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# Drivers of total and pathogenic soil-borne fungal communities in grassland plant species

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ABSTRACT

Soil-borne fungi are considered important drivers of plant community structure, diversity and ecosystem process in terrestrial ecosystems. Yet, our understanding of their identity and belowground association with different plant species in natural ecosystems such as grasslands is limited.

We identified the soil-borne fungal communities in the roots of a range of plant species representing the main families occurring in natural grasslands using next generation sequencing of the ITS1 region, alongside FUNGuild and a literature review to determine the ecological role of the fungal taxa detected. Our results show clear differences in the total and the pathogenic soil-borne fungal communities between the two main plant functional groups in grasslands (grasses and forbs) and between species within both functional groups, which could to a large extent be explained by plant phylogenetic structure. In addition, our results show that drought can increase the relative abundance of pathogenic fungi.

These findings on a range of plant species provide a baseline for future studies revealing the importance of belowground plant-fungal interactions in diverse natural grasslands.



## 1. Introduction

In recent decades, increasing concerns about biodiversity loss has led to a body of research investigating the role of biodiversity for ecosystem functioning (Tilman et al., 2001; Weisser et al., 2017), including the potential effects of diversity on mitigating fungal disease outbreaks (Allan et al., 2010; Rottstock et al., 2014). In contrast to agricultural systems, disease outbreaks that strongly reduce plant performance are rarely observed in species-rich natural ecosystems, such as grasslands (Thrall and Burdon, 1997; Gilbert, 2002; Alexander, 2010). This lack of disease outbreaks in natural grasslands suggests that these systems have mechanisms to keep pathogens in check compared to monoculture grasslands (Ampt et al., 2019, van Ruijven et al., 2020). Biodiversity experiments in common gardens suggest that plant diversity can dilute soil-borne fungal pathogens compared to monocultures (Maron et al., 2011; Schnitzer et al., 2011; Mommer et al., 2018). However, our understanding of soil-borne fungal pathogens and their associations with different grassland plant species is still rather limited. We hardly know what soil-borne fungal pathogens occur in grasslands and with what plant species they are associated (Wehner et al., 2014). Here, we aim to reveal the soil-borne fungi associated with a wide range of plant species representing the main families of natural grasslands (Schaminée et al., 1995), and identify the drivers of these (pathogenic) plant-fungal associations. Such basic knowledge is important to understand soil-borne pathogen dynamics in complex, natural plant communities, such as diverse grasslands.

Some studies have shown that soil-borne fungal communities mainly differ between plant functional groups, such as grasses and nitrogen-fixing legumes (Harrison and Bardgett, 2010; Cline et al., 2018) or grasses and forbs (Mommer et al., 2018; Francioli et al., 2020). Other studies have suggested that plant phylogeny is a better predictor of fungal community composition (Gilbert et al., 2012; Wehner et al., 2014; Burns et al., 2015; Koyama et al., 2019). The latter may be related to the fact that morphological and chemical root traits are also phylogenetically structured (Gilbert and Parker, 2016; Valverde-Barrantes et al., 2017). If these

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root traits affect the colonization of the roots by fungi, this can lead to closely related plant species being more likely to be colonized by the same soil-borne fungi. Indeed, several studies suggest that the probability of a pathogenic fungus infecting neighbouring plant species increases with decreasing phylogenetic distance between plant species (Gilbert and Webb, 2007; Liu et al., 2012). In contrast, phylogenetic distance between plant species may be a weak predictor of similarity in fungal community composition when molecular characteristics related to susceptibility (i.e. effector molecules and resistance-genes) are not phylogenetically conserved or highly plant species-specific (Mouquet et al., 2012; Pavoine et al., 2013; Zhang et al., 2013). Here, we aim to reveal the relative contributions of plant functional group and host phylogeny to species-specific variation in soil-borne (pathogenic) fungal communities in grassland species.

Abiotic factors like soil pH (Glassman et al., 2017; Canini et al., 2019), nutrient availability (Francioli et al., 2016; Guo et al., 2020), and temperature (Newsham et al., 2015; Birnbaum et al., 2019) are known drivers of fungal communities. Another important factor that can have considerable effects on the fungal community is soil water availability, directly (Choudhary et al., 2016) or indirectly via plant performance, since water deficit triggers changes in root architecture (Smith and De Smet, 2012) and root exudation profiles (Henry et al., 2007; Song et al., 2012). We focus on this aspect as droughts are predicted to occur more frequently in the future (Vicente-Serrano et al., 2014; Cook et al., 2018). Recent studies have found increases in pathogenic fungal abundance under severe drought stress (Ramegowda and Senthil-Kumar, 2015; Choudhary et al., 2016; Preece et al., 2019; Delgado-Baquerizo et al., 2020), while others have reported differential responses of the root associated microbes after drought stress (Fitzpatrick et al., 2018). However, the effect of drought on soil-borne fungal pathogens in natural grassland plant species in the field is largely unknown (de Vries et al., 2018).

This study aims to reveal the effects of plant species identity and drought on the diversity and composition of both the total and the pathogenic fungal community associated with the roots of 16 different grassland plant species in a common garden experiment in the Netherlands. Specifically, we address the following questions: (1) To what extent can plant phylogeny and functional group explain differences in total and pathogenic soil-borne fungal communities among plant species? (2) Does drought affect the total and pathogen soil-borne fungal communities? To answer these questions, we sequenced the fungal communities from roots of 16 grassland plant species affiliated to two plant functional groups (grasses and forbs), that have been grown for three years in monocultures. In the fourth year of the experiment, a drought treatment was applied, just before sampling the roots.

## 2. Materials and methods

### 2.1. Study site

We sampled plant roots from the monoculture plots from a common garden experiment established in April 2014 at an experimental field of Wageningen University, the Netherlands (51.99° N, 5.66° E) (Bakker et al., 2016). The 16 plant species used in this experiment were equally divided into two plant functional groups: the grasses *Agrostis stolonifera*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Briza media*, *Festuca pratensis*, *Festuca rubra*, *Phleum pratense*, *Trisetum flavescens* and forbs *Achillea millefolium*, *Centaurea jacea, Galium mollugo*, *Leontodon hispidus*, *Leucanthemum vulgare*, *Prunella vulgaris*, *Ranunculus repens*, and *Sanguisorba officinalis*. These two functional groups also represent a clear phylogenetic signal: the grasses represent a single plant family (Poaceae) within the monocots that is only distantly related to the forbs. The forbs, on the other hand, cover a relatively wide phylogenetic range within the dicots, representing five plant families from five orders (see Fig. S1).

