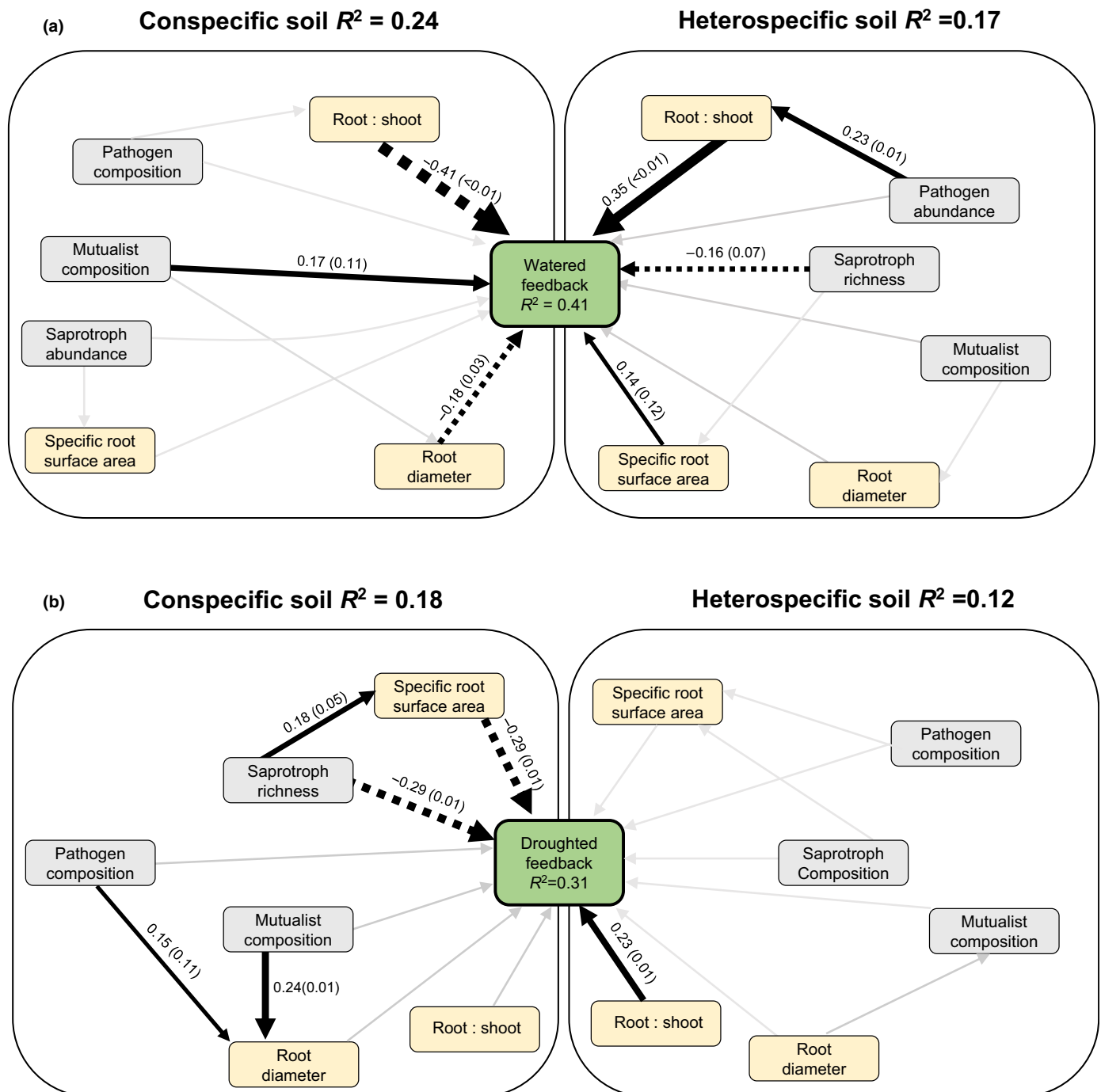
Fig. 4 Path analyses of the relationships between soil fungal communities, root traits, soil properties and (a) watered feedback (plant biomass in the feedback phase with inoculum from soil previously subjected to well-watered conditions) or (b) droughted feedback (plant biomass in the feedback phase 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 do not imply positive correlations but rather a relationship.

From the root economic spectrum perspective, traits positively associated with nutrient uptake capacity, such as high SRSA or SRL, should correlate negatively with root tissue investment (RTD) (Wright *et al.*, 2004; Reich, 2014). 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 as a consequence of 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, approximately 13 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 nonwoody 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 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 (Wahl & Ryser, 2000; Tjoelker *et al.*, 2005; Fort *et al.*, 2013), 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 also would be related to the legacy effect of soil biota in addition to the well-established adjustment to water scarcity (Fort *et al.*, 2013; De Vries *et al.*, 2016; Weemstra *et al.*, 2016; Lozano *et al.*, 2020). Drought can decline mutualist abundance and richness (Ochoa-Hueso *et al.*, 2018; Lozano *et al.*, 2021), explaining 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 RTD than on RAD (as the magnitude of the effect on RAD was around 0.1, whereas 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 & 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 resulting from drought play a key role promoting a negative PSF, via effects on root traits such as 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 as a consequence of 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 interactions of saprotrophs with other fungal groups. That is, the net outcome for 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 PSF. 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 PSF 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), higher-order roots may be preferentially protected from pathogens because the entire downstream branches depend upon them (Wells & Eissenstat, 2003), whereas first-order roots are most likely to encounter pathogen propagules (Emmett *et al.*, 2014). Thus, an increase in RAD in conspecific soils, would be associated with a greater pathogenic infection of first-order roots (finest roots). Likewise, RAD also was 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 resulting from previous drought also contribute to explaining the negative PSF. Our path analyses showed that mutualist composition appears to be a key fungal attribute determining PSF. Mutualistic fungi are known to promote drought resistance (Hartmann *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 (Bever, 1999; Lekberg & Koide, 2014), causing a negative PSF. Mutualists can drive negative PSF, especially in young plants, as the initial C drained to arbuscular mycorrhizal (AM) fungi can be costly for plants with little photosynthetic capacity (Jifon *et al.*, 2002; Schroeder & Janos, 2004), 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) also may arise from phosphorus deficiency which is thought to occur when AM fungi, while transferring phosphate to the root, impair or eliminate direct, root-mediated phosphate uptake (Lekberg & Koide, 2014). This phenomenon has been observed in plants poorly colonized by AM (Smith & 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 exposed frequently 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 in this regard. For example, some studies reported that during a drying/rewetting fluctuation, fungal richness and abundance decrease slightly (Meisner *et al.*, 2018), whereas 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-y-dried soil (Scheu & Parkinson, 1994; Lundquist *et al.*, 1999; Meisner *et al.*, 2013a; but see Gordon *et al.*, 2008, for a contrasting 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 from conspecific soils 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 also may play a role in modulating PSF (Van de Voorde *et al.*, 2012; Pugnaire *et al.*, 2019). 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 PSFs. 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 PSFs.

Our results showed strong linkages between fungal communities and root traits in modulating PSF. 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 in modulating PSF response to drought, via effects on root traits.

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Competing interests

None declared.

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; and YML analyzed the data and wrote the first draft. All authors contributed to the draft and gave final approval for publication.

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Data availability

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

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Plant–soil feedback as reflected by shoot mass, root mass and root : shoot.

Fig. S2 Soil fungal structure from the conditioned phase.

Table S1 Experimental design.

Table S2 Summary of plant–soil feedback responses.

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