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Research

Drought induces shifts in soil fungal communities that can be linked to root traits across 24 plant species

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Summary

- Root traits respond to drought in a species-specific manner, but little is known about how soil fungal communities and root traits respond to drought in concert.
- In a glasshouse experiment, we determined the response of soil pathogens, saprotrophs, and mutualistic and all fungi associated with the roots of 24 plant species subjected to drought. At harvest, soil fungal communities were characterized by sequencing. Data on root traits were extracted from a previously published work.
- Differences in fungal beta diversity between drought and control were plant species specific. For some species, saprotrophic fungi increased in relative abundance and richness with drought, whereas mutualistic fungi showed the opposite pattern. Community structure of pathogenic fungi was plant species specific but was slightly affected by drought.
- Pathogen composition was correlated with specific root surface area and root : shoot, saprotroph abundance with root tissue density, whereas mutualist composition was correlated with root : shoot. All these were the fungal attributes that best predicted shoot mass.
- Fungal response to drought depended highly on the fungal group and was related to root trait adjustments to water scarcity. This provides new insights into the role that root trait adjustments to drought may have in modulating plant–fungus interactions in grasslands ecosystems.

Introduction

Drought events are predicted to increase over the next few decades (Dai, 2013; Spinoni *et al.*, 2016; Hari *et al.*, 2020). Specifically, the European mid-latitudes are predicted to experience more frequent events of droughts in the near future (up to 2.5 more events per decade) (Spinoni *et al.*, 2016), and a sevenfold increase in the occurrence of consecutive droughts (Hari *et al.*, 2020), which likely may affect the structure of plant and soil microbial communities, their interactions, and the ecosystem functions associated with them (Carrão *et al.*, 2016; de Vries *et al.*, 2018). Given the tight relationship between plants and soil biota, it is necessary to understand how the feedback mechanisms between them are modified under drought conditions.

On the plant side, the drought-driven effects at the plant–soil interface are partly determined by species-specific trait responses to drought, particularly in root traits. These responses are linked to morphological adjustments or to changes in resource allocation patterns (Garrett *et al.*, 2006; Schimel *et al.*, 2007; Zufferey *et al.*, 2011; Mao *et al.*, 2018). For example, Lozano *et al.* (2020) showed that leaf trait response to drought is similar across plant species but that root trait responses strongly vary among plant species. Some species produce thinner roots with high specific root length (SRL) and specific root surface area (SRSA), which may improve soil moisture acquisition with a low plant investment (Debinski *et al.*, 2010; Comas *et al.*, 2013). By contrast, other species produce thick roots that may diminish the risk of hydraulic rupture (Zimmermann, 1983; Zufferey *et al.*, 2011) and are more prone to rely on mycotrophy (Brundrett, 2002; Comas *et al.*, 2012). In addition, changes in soil properties due to species-specific rhizodeposition patterns (Bardgett *et al.*, 2014; Williams & de Vries, 2020) would affect how plants influence soil biota during drought events (Fitzpatrick *et al.*, 2018; Ochoa-Hueso *et al.*, 2018).

On the soil biota side, by contrast, we have a rather limited understanding of how rhizosphere soil microorganisms change in concert with these species-specific root changes. This scarcity of data is likely in part due to the current paradigm that entire kingdoms of soil microorganisms would respond in a similar manner to drought. In particular, Fungi, as a whole, are expected to be inherently drought resistant (as opposed to Bacteria), remaining relatively more active under drought (Schimel *et al.*, 2007). This paradigm stems from studies that show that fungal communities and their networks are more stable under drought (de Vries *et al.*, 2018) and that fungi have several physiological and morphological features expected to be useful to avoid dehydration. For example, cells walls (Zhu, 2016) and synthesis of osmolytes (like glycerol) that increase water potential in the fungal cytoplasm (Davis *et al.*, 2000), reduced plasma membrane permeability (Dupont *et al.*, 2012), and filamentous growth that facilitates

utilization and redistribution of spatially heterogeneous water resources in soil (Jennings, 1987).

We expect that the response of the soil fungal community to drought may be plant species specific. We think this is the case because the large species diversity of soil fungi associated with roots is unlikely to respond homogeneously to the plant-species-specific root trait responses driven by drought (de Vries *et al.*, 2016; Lozano *et al.*, 2020). For example, drought usually reduces plant carbon (C) assimilation affecting the C exchange with fungal organisms (Rowland *et al.*, 2021) whose species specificity may have consequences for fungal composition. Besides, several studies show contrasting trends in fungal responses to drought: some found increases in fungal abundance (e.g. Allison *et al.*, 2013), whereas others have found the opposite pattern (e.g. Glomeromycota; Ochoa-Hueso *et al.*, 2018). These results show the average response of fungal communities to drought, without disentangling the specific response that each plant species and its associated fungal community may have.

Here, we focus on a 'fungal functional group' approach as a proxy to measure differential drought responses within fungal communities. We used this approach, rather than a taxonomic one, for two reasons. First, functional grouping allows us to find overall patterns among fungal species sharing a similar ecology (in contrast to tracking species-by-species differences). Second, it allows us to infer the potential feedback that changes in fungal community structure due to drought will have on plant performance. Although functional groups in fungi vary depending on the criteria (Treseder & Lennon, 2015), an informative grouping for soil fungi is one reflecting the degree of dependency on a plant host (Aguilar-Trigueros *et al.*, 2014; Lutzoni *et al.*, 2018). At one extreme are strict symbionts that can only use resources (such as C) from a living host (exemplified by arbuscular mycorrhizal fungi (AMFs)). At the other extreme are free-living saprotrophic fungi that can obtain resources from soil organic matter and cannot (or have limited abilities to) colonize living root tissue. In between these two extremes, there are fungi that alternate between symbiotic and free-living phases during their life cycle that can either have positive or neutral effects on plant performance (root endophytes) or that can harm the host (root pathogens) (Zanne *et al.*, 2020). Therefore, one might expect that strict symbionts with a close relationship to plant roots may be more affected by the adjustments of root traits to drought than free-living saprotrophs would be.

In particular, we expect that soil fungal groups and morphological root traits respond to drought in concert. For instance, drought may contribute to plant susceptibility to pathogens (Mayek-Pérez *et al.*, 2002), but it may also induce general defense pathways that increase resistance (Garrett *et al.*, 2006). Species with thin roots seem to have lower dependence on AMFs for water uptake (Hetrick *et al.*, 1990; Lin *et al.*, 2015), as thin roots can be efficient in water acquisition. However, fine roots likely interact intensively with saprotrophs by releasing easily degradable carbohydrates, which prime saprotrophic activity in the rhizosphere (Kuzyakov *et al.*, 2000). By contrast, species with thicker roots and low SRL often rely on mutualists to explore soil and acquire nutrients (Brundrett, 2002; Lin *et al.*, 2015). Likewise, plant trait relationships with fungal communities would be influenced by interactions with AMFs (Sweeney *et al.*, 2021), and plant species with thin roots would attract a diverse community of saprotrophs (Semchenko *et al.*, 2018), suggesting specific relationships between fungal groups and root traits.

Thus, in this study, we measured the response of fungal communities and their functional groups associated with the roots of 24 plant species (including grasses, forbs, and legumes) subjected to drought. To do this, we sequenced soil fungi associated with these roots and assigned functional group membership using online databases. We then tested: (1) whether fungal community resistance to drought depends on plant species identity; (2) how abundance, richness, and composition of pathogenic, saprotrophic, and mutualistic fungi respond to drought; and (3) whether the known adjustment in root traits to drought (Lozano *et al.*, 2020) is correlated with plant–fungus interactions in grasslands ecosystems.