The 16 plant species were grown in plots of 70 cm × 70 cm filled with a mixture of river sand and soil from an old field (3:1). These plots were distributed in 3 blocks, which contained 2 plots (2 replicates) of each of the 16 species per block (details in Bakker et al. (2016)). In the growing season of the fourth year a drought treatment was set up (10 June −14 July 2017). Each block was covered by rainout shelters (6.00 m × 36.00 m x 2.60 m (w x l x h)) made of an aluminium frame and a transparent plastic sheet (Solar EVA, 180 μm thick). We covered all plots to control for potential side effects of the rainout shelters (Kreyling et al., 2017). The plastic sheet was attached to aluminium gutters at 40 cm above the soil surface, to lead rain water away and to allow air circulation underneath the shelters. Light transmission of the plastic sheet was 90%. In each block, one plot was randomly allocated to the drought treatment and the other to the control. In total, this study included 16 species x two levels of the water treatment (drought and control) x three replicates equals 96 plots. The control plots were watered two to three times a week, whereas the drought plots did not receive any water for 34 days (see Bakker (2018) for details).

### 2.2. Root sampling and analysis

We collected root samples from all the monoculture plots in mid July 2017, immediately after the induced drought period. Root samples were taken to a depth of 10 cm with soil augers with a diameter of 4 cm. Four soil samples from each monoculture plot were pooled and sieved (2 mm mesh). Roots not passing through the sieve were washed free of soil. A subsample of the roots was immediately stored at −20 °C until the molecular analysis. The rest of the roots were stored at 4 °C for morphological and chemical analysis. We determined four important root traits (Bakker et al., 2016; Kong et al., 2019), being specific root length (SRL), root diameter, root tissue density (RTD) and root N, following standardized protocols (Perez-Harguindeguy et al., 2013). Briefly, root length, volume and diameter as required for SRL and RTD, were determined by scanning and analysing root samples with the WinRHIZO software (Regent Instruments Inc., Ville de Québec, Québec, Canada). Then, the root samples were weighed fresh and after drying at 70 °C for 48 h. Root N content was determined by dry combustion using a Vario EL III C/H/N analyser (Elementar, Hanau, Germany).

### 2.3. Fungal sequencing data

DNA was extracted from the root samples collected using the DNeasy Plant Mini kit (Qiagen). Fungal DNA amplification was performed using the forward primer ITS1F and reverse primer ITS2 (White et al., 1990) using the PCR protocol described in Mommer et al. (2018). The amplicons were sequenced on an Illumina MiSeq instrument with 2 × 300 base pair kits at the Plant Research International, Wageningen UR, Wageningen, the Netherlands. Amplicon sequence variants (ASV; also known as zero-radius operational taxonomic units; Callahan et al. (2017)) were determined from raw sequence data using the DADA2 pipeline (Callahan et al., 2016). ASVs were chosen over OTUs because they were found most effective for recovering the richness and composition of the soil-borne fungal community (Pauvert et al., 2019). Only ASVs that were detected in more than two plots were included in the data analyses. Fungal reads were rarefied to 9136 reads, the minimum number of sequence reads per root sample. For taxonomic assignment of the ASVs, the representative sequences were classified

using the dynamic version of the developer's full-length ITS reference sequences of the UNITE database (version 8, November 18, 2018; Nilsson et al. (2018)) with higher than 80% similarity, and the non-fungal ASVs were discarded. All sequences have been submitted to the European Nucleotide Archive (study accession number PRJEB36011). Since in this study we were also interested in the pathogenic component of the root-associated fungal community, the functional characterisation of the obtained fungal ASVs was determined in a two-step process. To create the pathogenic subset of the data we first made a rough selection of potential pathogens by selecting taxa that were classified as 'highly probable' and 'probable' fungal pathogens by FUNGuild (Nguyen et al., 2016). In the second step we further explored the potential pathogenicity of the pathogenic ASVs that were characterized at the species level using the literature (Domsch et al., 2007; Griffith and Roderick, 2008, Arnolds and van den Berg, 2013; Farr and Rossman, 2014; Dighton, 2016; Dighton and White, 2017; Mommer et al., 2018). This means that only ASVs that were identified to the species level and were reported in the literature to be plant pathogenic were included in our pathogenic subset. We acknowledge that the *modus operandi* used to attribute the ecological function of the fungal species identified in this study might introduce some biases, since the pathogenicity of a particular fungal taxa may depend on the host-fungus interaction and the environmental context (Gilbert and Parker, 2016). In addition, the presence of a pathogenic fungal species does not necessarily imply pathogenicity; for example, the identification of a well-described active pathogenic fungal species may not be sufficient to infer pathogenic effects on plants, which has to be assessed by specific testing. We further recognize that although the ITS regions may provide a high coverage of fungal diversity, they have limited taxonomic resolution for many fungal taxa. Therefore, the exclusion of fungal taxa that do not reach the species level in the taxonomic annotation may limit the coverage of the 'whole' pathogenic fungal population in our study. Nonetheless, the *modus operandi* we used in this study may contribute to further improve our understanding of the potential ecological function of the fungal species detected in field studies and common garden experiments (van Ruijven et al., 2020).

### 2.4. Statistical analyses

All statistical tests were performed using the software PERMANOVA + for PRIMER v7 (Anderson et al., 2008; Clarke et al., 2014) and R v3.5.0 (R Core Team, 2014).

#### 2.4.1. Plant phylogeny

To create the plant phylogenetic tree (Fig. S1) from the 16 plant species investigated in this study, plant phylogenetic information was obtained from the Daphne phylogenetic database (Durka and Michalski, 2012). Then, pairwise patristic distances (pairwise sum of the branch length connecting two terminal taxa) were generated using the *cophenetic. phylo* function in the R package "*ape*" (Paradis et al., 2004). Phylogenetic eigenvectors were derived from the pairwise patristic distance matrix based on principal coordinate analysis (PCoA) using the *cmdscale* command in the R package "*vegan*" (Oksanen et al., 2018). Significant PCoA vectors were forward selected ($\alpha = 0.05$) prior to subsequent analyses using the *forward. sel* command in the R package "*packfor*" (Dray et al., 2011).

#### 2.4.2. Fungal richness

To test the effects of drought and plant species on the richness of the root-associated fungi and to separate the effects of plant species identity, functional group and phylogeny, univariate PERMANOVA models were used (Anderson, 2017). As plant species identity, functional group and phylogeny are not independent, we used different models with sequential sums of squares (Hector et al., 2010). In the first model, only the effects of plant species identity and drought were included (model 1). Then, we ran two additional models in which plant functional group and phylogeny were fitted before plant species identity and drought. To take into account that plant functional group and host phylogeny are not independent, these terms were fitted in two different sequences. In model 2, plant functional group was fitted before phylogeny. In model 3, host phylogeny was fitted before plant functional group. To assess the extent to which plant functional group and phylogeny could explain the effect of plant species identity, the variance explained by plant species identity, functional group and phylogeny in each of the models was compared. These analyses were performed separately for total fungal richness and pathogenic fungal richness, using the PRIMER v7 software package (Clarke et al., 2014) with the PERMANOVA add-on package (Anderson et al., 2008).

#### 2.4.3. Fungal community structure

To assess differences in the root-associated fungal community structure across the 16 plant species, we first calculated Bray-Curtis dissimilarities using square-root transformed relative abundances (Hellinger transformation; Legendre and Gallagher (2001)). To understand the effect of plant identity, plant functional group, host phylogeny and drought on fungal community structure we used a similar approach to that described above for fungal richness. Hence, we constructed different PERMANOVA models using the following procedure: in the first model, only the effects of plant species identity and drought were included (model 1). To assess the extent to which plant functional group and host phylogeny capture the differences in the root mycobiome between plant species, we performed additional PERMANOVA analyses in which plant functional group and phylogeny were fitted before species identity and drought in multivariate models. To take into account the dependency between plant functional group and phylogeny, these terms were fitted in two different sequences. In model 2, plant functional group is fitted before phylogeny. In this model, a significant effect of phylogeny would indicate a phylogenetic signal within plant functional group. In model 3, host phylogeny is fitted before plant functional group. In both models, the effect of species identity then represents interspecific differences not captured by plant functional group and phylogeny. To assess the extent to which plant functional group and phylogeny could explain the effect of plant species identity, the variance explained by plant species identity, functional group and phylogeny in each of the models was compared. The effect of plant species identity, host phylogeny and drought within each plant functional group was further investigated constructing different PERMANOVA models as previously described. To determine to what extent the effect of plant phylogeny was related to the root traits measured in this study, we first tested for differences in root traits among the plant species and plant functional groups using linear mixed effect models for each root trait using the R packages "*lme4*" (Bates et al., 2015) with block as a random factor. Then, we created PERMANOVA models to assess how much variation in fungal community structure explained by host phylogeny could be captured by root traits. To take into account the dependency between host phylogeny and root traits, these terms were fitted in two different sequences. In model 4, root traits are fitted before host phylogeny. In model 5, host phylogeny is fitted before the root traits to assess whether root traits may explain additional variance that it is not captured by host phylogeny. To perform the PERMANOVA models 4 and 5, we first verified whether there was co-linearity between the root traits measured using the *varclust* function in the "*Hmisc*" package (Harrell and Dupont, 2017). Due to co-linearity between specific root length and root diameter we only included specific root length in the

analysis. The PERMANOVA analyses were performed separately for total and pathogenic fungal community structure, using the PRIMER v7 software package (Clarke et al., 2014) with the PERMANOVA add-on package (Anderson et al., 2008).

We tested whether plant functional group and drought had an effect on the relative abundance of the main fungal orders using factorial GLMs with negative binomial errors, building a separate model for each fungal order and including block as a random factor using the *glm. nb* function in the "*MASS*" R package (Venables and Ripley, 2002). To test whether the cumulative abundance of the pathogenic ASVs was affected by plant species and drought we built linear mixed effect models with plant species and drought as fixed factors and block as a random factor using the R packages "*lme4*" (Bates et al., 2015). The significance level was adjusted using the Benjamini-Hochberg correction to account for multiple comparisons. Using Kruskal-Wallis rank sum tests, we also examined the effect of drought on fungal pathogenic species that accounted for at least 0.1% of the total fungal reads and were found in at least four plant species and in both control and drought plots in each of those plant species. We further used the R package "*DESeq2*" (Love et al., 2014) to examine the differential representation of particular ASVs between the grass and forb plants using moderated shrinkage estimation for dispersions and fold changes as an input for a pairwise Wald test with Benjamini–Hochberg correction ($P < 0.05$) for multiple comparisons. For these analyses we used the un-rarefied ASVs counts as normalization is implemented in the R package "*DESeq2*" (Love et al., 2014). Total and pathogenic fungal community dissimilarity was visualized in a PCoA ordination plot by using the R packages "*ggplot2*" (Wickham, 2009) and "*vegan*" (Oksanen et al., 2018).

## 3. Results

### 3.1. Sequence summary

From the MiSeq run we recovered 2,879,404 high quality fungal reads, which clustered into 909 fungal ASVs (Table S1). Fungal sequences were associated with eight phyla, 23 classes, 49 orders, 86 families and 99 genera. Ascomycota were the most abundant phylum, comprising 57.4% of the reads across all samples (535 ASVs), followed by Basidiomycota (36.1% of reads, 274 ASVs, Fig. S2). Each of the other phyla represented less than 1% (Fig. S2). Nearly 4.5% of the fungal sequences (36 ASVs) could not be assigned to a fungal phylum.

From the 909 ASVs, we identified 59 ASVs that are known as soil-borne plant pathogenic fungal taxa (Table S2). These pathogenic fungal ASVs accounted for 9.6% of the total fungal reads and represented 6.7% of the total fungal ASVs identified in this study. They were affiliated to the Ascomycota (46 ASVs) and Basidiomycota (13 ASVs). These pathogenic ASVs clustered in 29 fungal species and were mainly affiliated to the genera *Fusarium*, *Paraphoma*, *Alternaria*, *Microdochium* and *Rhizoctonia* (Table S3).

### 3.2. Fungal richness

Total root-associated fungal richness ranged from 35 to 150 ASVs across the 16 plant species. It differed significantly among plant species ($F_{15,95} = 4.76$ $P < 0.001$, Table 1), with *F. rubra* and *G. mollugo* having on average the highest and *P. pratense* the lowest fungal richness (Fig. 1A). Plant species identity explained 45.9% of this variation in total fungal richness (Table 1, model 1), but host phylogeny was able to capture most of it (40 out of 45.9%; Table 1, model 3). Plant functional group did not have an effect on total fungal richness (Table 1, model 2). Similarly, drought did not affect total fungal richness and no interactions between drought and any of the plant variables used in this study were found (Table 1).

The root-associated pathogenic fungal richness was also significantly ($F_{15,95} = 3.78$, $P < 0.001$; Table 1) different between plant species, ranging from two to 17 (Fig. 1B). Plant species accounted for 40.5% of the variance (Table 1, model 1). Host phylogeny captured 75% of that variance (30 out of 40.5%, Table 1, model 3). A smaller portion could be explained by plant functional group (9.2 out of 40.5%; Table 1, model 2), with forbs showing significantly higher pathogenic richness than grasses. Drought significantly ($F_{1,95} = 12.077$, $P < 0.001$; 8.0% of variation) increased pathogenic richness (Table 1; Fig. 1B). No interaction between drought and any of the plant variables used in this study was observed for pathogenic fungal richness (Table 1).