Materials and Methods

Species selection

We selected 24 plant species: eight grasses (*Arrhenatherum elatius*, *Festuca brevipila*, *Holcus lanatus*, *Poa angustifolia*, *Anthoxanthum odoratum*, *Lolium perenne*, *Festuca rubra*, *Dactylis glomerata*), 13 forbs (*Achillea millefolium*, *Armeria maritima* ssp. *elongata*, *Artemisia* ssp. *campestris*, *Berteroa incana*, *Daucus carota*, *Galium verum*, *Hieracium pilosella*, *Hypericum perforatum*, *Plantago lanceolata*, *Potentilla argentea*, *Ranunculus acris*, *Rumex thyrsiflorus*, *Silene vulgaris*), and three legumes (*Trifolium repens*, *Vicia cracca*, *Medicago lupulina*). All these species are common, frequently co-occurring grassland species in central Europe. Seeds of these plant species were obtained from commercial suppliers in the region (Rieger-Hofmann GmbH, Blaufelden, Germany).

Experimental design

In September 2016, we collected sandy loam soil (nitrogen (N) 0.07%, C 0.77%, pH 6.66) from Dedelow, Brandenburg, Germany (53°37′N, 13°77′W) where these plant species naturally grow. Soil was sieved (4 mm) and homogenized to use as substrate in the experiment that was established in a climate-controlled glasshouse – see specific details of the setup in Lozano *et al.* (2020). Briefly, seeds were surface sterilized and transplanted after the third day of germination, into deep pots (11 cm diameter, 30 cm height) filled with 3 l of soil. One individual seedling per plant species was planted into the center of each pot (for a total of 10 replicate pots per plant species). Thus, the experimental design included 24 plant species × 2 water treatments × 5 replicates = 240 pots.

The experiment lasted for 3 months. All plants were well watered during the first month of growth. Then, half of the pots (i.e. five replicates of each plant species) were kept under drought (i.e. 30% water holding capacity (WHC)) while the other half were well watered and kept as control at 70% WHC for the remaining 2 months. Previous research found that these grasslands species experience water scarcity at 30% of soil WHC (de Vries *et al.*,

2016; Lozano *et al.*, 2020; see also Ahmed *et al.*, 2018). Soil moisture content was adjusted gravimetrically to keep pots at their respective WHC. Pots were randomly distributed in the glasshouse, and their position was shifted twice to homogenize environmental conditions during the experiment. At harvest, rhizosphere soil was sampled and kept at −80°C for molecular analyses.

Molecular analyses of fungal communities

DNA was extracted from 0.25 g of homogenized soil from each of the 240 soil samples using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's directions. Fungal sequences were amplified from soil DNA extracts using the fITS7 and ITS4 primers, which in combination yield amplicons that span the fungal ITS2 region (Ihrmark *et al.*, 2012). These are general primers for fungi; thus, they do not amplify any specific fungal group. We added 1 µl DNA to 25 µl reaction mixture (7 µl buffer (10 mM magnesium chloride), 1 ml deoxynucleoside triphosphate (10 mM), 1 µl fITS7 (10 µM), 0.5 µl ITS4 (10 µM), and 0.5 µl DNA polymerase (1 U μl^{-1}) (Kapa Biosystems Wilmington, MA, USA). PCR consisted of initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 98°C for 20 s, annealing at 55°C for 30 s and elongation at 72°C for 30 s, and then final elongation at 72°C for 5 min. PCR products were purified using magnetic beads (CleanNA; GC Biotech B.V., Waddinxveen, the Netherlands) following the manufacturer's directions. Then, purified products were indexed for multiplexing following the same protocol as before, but using 12 cycles. DNA concentration of purified amplicons was measured using Quant-IT PicoGreen dsDNA Reagent (Invitrogen) to ensure an equimolar pooling. Afterwards, amplicons were sequenced on an Illumina MiSeq instrument using v.3 2 × 300 bp cycles chemistry at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv).

Plant traits

Data on shoot and root mass, root diameter, SRL, SRSA, root tissue density (RTD), root N, and root C were taken from Lozano *et al.* (2020).

Microbial sequencing data

Exact sequence variant (ESV; also known as amplicon sequence variants and zero-radius operational taxonomic units) counts were determined from raw sequence data using the DADA2 pipeline (Callahan *et al.*, 2016). *Festuca brevipila*, *L. perenne*, *R. acris*, and *S. vulgaris* had four replicates in the control treatment. The rest of the plant species and treatments had five replicates. Singletons were excluded from the data set. Most of the microbial data were rarefied to 8025 sequences per soil sample. Only 28 out of the 236 samples had less than 8025 sequences: 24 of them had an average of 5983 sequences within a range between 1749 and 7999 sequences, and four samples had an average of 222 ± 37 sequences. The low read count of some samples should not be a concern, as these samples were randomly distributed in the experiment and the number of retained sequences per sample did not show differences among plant species or water treatment after a linear model (see rarefaction curves in Supporting Information Fig. S1). Taxonomic affiliation to the sequences was determined using the naive Bayesian classifier (Wang *et al.*, 2007) against UNITE 8.3 (Nilsson *et al.*, 2018). We kept sequences that were taxonomically assigned at least to at phylum level.

Functional group assignment

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). Sequences were assigned to each of the three fungal groups if they exclusively belonged to that group. That is, fungal sequences that were assigned to multiple functional groups (e.g. pathogenic–saprotrophic) were not included in any of the three fungal groups. However, we included pathogen genera that are reported in FUNGUILD in combination with the endophytic guild (i.e. pathogenic–endophytic) because almost all root pathogens described to date initiate infection through an endophytic phase (Aguilar-Trigueros *et al.*, 2014). We further cross-checked these pathogenic organisms against the Fungus-Host distribution database of the US Department of Agriculture (https://nt.arsgrin.gov/fungaldatabases/fungushost/fungushost.cfm) and *Wescott's Plant Disease Handbook* (Horst, 2013) to ensure to include pathogens that are associated with an established disease (Table S1). However, we acknowledge that there will always be a level of uncertainty with the categorization of ESVs as pathogens because disease development is extremely context dependent (Agrios, 1997). By comparison, the other two fungal functional groups (saprotrophic and mutualistic fungi) were more easily identified. Saprotrophic fungi are simply those that have only been reported as free-living or in combination with an endophytic guild, whereas mutualistic fungi were those reported as arbuscular mycorrhizal (obligate symbionts) or in exclusively endophytic guilds (i.e. endophytic fungi that have not been reported as pathogenic or saprotrophic). Sequences reported as ectomycorrhizal fungi were not included in this classification as they differ greatly from AMFs and could have contrasting responses to drought. Through this approach, we obtained functional information for 35% of the ESV. That is, pathogens (4%), saprotrophs (22%), and mutualists (9%). In addition to the ESVs assigned to the three fungal groups, the total fungal community included those ESVs assigned to multiple functions (e.g. pathogenic–saprotrophic) and all the ESVs that were not assigned to any fungal group by FUNGUILD but that were classified at least at the phylum level. The low percentage of ESVs assigned to these fungal functional groups, which is common in these types of studies, is evidence of a pressing need to improve FUNGUILD content and/or similar database information.