### 3.3. Total fungal community structure

Total fungal community structure differed significantly between plant species (Table 2). Plant species identity explained 35.2% of the variation in total fungal community structure (Table 2, model 1). When plant functional group and host phylogeny were fitted before plant identity (Table 2, model 2 and 3), they captured the majority of the variance explained by plant identity (27.6 out of 35.2%). When plant functional group was fitted first in the model (Table 2, model 2) it captured 14.1% of that variation and phylogeny captured the remaining 13.5%. However, when phylogeny was fitted first (Table 2, model 3), it accounted for almost all of the captured variance (26 out of 27.6%) and plant functional group accounted for only 1.6%.

Also within the two functional groups, plant species identity was a major driver, explaining 21.8 and 27.1% of the variance in the total fungal community structure in grasses and forbs, respectively (Tables S4 and S5). Host phylogeny captured the variance explained by plant identity within functional groups to a large extent, ranging from 75% for the grasses (Table S5) to more than 90% for the forbs (Table S4).

The four root traits were significantly different among the 16 plant species (Fig. S3). Specific root length was significantly higher ($F_{1,95} = 84.93$, $P < 0.001$) and root diameter significantly lower ($F_{1,95} = 109.87$, $P < 0.001$) in grasses than in forbs (Fig. S3) whereas root tissue density and root nitrogen were not different between plant functional groups. The variation in root traits explained 11.2% of the variance in fungal community structure when fitted before host phylogeny, but phylogeny remained significant, explaining a further 14.8% (Table S6, model 4). Specifically, specific root length, root tissue density and root nitrogen accounted for 8.1, 1.6 and 1.5% of variance, respectively. When phylogeny was fitted before the root traits, it captured most of the variance explained by root traits in the previous model and root traits were no longer significant (Table S6, model 5), indicating a strong phylogenetic signal in the root traits measured.

The differences in fungal community structure between functional groups (Fig. 2A; Fig. 3A and B) were detected in several fungal orders. The fungal communities of the grass species were characterized by a significantly ($P < 0.05$) higher proportion of reads affiliated to the orders Agaricales, Auriculariales, Chaetothyriales, Sordariales and Xylariales, while the fungal communities of the forb species were mainly composed of fungal taxa associated to Cantharellales, Helotiales, Pleosporales and Sebacinales. At the ASV level, the 16 plant species were generally dominated by plant functional group-specific ASVs. We found that 93 fungal ASVs were differentially abundant between grasses and forbs. Most of these taxa were affiliated to the above-mentioned fungal orders (Figs. S4 and S5, Table S7) and accounted for a large proportion of the total fungal reads (ranging from 54.8 to 69.8% across the 16 species).

**Table 1**
The relative importance of plant identity (PI), plant functional group (PF), host phylogeny (HP) and water treatment (WT) for the total and pathogenic root-associated fungal richness across the sixteen plant species as revealed by PERMANOVA.

| | | Total fungal community | | | | Pathogenic fungal community | | | |
|---|---|---|---|---|---|---|---|---|---|
| Model[a] | Parameter[b] | Df | Pseudo-F | $R^2$ | P-value | df | Pseudo-F | $R^2$ | P-value |
| 1 | PI | 15 | 4.759 | 0.459 | 0.001 | 15 | 3.787 | 0.405 | 0.001 |
| | WT | 1 | 0.483 | 0.003 | 0.469 | 1 | 12.077 | 0.080 | 0.002 |
| | PI x WT | 15 | 1.370 | 0.132 | 0.191 | 15 | 0.570 | 0.061 | 0.866 |
| 2 | PFG | 1 | 1.640 | 0.010 | 0.216 | 1 | 12.962 | 0.092 | 0.001 |
| | HP | 8 | 7.73 | 0.401 | 0.001 | 5 | 5.893 | 0.209 | 0.001 |
| | PI | 7 | 1.161 | 0.083 | 0.314 | 10 | 1.607 | 0.114 | 0.122 |
| | WT | 1 | 0.632 | 0.018 | 0.002 | 1 | 11.265 | 0.080 | 0.003 |
| | PFG x WT | 1 | 2.571 | 0.016 | 0.092 | 1 | 1.549 | 0.011 | 0.213 |
| | HP x WT | 8 | 1.178 | 0.061 | 0.411 | 5 | 0.760 | 0.023 | 0.341 |
| | PI x WT | 6 | 0.884 | 0.052 | 0.523 | 9 | 0.441 | 0.0282 | 0.911 |
| 3 | HP | 8 | 8.291 | 0.398 | 0.001 | 5 | 8.498 | 0.302 | 0.001 |
| | PFG | 1 | 2.089 | 0.013 | 0.143 | 1 | 0.2033 | 0.001 | 0.621 |
| | PI | 7 | 1.162 | 0.052 | 0.332 | 10 | 1.607 | 0.114 | 0.129 |
| | WT | 1 | 0.632 | 0.004 | 0.422 | 1 | 11.265 | 0.080 | 0.001 |
| | HP x WT | 8 | 1.199 | 0.062 | 0.417 | 5 | 1.072 | 0.034 | 0.533 |
| | PFG x WT | 1 | 2.389 | 0.016 | 0.093 | 1 | 0.081 | 0.001 | 0.785 |
| | PI x WT | 6 | 0.885 | 0.03 | 0.514 | 9 | 0.441 | 0.028 | 0.925 |

[a] Model 1, PERMANOVA analysis with plant identity and water treatment as explanatory variables; model 2, PERMANOVA analysis with plant functional group, before host phylogeny, followed by plant identity and water treatment as explanatory variables; model 3, PERMANOVA analysis with host phylogeny before plant functional group, followed by plant identity and water treatment as explanatory variables.
[b] PI = plant identity; WT = water treatment; PFG = plant functional group; HP = host phylogeny.