Statistical analyses

The effects of plant species identity and drought treatment on fungal community structure were evaluated for the total fungal

community and for each functional group (pathogens, saprotrophs, mutualists) by two-way permutational multivariate ANOVA (PERMANOVA) with Bray–Curtis dissimilarity index and 999 permutations using the function 'adonis' from the VEGAN R package (Oksanen *et al.*, 2019). We also evaluated the effect of plant functional group and drought using plant species as random factor. The function 'pairwise.adonis' was used to establish the *P*-values of the pairwise comparisons. Dissimilarity (i.e. beta-diversity) among fungal communities with respect to plant species and drought was visualized with principal-coordinate analyses (PCoAs) using the Bray–Curtis dissimilarity index. We performed a PCoA including all the ESVs (beta-diversity: total fungal community) and an additional one including only the ESVs assigned to a fungal group (beta-diversity: fungal functional groups). In further analysis, we used the first two axes of each of these two PCoAs as variables that represent fungal composition. In addition, given the high number of data points, we also visualized the effect of drought on fungal community structure in separate PCoAs (one for total fungal community and the other for fungal functional groups) for each plant species. To indicate whether the effect of drought was strong enough to separate fungal community structure, we indicated in each PCoA the results on independent PERMANOVA tests per plant species. We tested whether the effects of drought on fungal communities in terms of relative abundance, richness (number of ESVs assigned to each group), and composition (PCoA axes as described herein) depended on plant species identity and on plant functional group. Each fungal community attribute was analyzed separately. Plant species or plant functional group, drought, and their combined effect were used as fixed factors. When plant functional group was the fixed factor, plant species was established as a random factor. Fungal abundance (i.e. relative abundance) was calculated based on the total number of reads within each sample divided by the total number of sequences that were taxonomically assigned at least at phylum level. The relative abundances of pathogens, saprotrophs, and mutualists were log-transformed, and along with richness were analyzed with linear models using the function 'gls' from the NLME R package (Pinheiro, 2018). Model residuals were checked for homoscedasticity, normal distribution, and, when necessary, corrected using the function 'varIdent' from the same R package. In addition, we performed multiple comparisons to assess whether fungal attributes (i.e. richness and abundance of pathogens, saprotrophs, and mutualists, and total richness) differed within each plant species due to drought. For that, we used the function 'glht' from the MULTCOMP R package, along with the Tukey test and the function 'sandwich' from the eponymous R package. This last function provided a heteroscedasticity-consistent estimate of the covariance matrix (Zeilais, 2006; Bretz *et al.*, 2011).

We also evaluated the importance of plant species and drought for explaining the variation in fungal composition, through variance partitioning using the function 'varpart' from the VEGAN R package. Plant species and drought were tested with the 'anova.cca' function, as they were expressed as a redundancy analysis model (Oksanen *et al.*, 2019).

We assessed the relative importance of each fungal attribute to shoot mass and of each root trait to fungal abundance, richness, and composition by using the metric 'pmvd' from RELAIMPO R package (Grömping, 2006). This metric is based on sequential R^2 values but takes care of the dependence on orderings by weighted averages with data-dependent weights and also guarantees that a regressor with a coefficient of zero is assigned a relative importance of zero (Grömping, 2006).

Finally, we performed a path analysis in order to understand the links between root traits and fungal community attributes (abundance, richness, and composition), and how they may predict plant performance (i.e. shoot mass). For that, we assumed a chain reaction where root trait adjustments to drought may modify fungal communities, which in turn may affect shoot mass. Nonetheless, we acknowledge that soil fungal communities and root traits respond to drought in concert and that continuous feedback processes occur among them. For this analysis, the best predictors of shoot mass were selected based on the Akaike information criterion (AIC) by using the 'stepAIC' function from the MASS R package (Venables & Ripley, 2002), from three sets of variables: abundance, richness, and composition of (1) pathogens; (2) saprotrophs; and (3) mutualists. The selected predictors were retained for use in the path analysis. Then, by using the same method, we selected the root traits that best predicted those fungal attributes (abundance, richness, or composition). By comparing the AIC, we selected the most parsimonious model. 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 (< 0.1) (Pugesek *et al.*, 2003; Grace, 2006), and high CFI (> 0.95, Byrne, 1994). Similarly, by using the 'stepAIC' function, we selected for each plant species the fungal attributes that predicted shoot mass and the root traits that predicted those fungal attributes. 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

Total fungal community

A total of 2113 fungal ESVs were identified at least to the phylum level. The effect of drought on total fungal richness and community structure depended strongly on plant species identity (PERMANOVA and PCoA; Table 1). For example, fungal richness associated with plant species including *B. incana*, *A. millefolium*, *H. lanatus*, or *H. perforatum* decreased with drought, whereas *P. angustifolia*, *H. pilosella*, *A. elatius*, or *P. lanceolata* had the opposite pattern (Fig. 1). Similarly, the fungal communities associated with *B. incana*, *H. lanatus*, and *H. perforatum* along with *T. repens*, *P. argentea*, and *D. glomerata* had the strongest shift in community structure in response to drought (i.e. based on total fungal community, these plant species had the largest dissimilarities between drought and control treatment; Figs 2a, S2a). Plant species also differed in terms of fungi belonging to the different functional groups (i.e. pathogens, saprotrophs, and mutualists) in response to drought. This can be visualized in the

Table 1 Plant species and drought effects on fungal attributes (richness, composition, and relative abundance) of the total community, pathogens, saprotrophs, and mutualists.

Fungal group	Attribute	Plant species (Ps)	Drought (D)	Ps × D
Total community	Richness	**28.37 (< 0.01)**	0.05 (0.82)	**2.82 (< 0.01)**
	Structure (PERMANOVa)	**3.68 (< 0.01)**	1.31 (0.16)	**1.13 (0.04)**
	Composition (PCoA1)	**10.61 (< 0.01)**	0.23 (0.62)	0.90 (0.59)
	Composition (PCoA2)	**10.99 (< 0.01)**	**16.84 (< 0.01)**	**2.49 (< 0.01)**
Pathogens	Richness	**8.14 (< 0.01)**	0.09 (0.76)	1.32 (0.1)
	Structure (PERMANOVA)	**2.49 (< 0.01)**	0.72 (0.67)	0.91 (0.76)
	Composition (PCoA1)	**4.61 (< 0.01)**	0.04 (0.83)	0.77 (0.76)
	Composition (PCoA2)	**4.65 (< 0.01)**	***3.19 (0.07)***	1.23 (0.21)
	Abundance	***1.47 (0.08)***	0.42 (0.52)	0.97 (0.50)
Saprotrophs	Richness	**27.40 (< 0.01)**	0.98 (0.32)	**2.46 (< 0.01)**
	Structure (PERMANOVA)	**3.59 (< 0.01)**	0.91 (0.49)	1.09 (0.17)
	Composition (PCoA1)	**4.92 (< 0.01)**	0.33 (0.56)	1.05 (0.40)
	Composition (PCoA2)	**1.79 (< 0.01)**	***3.22 (0.07)***	1.39 (0.11)
	Abundance	**13.47 (< 0.01)**	**10.14 (0.01)**	**1.62 (0.04)**
Mutualists	Richness	**3.46 (< 0.01)**	0.11 (0.74)	***1.31 (0.10)***
	Structure (PERMANOVA)	**2.98 (< 0.01)**	0.95 (0.44)	**1.28 (< 0.01)**
	Composition (PCoA1)	**5.52 (< 0.01)**	**22.51 (< 0.01)**	**3.61 (< 0.01)**
	Composition (PCoA2)	**3.07 (< 0.01)**	0.07 (0.79)	**1.61 (0.04)**
	Abundance	**7.98 (< 0.01)**	***3.38 (0.06)***	***1.53 (0.06)***

Plant species (Ps), drought (D) and their interactions were considered as fixed factors. Composition was analyzed through permutational multivariate ANOVA (PERMANOVA) and by linear models using the first two principal coordinate axes (PCoAs) as representative of the fungal composition. *F* and *P* values (in parentheses) are shown. $P < 0.05$ in bold; $P < 0.1$ in bold italic.

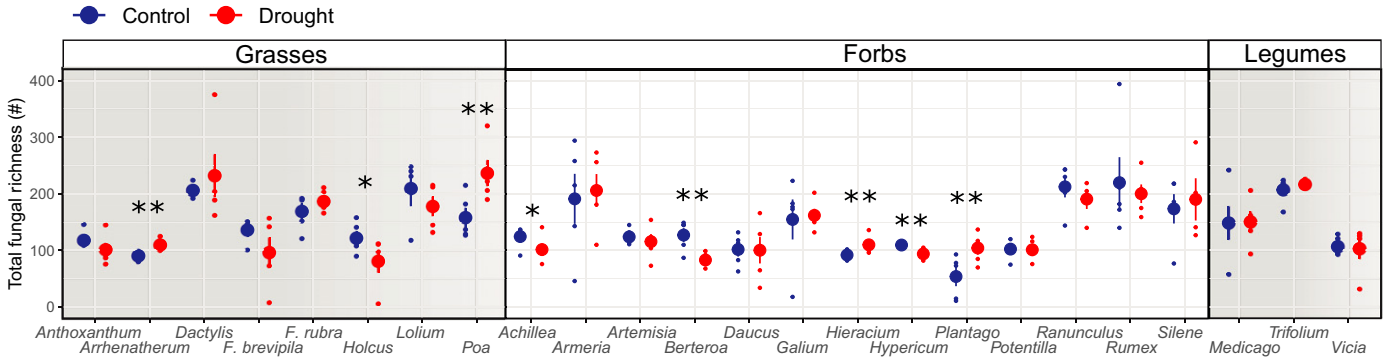

Fig. 1 Total fungal richness associated with different plant species growing under well-watered (control) and drought conditions. Data are represented by mean and SE. Data points are shown as circles. $n = 5$ (*, $P < 0.1$; **, $P < 0.05$). Plant species are referred to by their genus name (except for the two *Festuca* species to which we refer as *F. brevipila* and *F. rubra*). See full names in the Materials and Methods, subsection Species selection.

ordination along the PCoA axes (Fig. 2b), where again functional groups associated with *B. incana* as well as associated with *P. lanceolata* and *T. repens* shifted the most in response to drought (Figs 2b, S2b). Overall, our results showed that pathogens were slightly affected by drought, saprotrophs increased with drought, and fungi with mutualistic attributes decreased with drought (Fig. 3).

Pathogens

Pathogens were represented by 86 ESVs. Of those ESVs, 78 were taxonomically identified to genus level, comprising 35 genera, and 40 of these ESVs were identified to species level, comprising 24 species); the remaining eight ESVs were identified only to family level. The cross-checking of these pathogens against plant pathology databases revealed that most of them are reported to be soil-borne ascomycete fungi causing diseases that induce cell death (necrosis) of plant tissue or the entire plant (including the most common species *Gibberella tricintata* reported to cause rots, lesions, or wilts on plants). We also detected biotrophic pathogens (pathogens that do not cause plant death but instead use resources of living cells) causing aboveground diseases (e.g. in leaves or fruits), such as smuts (*Urocystis agropyri*; Basidiomycota). Although these fungi are well-known pathogens, it is known that they have a saprotrophic phase (McLaughlin & Spatafora, 2014). Finally, we also captured common chytrid fungi (e.g. *Powellomyces hirtus*), which are reported to be associated with roots of different hosts. Pathogen richness changed

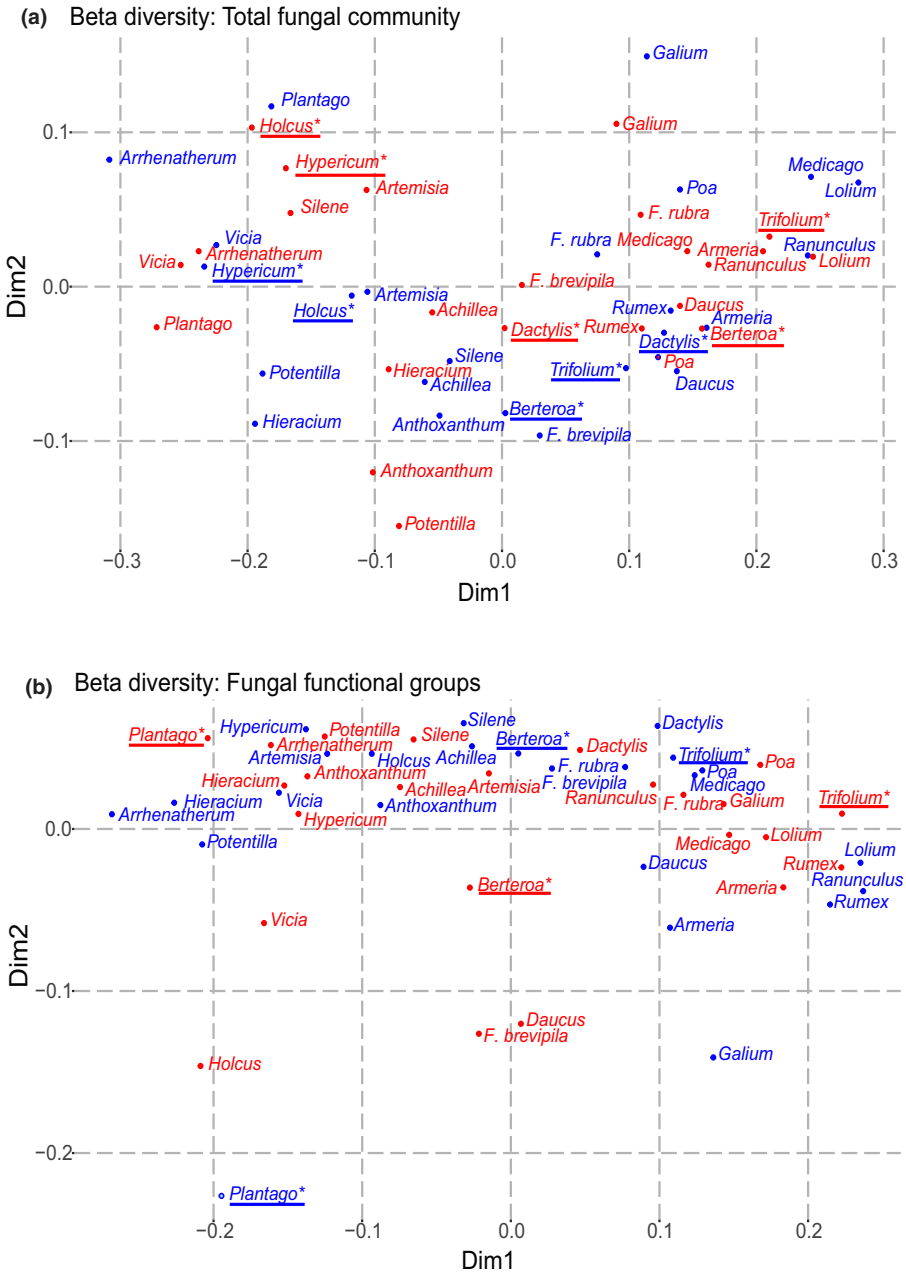

Fig. 2 Community structure dissimilarity (beta-diversity) of (a) total fungal communities and (b) fungal functional groups (b) using Bray–Curtis dissimilarity metric. Principal coordinate analyses (PCoAs) show centroids of plant species under control and drought conditions. Plant species differing in their fungal community due to drought are underlined (*, $P < 0.05$). Plant species are referred to by their genus name (except for the two *Festuca* species, to which we refer as *F. brevipila* and *F. rubra*). See full names in the Materials and Methods, subsection Species selection.