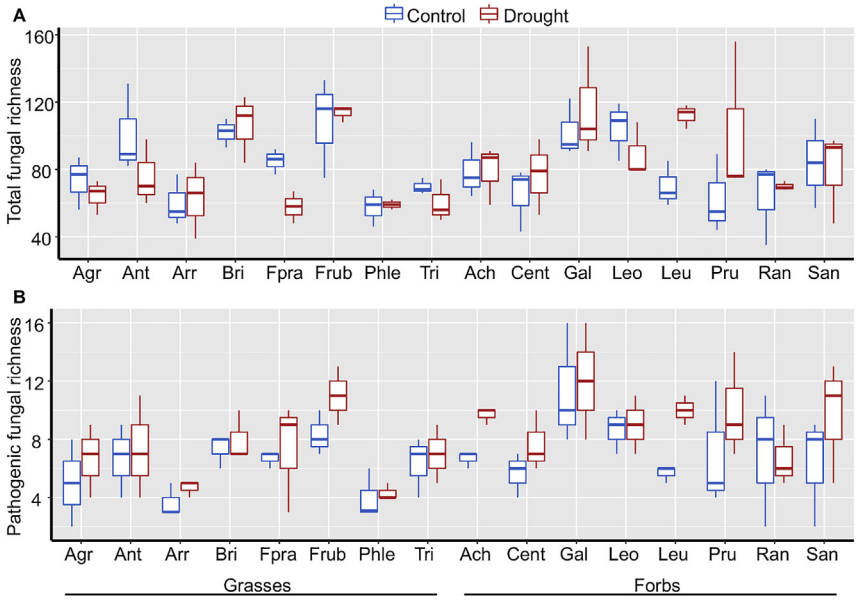


**Fig. 1.** Box plots of the observed richness of the **(A)** total and **(B)** pathogenic fungal communities across the sixteen plant species in the control and drought plots. Species abbreviations are *Agrostis stolonifera* (Agr), *Anthoxanthum odoratum* (Ant), *Arrhenatherum elatius* (Arr), *Briza media* (Bri), *Festuca pratensis* (Fpra), *Festuca rubra* (Frub), *Phleum pratense* (Phle), *Trisetum flavescens* (Tri), *Achillea millefolium* (Ach), *Centaurea jacea* (Cent), *Galium mollugo* (Gal), *Leontodon hispidus* (Leo), *Leucanthemum vulgare* (Leu), *Prunella vulgaris* (Pru), *Ranunculus repens* (Ran) and *Sanguisorba officinalis* (San).

### 3.4. Pathogenic fungal community structure

Similar to the total fungal community, plant identity had a significant effect on the pathogenic community, explaining 34.7% of the variation in community structure (model 1, Table 2; Fig. 2B; Fig. 4). When plant functional group and host phylogeny were fitted before plant identity (model 2 and 3, Table 2), they captured two-thirds of the variance explained by plant identity (26.1% of the 34.7%). This was almost entirely due to host phylogeny, capturing 24.1 out of 26.1% (compare models 2 and 3, Table 2). Also within functional groups, plant identity was a main factor driving fungal community structure, explaining 26.6 and 20.3% of the variance within forbs and grasses, respectively (Tables S4 and S5). Host phylogeny captured approximatively 60% of the variance explained by plant identity in the forbs (Table S4), while in the grasses it captured 40% (Table S5).

When fitted before host phylogeny, root traits explained 11% of the variance in fungal pathogenic community structure (Table S6, model 4). Specifically, specific root length, root tissue density and root nitrogen accounted for 7.2, 2.5 and 1.3% of the variance, respectively. However, phylogeny remained significant and explained an additional 13.1%. When fitted first, phylogeny

**Table 2**
The relative importance of plant identity (PI), plant functional group (PF), host phylogeny (HP) and water treatment (WT) for the total and pathogenic root-associated fungal community structure across the sixteen plant species as revealed by PERMANOVA.

| | | Total fungal community | | | | Pathogenic fungal community | | | |
|---|---|---|---|---|---|---|---|---|---|
| Model[a] | Parameter[b] | df | Pseudo-F | $R^2$ | P-value | df | Pseudo-F | $R^2$ | P-value |
| 1 | PI | 15 | 3.14 | 0.352 | 0.001 | 15 | 3.000 | 0.347 | 0.001 |
| | WT | 1 | 2.356 | 0.018 | 0.001 | 1 | 3.115 | 0.023 | 0.001 |
| | PI x WT | 15 | 1.434 | 0.161 | 0.001 | 15 | 1.237 | 0.143 | 0.023 |
| 2 | PFG | 1 | 18.912 | 0.141 | 0.001 | 1 | 19.803 | 0.164 | 0.001 |
| | HP | 8 | 2.270 | 0.135 | 0.001 | 4 | 2.938 | 0.097 | 0.001 |
| | PI | 7 | 1.596 | 0.083 | 0.001 | 11 | 1.510 | 0.127 | 0.001 |
| | WT | 1 | 2.383 | 0.018 | 0.002 | 1 | 3.045 | 0.023 | 0.001 |
| | PFG x WT | 1 | 1.617 | 0.012 | 0.039 | 1 | 1.680 | 0.013 | 0.048 |
| | HP x WT | 8 | 1.611 | 0.096 | 0.001 | 4 | 1.270 | 0.039 | 0.077 |
| | PI x WT | 6 | 1.156 | 0.052 | 0.069 | 10 | 1.190 | 0.091 | 0.047 |
| 3 | HP | 8 | 4.360 | 0.260 | 0.001 | 4 | 7.679 | 0.241 | 0.001 |
| | PFG | 1 | 2.191 | 0.016 | 0.001 | 1 | 2.662 | 0.020 | 0.002 |
| | PI | 7 | 1.596 | 0.083 | 0.001 | 11 | 1.510 | 0.127 | 0.002 |
| | WT | 1 | 2.383 | 0.018 | 0.001 | 1 | 3.045 | 0.023 | 0.001 |
| | HP x WT | 8 | 1.564 | 0.093 | 0.001 | 4 | 1.319 | 0.040 | 0.048 |
| | PFG x WT | 1 | 1.992 | 0.015 | 0.002 | 1 | 1.482 | 0.011 | 0.010 |
| | PI x WT | 6 | 1.156 | 0.052 | 0.058 | 10 | 1.191 | 0.077 | 0.048 |

[a] Model 1, PERMANOVA analysis with plant identity and water treatment as explanatory variables; model 2, PERMANOVA analysis with plant functional group, before host phylogeny, followed by plant identity and water treatment as explanatory variables; model 3, PERMANOVA analysis with host phylogeny before plant functional group, followed by plant identity and water treatment as explanatory variables.
[b] PI = plant identity; WT = water treatment; PFG = plant functional group; HP = host phylogeny.