across plant species ($P < 0.01$; Table 1), where *T. repens*, *S. vulgaris*, and *R. thyrsiflorus* had the highest pathogen richness (*c.* 12 ESVs) and *P. lanceolata* and *D. carota* had the lowest (*c.* 5 ESVs). Likewise, multiple comparisons showed that *P. angustifolia*, *A. elatius*, and *P. lanceolata* had increased pathogen richness under drought, whereas *B. incana* and *A. millefolium* had increased pathogen richness under control conditions (Fig. 4a; Table S2). Similarly, pathogen composition differed between plant species ($P < 0.01$) and was affected by drought (PCo2, $P = 0.07$; Table 1). Pathogen abundance differed among plant species ($P = 0.08$) but did not differ as a function of drought (Table 1). Here, *P. angustifolia*, *F. rubra*, and *V. cracca* had the highest values of pathogen abundance (*c.* 6%), and *P. lanceolata* had the lowest (*c.* 4%; Fig. 4a). Regarding plant functional group, we found that grasses and forbs differed in the structure of fungal pathogens (Table S3). Plant functional group showed no effect on the other pathogens' attributes and on any of the attributes of the other fungal groups or the total fungal community.

Saprotrophs

Saprotrophs were represented by 470 ESVs (278 ESVs were identified to the genus level, comprising 68 genera, of which 129 were identified to species level, comprising 65 species; 173 ESVs were identified to family level and the remaining 19 ESVs to order level); the most common species were *Mortierella minutissima*, *Mortierella sarnyensis*, *Trichocladium opacum*, and *Preussia flanaganii*. Saprotroph richness changed due to the combined effect of drought and plant species identity ($P < 0.01$; Table 1). Richness increased with drought for some species (i.e. *P. angustifolia*,

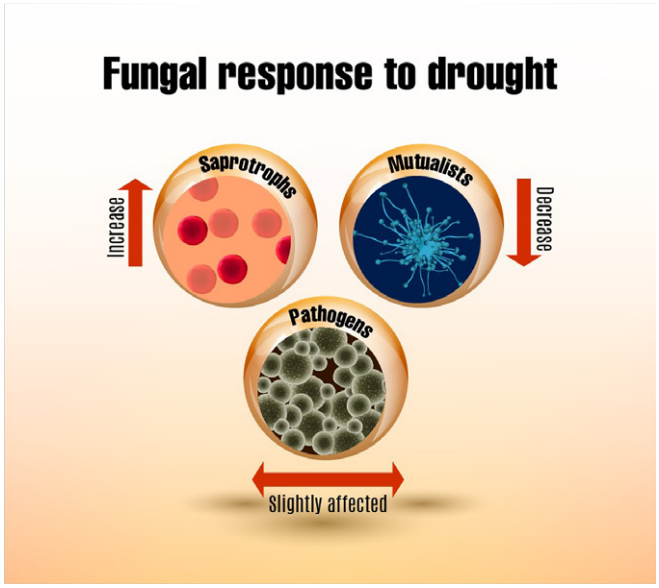

Fig. 3 Overall fungal response to drought. Saprotrophs increased with drought, whereas mutualists showed a contrary pattern. Pathogens were slightly affected by drought.

H. pilosella, A. elatius, or *P. lanceolata*) and decreased for others (i.e. *B. incana*, *H. lanatus*, or *H. perforatum*; Fig. 4b). Saprotroph composition differed between plant species ($P < 0.01$), whereas relative abundance of saprotrophs changed with plant species identity and drought ($P = 0.04$; Table 1). Saprotroph abundance increased with drought for *T. repens*, *P. argentea*, *H. pilosella*, and *P. lanceolata* and decreased for *L. perenne* and *H. lanatus* (Fig. 4b).

Mutualists

Mutualists were represented by 182 ESVs. Of those ESVs, 139 were assigned to AMFs (phylum Glomeromycota). The remaining 43 ESVs correspond to root endophytic fungi in the Ascomycota and Basidiomycota (including seven ESVs belonging to the Sebacinales, a common group of root endophytes in grasslands with known positive effects on plant fitness; Weiß *et al.*, 2016). Richness depended on plant species ($P < 0.01$) and was marginally affected by the combined effect with drought ($P = 0.1$; Table 1). For some plant species, mutualist richness declined with drought (*F. brevipila*, *B. incana*, *H. lanatus*, *H. perforatum*; Fig. 5a), whereas the opposite pattern was found for *T. repens*, *A. millefolium*, *H. pilosella*, and *P. lanceolata*. Similarly, composition and relative abundance of mutualists differed between plant species and was affected by drought ($P < 0.01$; Table 1). Abundance of mutualists decreased with drought for *A. maritima, Festuca rubra*, *B. incana*, and *H.pilosella*, while increasing for *G. verum* and *A. elatius* (Fig. 4c).

Plant species identity and root traits strongly correlate with fungal communities

Plant species identity was a stronger predictor than drought in explaining the variation in fungal community structure (Fig. S3). That is, plant species identity alone explained a considerable amount of that variation ($R^2 = 0.41$, $P < 0.01$); by contrast, drought alone did not explain the variation in fungal community structure to a great extent ($R^2 = 0.001$, $P = 0.63$). Our results show that pathogen and mutualist composition, along with saprotroph abundance, were the fungal attributes that better predicted shoot mass (Fig. S4). Likewise, in terms of root traits, fungal attributes were best explained as follows: pathogen composition was most explained by SRSA (29.78%) and root : shoot (22.68%), saprotroph abundance was most explained by RTD (46.58%), and mutualist composition was better explained by root : shoot (84.29%; Fig. 5). In addition, we found that the contribution of each fungal attribute to explain variation in shoot mass depended on species, as well as the root traits associated with these fungal attributes (Table S4). For instance, *A. odoratum* was related to pathogen abundance, saprotroph abundance, and mutualist composition, fungal attributes that, in turn, were related to root diameter, RTD, and SRSA, respectively, whereas *A. millefolium* was related to pathogen and saprotroph richness and to mutualist abundance, all of them in turn being related to root : shoot.

We found that pathogen, saprotroph, and mutualist composition were the attributes that best explained variation in shoot mass of grasses (when considering functional group as an explanatory variable). These attributes were in turn related to SRL, diameter, and SRSA, respectively (Table S4). Pathogen, saprotroph, and mutualist composition were also the attributes that best explained forb biomass, although the root traits related to these attributes were SRSA, RTD, and SRL, respectively. By contrast, shoot biomass variation of legumes was explained by pathogen richness, saprotroph richness, and mutualist abundance, which in turn were related to root : shoot, SRSA, and SRL, respectively (Table S4).

Root traits and fungal communities as predictors of plant performance

The path analyses that include root traits, fungal attributes, and shoot mass showed that, among fungal attributes, those that had a higher influence on shoot mass, a proxy of plant performance, were pathogen composition ($\beta = 0.18$, $P < 0.01$), mutualist composition ($\beta = 0.12$, $P = 0.06$), and saprotroph abundance ($\beta = -0.12$, $P = 0.05$), which in turn were affected by different root traits (Fig. 6). In particular, pathogen composition was linked to SRSA ($\beta = 0.14$, $P = 0.02$) and with root : shoot ($\beta = 0.09$, $P = 0.1$), whereas mutualist composition was mainly linked to root : shoot ($\beta = 0.23$, $P = <0.01$). Saprotroph abundance was linked to RTD ($\beta = 0.18$, $P < 0.01$).

Discussion

Though soil fungal communities of the 24 different grassland species examined were affected by drought, the exact magnitude and direction of these effects depended, as expected, on the fungal functional group and plant species identity. For example, saprotrophic and mutualistic fungi increased or decreased in

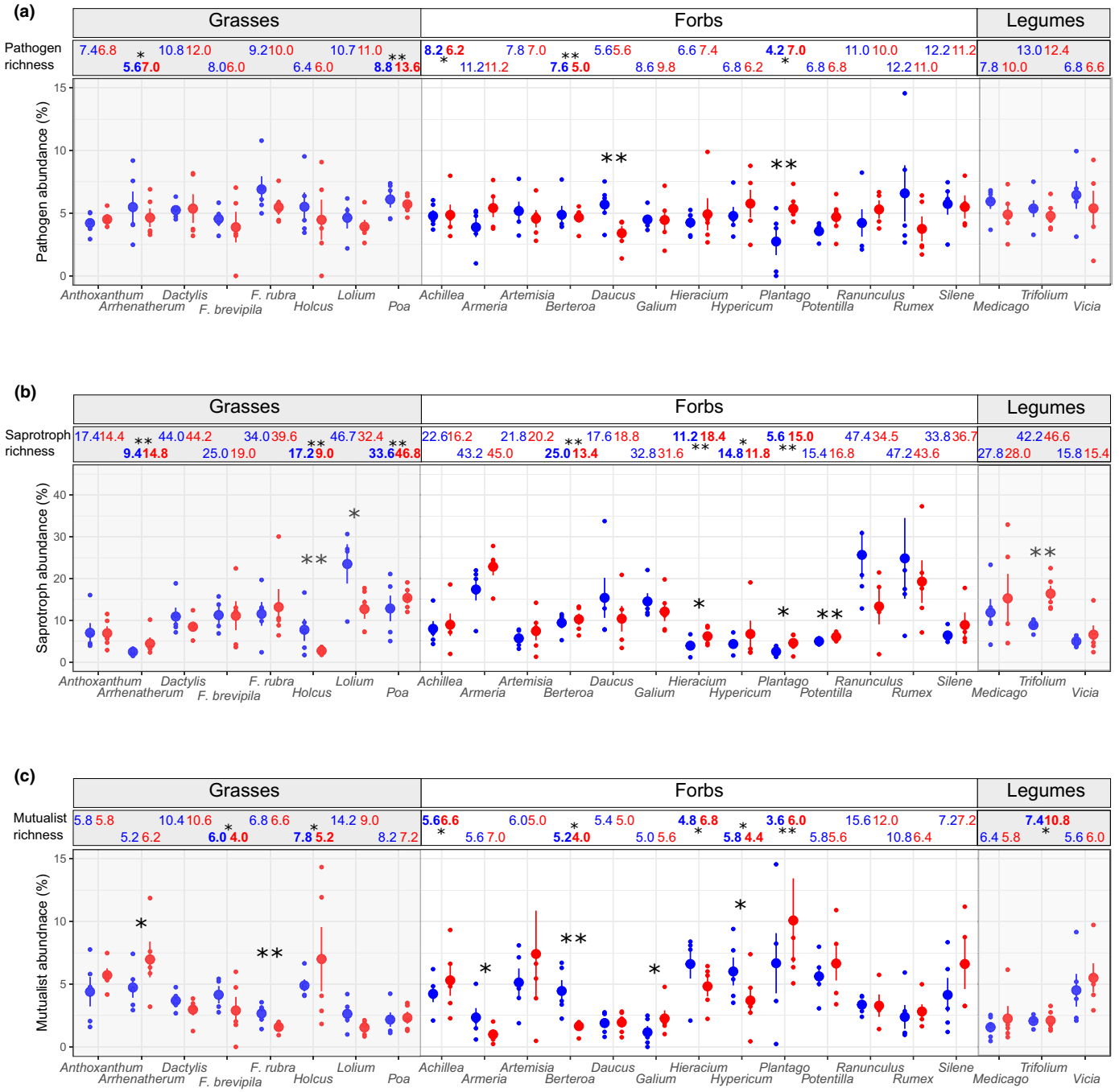

Fig. 4 Richness and relative abundance (percentage calculated based on the total number of sequences) of (a) pathogens, (b) saprotrophs, and (c) mutualists associated with different plant species growing under well-watered (control) and drought conditions. Relative abundance is represented by mean and SE. Data points are shown as circles. $n = 5$. (*, $P < 0.1$; **, $P < 0.05$). Plant species are referred to by their genus name (except for the two *Festuca* species, to which we refer as *F. brevipila* and *F. rubra*). See full names in the Materials and Methods, subsection Species selection.

abundance and richness under drought conditions depending on plant species identity, whereas community structure of pathogenic fungi was less affected by drought. We found that fungal responses were linked with the morphological adjustments of root traits to drought, especially with root : shoot, SRSA, and RTD. Note that the direction of these effects cannot be disentangled in our experiment as the fungal communities and root traits respond to drought in concert. However, our results suggest that root trait adjustment to drought may affect plant performance through its effects on fungal communities.

Resistance of fungal communities to drought depended on plant species identity

Drought affected soil fungal richness and composition, in agreement with previous studies (Meisner *et al.*, 2018; Ochoa-Hueso