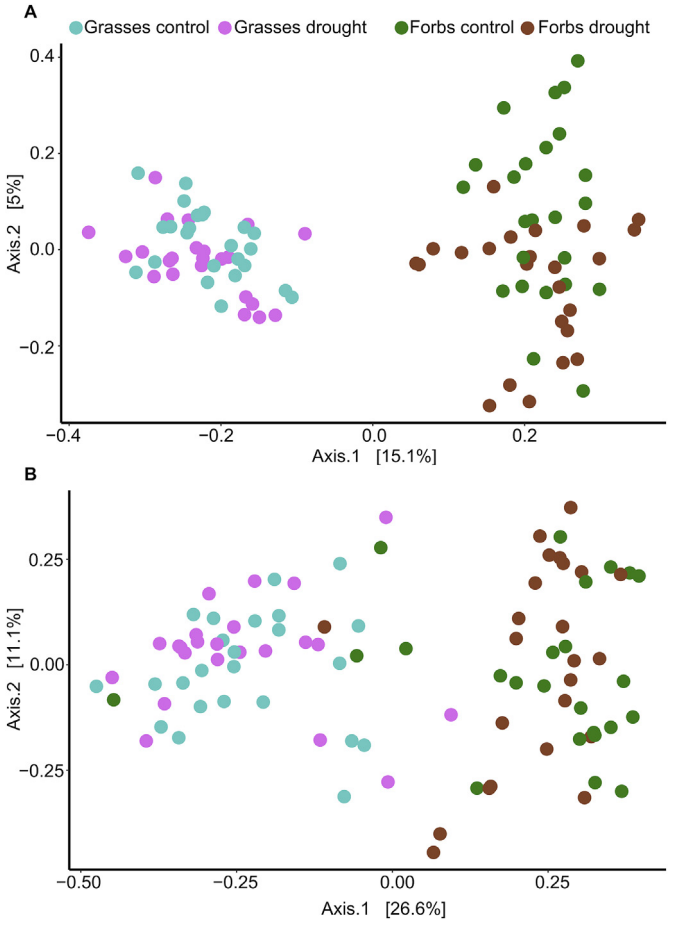


**Fig. 2.** Principal Coordinates Analysis (PCoA) of the **(A)** total and **(B)** pathogenic fungal community associated with the sixteen plant communities in the control and drought plots.

captured the variance previously explained by roots almost entirely and the root traits no longer had significant effects on pathogenic fungal community composition (Table S6, model 5). These analyses together suggest that plant phylogeny is the main driver of plant species identity effects on soil-borne pathogenic fungal communities.

Several pathogenic taxa were predominantly associated with a single plant species or a particular functional group (Fig. 5). For example, *Slopeiomyces cylindrosporus* was present in all grasses but hardly detected in the forbs. *Fusarium culmorum* was identified only in four grass species (i.e. *B. media*, *F. pratensis*, *F. rubra*, *T. flavescens*), while *Alternaria solani, Boeremia exigua*, and *Phoma herbarum* were identified predominantly in the forbs and rarely in the grasses. *Stemphylium vesicarium* was found only in two forb species (*A. millefolium* and *L. hispidus*). In contrast, the pathogens *Alternaria alternata, Alternaria infectoria, Didymella americana*, *Microdochium bolleyi*, *Epicoccum nigrum*, *Fusarium oxysporum*, *Fusarium solani*, *Paraphoma chrysanthemicola* and *Rhizoctonia solani* were found in almost every plant species investigated.

### *3.5. Drought effects on total and pathogenic fungal community structure*

The drought treatment had a significant effect on the total and pathogenic fungal community, although it explained only approximatively 2% of the variation in these communities (Table 2, model 1). Interestingly, we found a significant interaction between plant identity and drought that accounted for an additional 16.1% and 14.3% of variation for the total and pathogenic fungal community, respectively (Table 2, model 1), suggesting a potential differential response to drought across plant species. Host phylogeny captured approximatively half of the variance explained by the significant interaction between plant identity with drought (Table 2, model 3), while in the pathogenic community it captured only one third of this variance (Table 2, model 3). Plant functional group was only able to capture a small proportion of the variance explained by the significant interaction between plant identity with drought which was around 1% in both the total and pathogenic fungal community (Table 2 model 2). A significant and comparable drought effect on

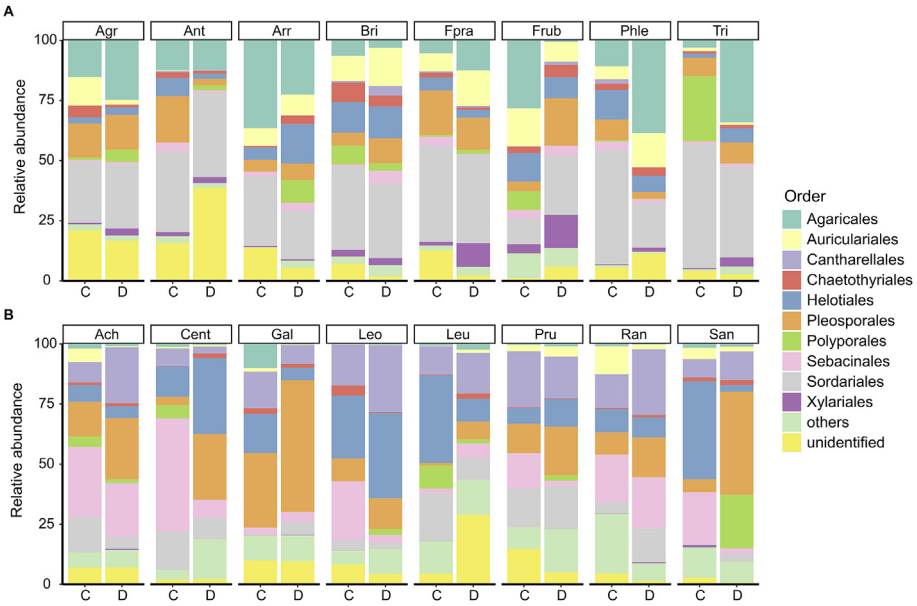


**Fig. 3.** Mean relative abundance of the main orders of the total root associated fungal taxa detected in the **(A)** grass and **(B)** forbs species in the control and drought plots. Species abbreviations are *Agrostis stolonifera* (Agr), *Anthoxanthum odoratum* (Ant), *Arrhenatherum elatius* (Arr), *Briza media* (Bri), *Festuca pratensis* (Fpra), *Festuca rubra* (Frub), *Phleum pratense* (Phle), *Trisetum flavescens* (Tri), *Achillea millefolium* (Ach), *Centaurea jacea* (Cent), *Galium mollugo* (Gal), *Leontodon hispidus* (Leo), *Leucanthemum vulgare* (Leu), *Prunella vulgaris* (Pru), *Ranunculus repens* (Ran) and *Sanguisorba officinalis* (San). Treatment abbreviations are control (C), drought (D).