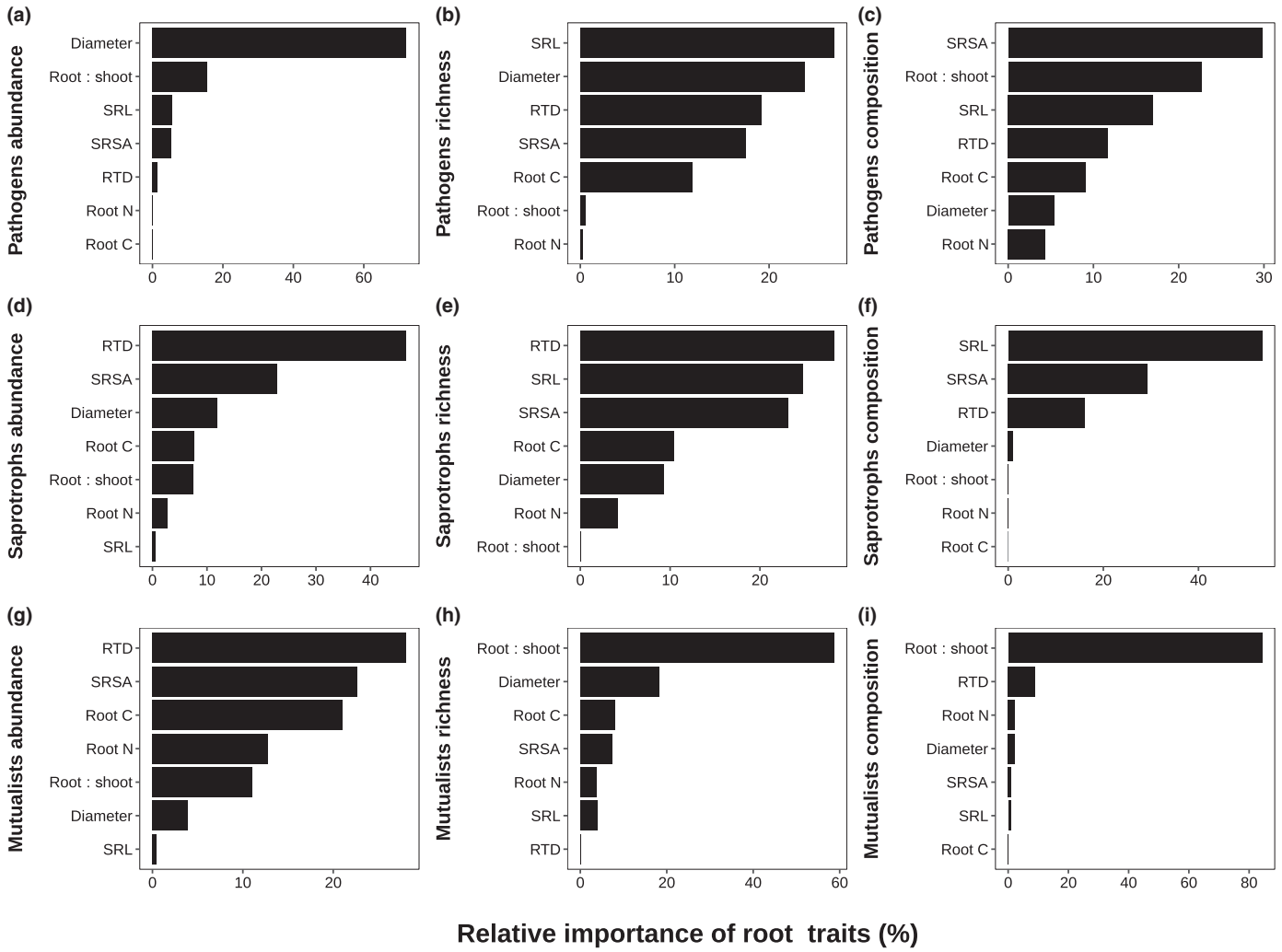

Fig. 5 Relative importance of each root trait on (a–c) pathogens', (d–f) saprotrophs', and (g–i) mutualists' attributes (abundance, richness' and composition). C, carbon; N, nitrogen; SRSA, specific root surface area; RTD, root tissue density; SRL, specific root length. Fungal composition corresponds to the principal coordinate analysis 2, as this was the axis that best explained shoot mass. The proportionate value of each trait considered both its direct effect (i.e. its correlation with the fungal attribute) and its effect when combined with the other variables in the regression equation. The metric for assessing relative importance of regressors in the linear model was 'pmvd'.

et al., 2018). However, this depended highly on the plant species involved. That is, fungal communities associated with species such as *H. lanatus*, *B. incana*, *H. perforatum*, *T. repens*, *P. argentea*, or *D. glomerata* showed the lowest resistance to drought, evidenced by the high dissimilarity in their communities between control and drought treatments, whereas species such as *M. lupulina*, *V. cracca*, *R. thyrsiflorus*, or *F. brevipila* showed a high resistance to drought as fungal communities were similar between treatments. The morphological root trait adjustment to drought conditions, which is species specific (Lozano *et al.*, 2020), may cause changes in the quality and quantity of root exudates, in line with Warembourg *et al.* (2003) and Williams & de Vries (2020), which may help explain the observed variation in fungal communities. In addition, studies have shown that C assimilation and storage could be affected differently by water shortage, as some plant species have greater water uptake than others. Thus, plant species might experience drought conditions differently. In this regard, it has been observed that in a field zone, subjected to drought conditions, different plant species experience different drought patterns (Chitra-Tarak *et al.*, 2018). Likewise, drought can also affect plant C storage in the form of nonstructural carbohydrates (mainly sugars and starch), which, among other functions, provide substrates for the synthesis of defense compounds against pathogens or exchange with symbionts (e.g. mycorrhizal fungi) involved in nutrient acquisition (Hartman & Trumbore, 2016), which in the end help explain the differences in fungal resistance to drought within each plant species.

Pathogen response mainly depended on plant species identity

Pathogen abundance and richness were plant species specific. This result suggests that the soil-borne fungi present in the natural environment where these plant species grow could regulate plant diversity and productivity. Such a scenario is congruent with the current view that fungal pathogens drive biodiversity–

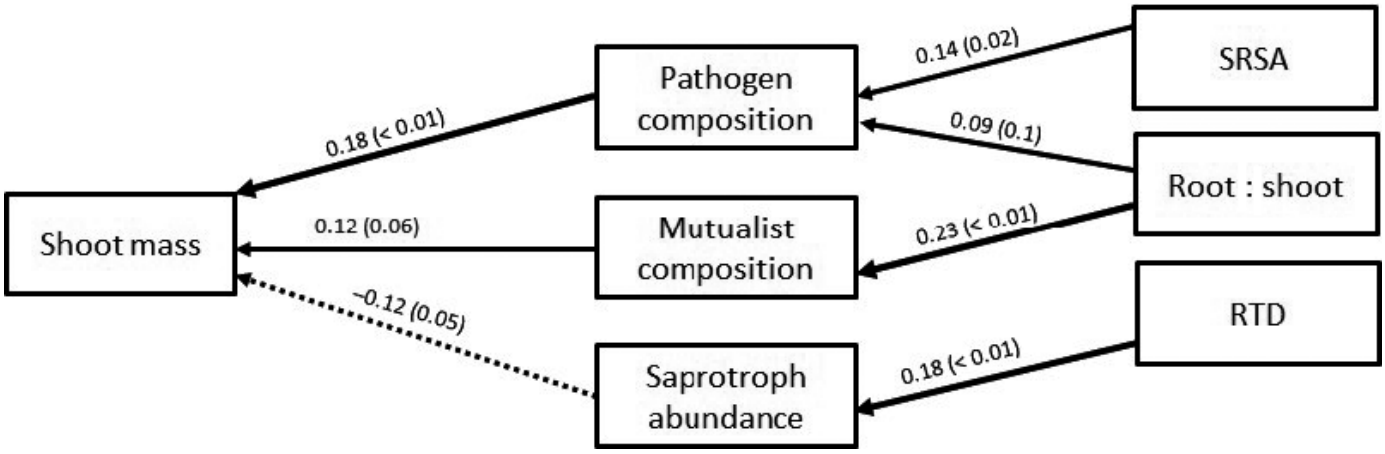

Fig. 6 Path analyses of the relationships between root traits, fungal communities, soil properties, and shoot mass. The coefficient adjacent to each arrow is the strength of the effect of each standardized path and its significance (*P* value). The width of the arrows is proportional to the magnitude 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 a relationship. SRSA, specific root surface area; RTD, root tissue density. See linkages for each plant species and plant functional group in Supporting Information Table S4.

ecosystem functioning relationships (Mommer *et al.*, 2018). We also found that pathogen composition was affected by drought ($P = 0.07$) and was linked to SRSA and root : shoot (Fig. 6). This suggests that a reduction in SRSA (Lozano *et al.*, 2020), and an increase in root C allocation due to drought (Fig. S5), affect pathogen composition with consequences for shoot mass, as observed in our path analyses. The decrease in SRSA may reduce the probability of pathogenic infection, whereas the increase in root : shoot may cause a higher production of secondary metabolites for defense against this fungal group (Hartman *et al.*, 2020). Thus, these root trait adjustments to drought may help explain our findings that fungal pathogen abundance and richness did not change due to drought, which is opposite to the suggestion that soil pathogenicity would increase under climate change scenarios (Van der Putten *et al.*, 2010). We also found that plant functional group played a role determining the structure of pathogen communities, but not those of saprotrophs or mutualists. For example, grasses had a different pathogen structure than forbs did. These results are in accordance with the findings by Francioli *et al.* (2020), supporting the idea that soil-borne pathogen communities can at least partially be predicted by plant phylogeny.