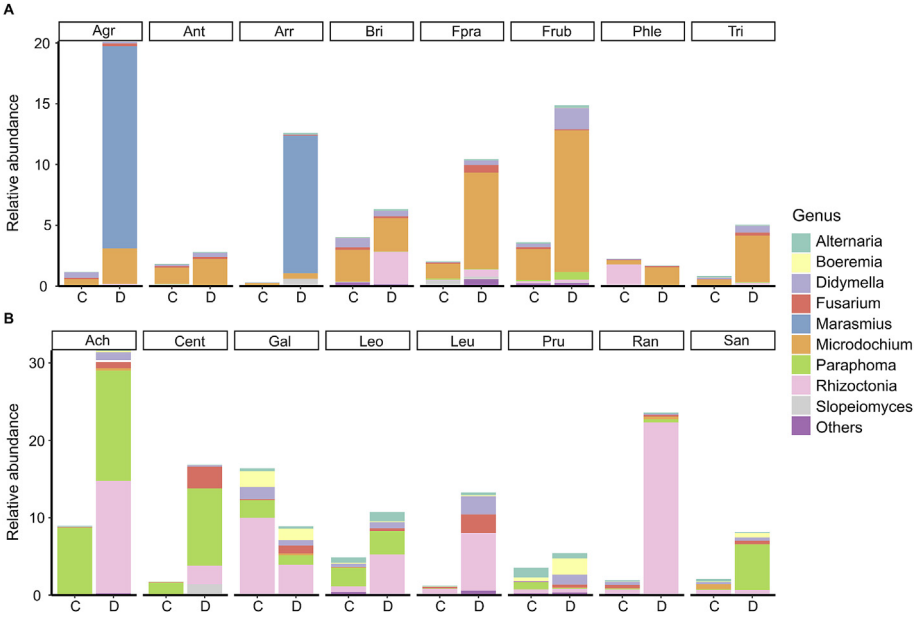


**Fig. 4.** Relative abundance of the main genera of the pathogenic fungal taxa detected in the **(A)** grass and **(B)** forb species in the control and drought plots. Species abbreviations are *Agrostis stolonifera* (Agr), *Anthoxanthum odoratum* (Ant), *Arrhenatherum elatius* (Arr), *Briza media* (Bri), *Festuca pratensis* (Fpra), *Festuca rubra* (Frub), *Phleum pratense* (Phle), *Trisetum flavescens* (Tri), *Achillea millefolium* (Ach), *Centaurea jacea* (Cent), *Galium mollugo* (Gal), *Leontodon hispidus* (Leo), *Leucanthemum vulgare* (Leu), *Prunella vulgaris* (Pru), *Ranunculus repens* (Ran) and *Sanguisorba officinalis* (San). Treatment abbreviations are control (C), drought (D).

the total and pathogenic fungal community structure was found within the forbs and grasses (see Tables S4 and S5). In the total fungal community we observed a significant interaction between plant identity and drought (grasses 16.7%; forbs 17.8%), which was mainly captured by host phylogeny (Tables S4 and S5). In contrast, in the pathogenic community a significant interaction between plant identity and drought was only detected in the forbs (14.9% of variance; Table S4).

Despite the fact that the drought response of the total and pathogenic fungal community varied significantly across the 16 plant species (Figs. 2 and 3, Table 2), for the total community we found some general patterns in the relative abundance shifts at high taxonomic level within the two plant functional groups. For instance, drought caused a significant increase ($P < 0.05$) in the abundance related to members of the Hypocreales and Xylariales orders in the grass monocultures. Within forb species, drought significantly ($P < 0.05$) increased the proportion of sequences affiliated to the order Pleosporales, while a significant ($P < 0.05$)

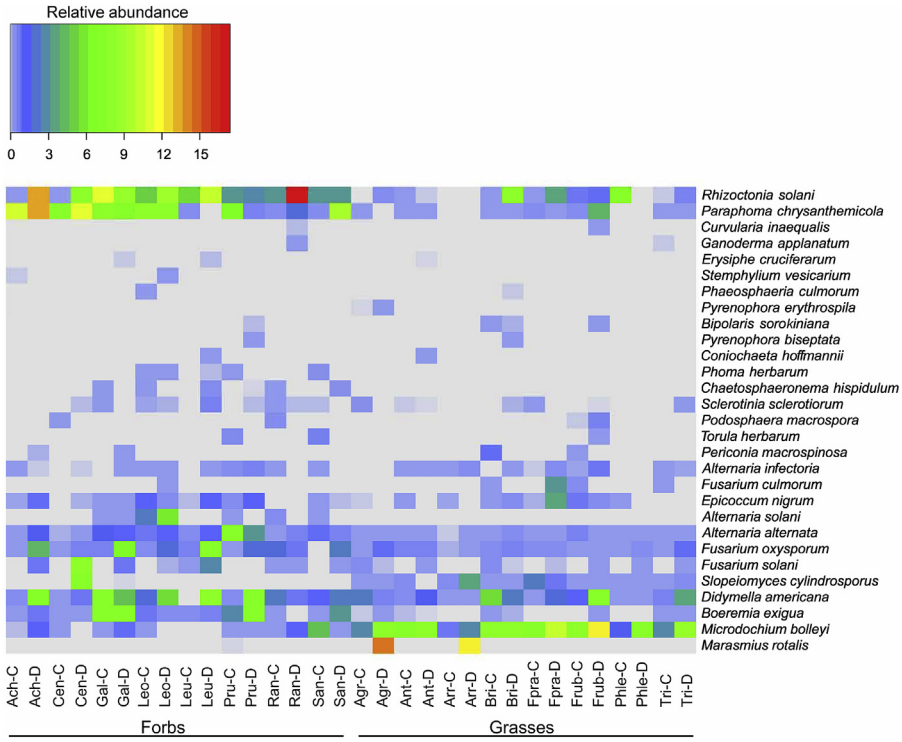


**Fig. 5.** Mean relative abundance of the 29 pathogenic species found across the plant species in the control (C) and drought (D) plots. Species abbreviations are *Agrostis stolonifera* (Agr), *Anthoxanthum odoratum* (Ant), *Arrhenatherum elatius* (Arr), *Briza media* (Bri), *Festuca pratensis* (Fpra), *Festuca rubra* (Frub), *Phleum pratense* (Phle), *Trisetum flavescens* (Tri), *Achillea millefolium* (Ach), *Centaurea jacea* (Cent), *Galium mollugo* (Gal), *Leontodon hispidus* (Leo), *Leucanthemum vulgare* (Leu), *Prunella vulgaris* (Pru), *Ranunculus repens* (Ran) and *Sanguisorba officinalis* (San).

opposite effect was observed for the Sebacinales. Exploring the pathogenic community, we observed that the cumulative abundance of the pathogenic taxa did not differ between the 16 plant species, but it significantly increased ($F_{15,58} = 28.56$, $P < 0.05$) with drought (Fig. 4). Particularly in the grasses *A. stolonifera*, *F. pratensis* and *T. flavescens* and in the forbs *A. millefolium* and *R. repens* drought induced a dramatic increase of pathogenic fungal abundance, since the pathogenic taxa accounted for the 20–32% and 3–10% of the total fungal reads affiliated to these plants in the drought and control plots, respectively (Fig. 4). At the species level, the fungal pathogen *M. rotalis* was only observed in the drought plots of the grasses *A. stolonifera* and *A. elatius* (Fig. 5). *F. solani* was mainly detected only in the drought plots of all the 16 plants with few exceptions (Fig. 5). Interestingly, the relative abundance of the pathogen *M. bolleyi* significantly ($P < 0.05$) increased in the drought plots in all the grasses showing a notable presence in *F. pratensis* and *F. rubra* (Fig. 5). The fungal pathogen *R. solani* increased significantly ($P < 0.05$) its abundance in all drought plots of the forbs plants and the grasses *F. pratensis* and *B. media* (Fig. 5). A similar pattern, but restricted to the forb species, was observed for *P. chrysanthemicola* which was significantly ($P < 0.05$) more abundant in the drought than the control plots (Fig. 5).