Saprotrophs increased in abundance and richness with drought for some plant species

Saprotrophs are less dependent on plants than other fungal groups are, and most of their activity occurs around the rhizosphere because of the release of root exudates. We found that saprotroph abundance and richness increased with drought for species such as *H. pilosella* or *P. lanceolata*, which are species that develop thinner roots under drought conditions, as reported by Lozano *et al.* (2020). This suggests a link among fine roots (high SRL or SRSA) and saprotrophs (as observed in Fig. 6), which is likely the case, as fine roots are thought to interact intensively with saprotrophs by releasing easily degradable carbohydrates, 'priming' saprotrophic activity in the rhizosphere and releasing nutrients held in soil organic matter (Kuzyakov *et al.*, 2000). Our results also shown a positive relationship between saprotroph abundance and RTD, a root trait that could be positively correlated with root diameter. Thus, the drawback of building an expensive root system with thicker roots under drought (Zhou *et al.*, 2018; Lozano *et al.*, 2020) may be further compensated by long-lived roots (Weemstra *et al.*, 2016; Kong *et al.*, 2017) and by a stimulation of saprotroph communities. Nonetheless, saprotrophs would have antagonistic or competitive effects on mutualistic fungi as they partly depend on the same nutrient sources and share habitats (Verbruggen *et al.*, 2017). Indeed Leake *et al.* (2001) found that the vigor of mutualists was reduced when they encountered saprotrophs, and that this was accompanied by a reduction of C allocation to the mutualistic fungus.

Abundance and richness of mutualists decreased with drought for some plant species

Under drought, mutualists decreased in their relative abundance and richness for some plant species. In comparison with the saprotrophic and pathogenic fungal species, which show some level of independence from the host, the low richness and abundance of mutualists can be tightly coupled with the morphological adjustment of root traits to drought. Indeed, mutualistic composition was mainly explained by root : shoot (84.29%). In that regard, it has been found that most C allocated belowground is transferred to rhizosphere mycorrhizal fungi (Pickles *et al.*, 2017; Verbruggen *et al.*, 2017), which can contribute to promoting drought resistance (Hartman *et al.*, 2020). Related to this, mutualistic richness was again mainly explained by root :shoot (58.73%). Overall, plant species increased root : shoot with drought. Specifically, plant species that increased mutualistic richness with drought (e.g. *P. lanceolata*, *A. millefolium*, *H. pilosella*) also increased root : shoot with drought (Fig. S5).

The decrease in mutualistic richness with drought was evident for some grasses (e.g. *F. brevipila*, *H. lanatus*), which in turn

showed higher SRSA with drought (Lozano *et al.*, 2020). Species with thinner roots may be less responsive to mutualistic fungi than species with thicker roots are, as the former are more efficient in nutrient uptake (Brundrett, 2002; Valverde-Barrantes & Blackwood, 2016) – but see Maherali (2014) for a challenge to this view. Thus, the patterns in these two traits could explain why some graminoids hosted a less diverse mutualistic community particularly under drought, suggesting that under stressful conditions these plants may not depend as much as other species on mutualistic relationships but rather on root morphological trait adjustments.

In a response deviating from this overall pattern, species such as the legume *T. repens* had increased mutualistic richness under drought conditions. Although not clearly observed for the other legumes species, mycorrhizal symbiosis seems to be a key mechanism that *T. repens* uses in response to drought, as AMFs can alleviate plant drought stress (Ruiz-Lozano *et al.*, 2012).

Root trait adjustments to drought predict fungal community structure and plant performance

Plant trait variation influences the relative abundance and composition of different fungal groups (Semchenko *et al.*, 2018; Sweeney *et al.*, 2021). As root traits may adjust to drought conditions (de Vries *et al.*, 2016; Zhou *et al.*, 2018; Lozano *et al.*, 2020), we propose a chain reaction where these changes in root traits modify fungal communities, which in turn affect plant performance (i.e. shoot mass). Specifically, changes in root : shoot affect mutualist composition and, added to changes in SRSA, affect pathogen composition. Likewise, changes in RTD might modify saprotroph abundance. All of these changes at the end will have consequences on shoot mass.

Our study not only identifies which root traits play a key role in predicting responses of rhizosphere and root-colonizing fungi, but also how the predictive importance of those traits varies depending on plant functional groups (though we acknowledge that there is still a large amount of variation in fungal communities not fully explained by plant traits – see also Semchenko *et al.* (2018) and Sweeney *et al.* (2021). Even though we sampled the rhizosphere, we expect that changes in root traits would impact root-colonizing fungi, particularly arbuscular mycorrhizal mutualists. This is because arbuscular mycorrhizal fungal mycelia found in the rhizosphere (the extraradical mycelia) are connected to the mycelia growing inside the root (the intraradical mycelia). Thus, changes in root traits will impact not only the rhizosphere but also the root compartment (Smith & Read, 2010). For example, it has been reported that plants with thinner roots had lower colonization by AMFs than those plants with thicker roots did (Wen *et al.*, 2019). Likewise, the variation in stele : root diameter ratio can also be a good predictor of AMF colonization (Valverde-Barrantes *et al.*, 2016). Finally, we also showed that these root trait–fungal attribute relationships are influenced by plant functional group and plant species identity. This variation was expected, as soil fungal diversity associated with roots is large and, thus, is unlikely to respond homogeneously to the plant-species-specific root trait responses driven by drought (de Vries *et al.*, 2016; Lozano *et al.*, 2020).

We cannot discount alternative flows of causality; it is also possible that root traits and fungal communities are correlated, independently responding to drought and drought-related effects. Our results propose relationships between drought, root traits, fungal communities, and plant performance (shoot mass) and provide new insights into the role that root trait adjustments to drought may have in modulating plant–fungus interactions in grasslands ecosystems.

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Author contributions

YML conceived the ideas and designed the methodology with the help of CAA-T and MCR; YML and CAA-T established and maintained the experiment in the glasshouse; JR performed the bioinformatic analysis; YML analyzed the data with helpful input from CAA-T; YML wrote the first draft of the manuscript. All authors contributed critically to the draft and gave final approval for publication.

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Data availability

Results from Illumina MiSeq were deposited in the ENA Sequence Read Archive with the accession no. PRJEB47271. http://www.ebi.ac.uk/ena/data/view/PRJEB47271.

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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 Rarefaction curves for the 236 samples.

Fig. S2 Community structure dissimilarity (beta-diversity) of fungal communities based on taxonomic and functional diversity using Bray-Curtis dissimilarity metric.

Fig. S3 Variation in total fungal composition explained by plant species and drought.

Fig. S4 Relative importance of pathogens, saprotrophs and mutualists attributes on shoot mass.

Table S1 List of exact sequence variants (ESVs) classified as pathogens and its associated disease.

Table S2 Multiple comparisons indicating whether fungal attributes (i.e. richness and abundance) of pathogens, saprotrophs, mutualists, and total richness, differed within each plant species due to drought.

Table S3 Plant group and drought effects on fungal attributes (richness, composition and relative abundance) of the total community, pathogens, saprotrophs and mutualists.

Table S4 List of fungal attributes that best predicted shoot mass for each plant functional group and each plant species within.

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