## 4. Discussion

Our study demonstrates clear differences in soil-borne fungal communities between a wide range of plant species in grasslands. To a large extent, these differences could be captured by plant phylogeny. This phylogenetic signal was not limited to differences between the two main plant functional groups in grasslands (grasses and forbs), but was also evident within both groups. Our study also highlights that drought can significantly affect the fungal community, and the pathogenic fungi in particular. Under drought, the relative abundance of pathogenic fungi in the different plant species increased. These results support the idea that soil-borne pathogen communities can at least partially be predicted by plant phylogeny. Our results also highlight the importance of environmental conditions - drought in this case - when studying the soil-borne fungal communities (Guo et al., 2020).

### 4.1. Plant predictors of the total and pathogenic fungal community structure

In contrast to aboveground fungal communities (Parker et al., 2015), we have limited understanding of the belowground fungal communities and their belowground associations with different plant species inhabiting natural grasslands. Here, we show that in a common garden experiment, where sixteen different grassland plants have grown for 3 y in the same soil, the roots of these plant species harboured distinct fungal communities. Plant phylogeny was the main predictor of interspecific differences in the total and pathogenic fungal community structure and could also explain the observed differences between plant functional groups (Mommer et al., 2018; Francioli et al., 2020).

Root traits could only partly capture the effect of plant phylogeny on the total and pathogenic fungal community in our study. Root traits have previously been shown to affect the fungal community (Lugo et al., 2015; Wang et al., 2019). For example, root diameter and specific root length can significantly affect the fungal saprotrophic community associated with living roots in grasslands species (Francioli et al., 2020). However, the effects of root traits on the pathogenic fungal community have rarely been investigated. Root architecture and morphology have been hypothesized as prominent traits in relation to pathogen susceptibility (Wehner

et al., 2014; Deveautour et al., 2018), but we found only small effects for the root traits we measured. Perhaps other root traits, such as specific root tip number, which was recently found to be positively correlated with fungal pathogenic richness (Wang et al., 2019) or exudation profiles that may act as signals for pathogens (Doornbos et al., 2012; Yuan et al., 2018) may have a stronger impact on the fungal pathogenic community.

The soil-borne fungal plant pathogens in our study varied along a specialist-to-generalist continuum (Barrett et al., 2009; Koyama et al., 2019). Several of the pathogens were detected in the roots of all the 16 plant species. The presence and abundance of other fungal species were related to either grasses or forbs. Most of the pathogens were only abundant in one or few plant species. Overall, the host ranges of the pathogenic fungi in our study match the reported host ranges in the literature. For instance, in grasses in our study we found *S. cylindrosporus* (synonym *Gaeumannomyces cylindrosporus*) and *M. bolleyi*, which are known as specific pathogens of Poaceae (Klaubauf et al., 2014; Hernández-Restrepo et al., 2016). *P. chrysanthemicola*, known to be specific for the genus *Chrysanthmum* within the Asteraceae, was found in other species of that family in our study (Garibaldi and Gullino, 1981, de Gruyter et al., 2010).

### 4.2. Effect of drought on the fungal community

Drought significantly affected the total and pathogenic fungal community in the roots of the 16 grassland species. These results are in line with other research that has demonstrated that drought can have considerable effects on soil borne microbial communities (Barnard et al., 2013; Santos-Medellín et al., 2017; Ochoa-Hueso et al., 2018; Preece et al., 2019). Recent studies on soil microbes - both fungi and bacteria - reported that soil-borne fungi are generally more resistant to drought than bacteria (Bapiri et al., 2010; Kaisermann et al., 2017, de Vries et al., 2018).

Intriguingly, we found a notable differential drought response of the total and pathogenic fungal community across the sixteen plant species. The drought shifts observed in fungal community structure showed a phylogenetic signal, while plant functional group was able to capture only a fraction of the fungal community variation caused by the induced drought stress. This differential drought response of the root-associated fungi across the sixteen plant species may be due to plant-specific responses to a water deficit, since drought triggers a complex molecular, physiological and morphological response to which associated microbes can actively react (de Vries et al., 2016; Santos-Medellín et al., 2017).

Furthermore, we observed a significant increase in pathogenic fungal reads in almost all 16 plant species under drought. These findings are in accordance with recent studies that have reported increments in pathogenic fungal abundance and incidence of fungal diseases during drought (Choudhary et al., 2016; Preece et al., 2019; Delgado-Baquerizo et al., 2020), probably due to reduced plant performance under this abiotic stress (Chakraborty et al., 2000; Garrett et al., 2006). Further, we found pathogens that have been described to increase in abundance under drought in agricultural systems. For example, *D. americana*, known as pathogens of wheat (*Triticum aestivum*), maize (*Zea mays*) (Boerema, 2004) and beet (*Beta vulgaris* ssp. *vulgaris)* (Vaghefi et al., 2016) was detected in all the 16 plant species and showed a significant increase in its abundance in the drought plots compared to the control ones. A similar result was found for the ubiquitous soil-borne fungal pathogens *R. solani* and *P. chrysanthemicola* (Garibaldi and Gullino, 1981; Lübeck, 2004; Hiddink et al., 2005; de Gruyter et al., 2010; Sturrock et al., 2015; Spurlock et al., 2016; Kevan and Shipp, 2017) and for the grass pathogen *M. bolleyi* (Kane and Smiley, 1987; Hong et al., 2008).

In conclusion, our study demonstrates the central role of host phylogeny in structuring the soil-borne fungal communities associated with the roots of grassland plant species. Our study also demonstrates that abiotic factors, such as drought can affect fungal community composition. Specifically, drought significantly increased both the richness and abundance of the pathogenic fungi in most of the 16 grassland plants, which may lead to enhanced disease risks when climates get drier. However, we also found differential responses of the total and pathogenic community structure to drought across plant species. The challenge for future studies will be to determine the consequences of the species-specific differences in fungal community composition for plant performance. Including variation in environmental factors, such as drought, in these studies will be instrumental to enhance our understanding of plant-pathogen interactions in different environments.

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## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2020.100987.

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