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Effects of rainfall exclusion on soil fungi in a boreal forest landscape

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ABSTRACT

Due to climate change, droughts are increasingly frequent and intense. Yet, their impact on boreal forest fungal communities remains unclear, especially across different fungal functional and taxonomic groups. We induced an experimental rainfall exclusion for 45 summer days, using a paired design of 1 × 1 m treatment and control plots replicated in 25 sites in a boreal forest landscape in Sweden. Immediately after the experiment, we assessed the effects on soil fungal biomass, community composition and, after 2 months, sporocarp production. We did not detect significant effects of the rainfall exclusion on soil fungal biomass, but the fungal community composition was affected. In the rainfall exclusion plots, richness of ectomycorrhizal species with extensive extramatrical mycelia and saprotrophic basidiomycetes was reduced, while richness of ascomycetes was not affected. Sporocarp production of both saprotrophic and ectomycorrhizal fungi was reduced. The clear effects of a small-scale rainfall exclusion demonstrated in our study suggest that belowground fungal communities in boreal forests may be vulnerable to drought.

1. Introduction

Soil fungi play pivotal roles in forest ecosystems, where they mediate decomposition and soil nutrient cycling, and thereby influence resource supply, growth and health of plants. An increase in the frequency and intensity of extreme weather events, such as droughts and heatwaves (von Rein et al., 2016; IPCC, 2023), may alter fungal communities, potentially impacting soil processes and biodiversity (Kaisermann et al., 2015; Li et al., 2019; Liu et al., 2022; Baldrian et al., 2023). Still, studies on drought effects on fungal communities in boreal forests, and how such effects vary across gradients in soil conditions, are surprisingly scarce, especially considering the dominance of fungi in boreal forest soils (Lindahl and Clemmensen, 2016; Pérez-Izquierdo et al., 2021).

Saprotrophic and mycorrhizal fungi are two major fungal guilds with contrasting ecologies that largely dominate boreal forest soils (Lindahl and Clemmensen, 2016). Species within both guilds can play important roles in decomposition and nutrient cycling, but while saprotrophic fungi are free-living and decompose dead organic matter to access both carbon and nutrients, mycorrhizal fungi form close mutualistic relationships with plants to access photosynthetic carbon directly in exchange for soil nutrients and water. Mycorrhizal fungi thereby more directly influence ecosystem productivity and diversity (Mohan et al., 2014; Schmidt et al., 2018; Liu et al., 2022), and can reduce negative effects of abiotic stress, including droughts (Mohan et al., 2014; Liu et al., 2022). While it is well established that drought alters fungal community composition, it remains unclear how fungal taxa differ in susceptibility, and whether differences are linked to ecological guild (e.g., saprotrophs vs. mycorrhizal fungi), functional traits, and phylogenetic relatedness.

Saprotrophic and ectomycorrhizal fungi may differ in their general drought susceptibility for several reasons. One such reason might be that they dominate in different soil layers (Lindahl and Clemmensen, 2016); saprotrophic fungi primarily inhabit the upper organic soil layer with high-energy litter which is the first to dry out, while mycorrhizal fungi reside both in the uppermost litter layer, and further down into the lower organic and mineral soil layers which stay moist longer.

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Furthermore, water limitation directly reduces saprotrophic decomposition activity and carbon assimilation from organic matter, leading to less mycelial growth and sporocarp production (Boddy et al., 2013; Ágreda et al., 2015). In contrast, even if some plants may limit carbon allocation to roots and associated microbial communities during drought events (Ruehr et al., 2009; Fuchslueger et al., 2014), carbon provision from trees to ectomycorrhizal fungi may be sustained through infavourable periods due to large internal carbon stores in tree roots and trunks (Parker et al., 2022). Trees may also access groundwater via hydraulic lift during drought conditions (Voltas et al., 2015), which may benefit ectomycorrhizal symbionts (Querejeta et al., 2003, 2017), although the relevance of this mechanism for tree symbionts under field conditions is still unclear. Some studies indeed suggest larger drought effects on saprotrophs (Castaño et al., 2018; Pérez-Izquierdo et al., 2021). However, greater effects on mycorrhizal fungal biomass and community composition, compared to saprotrophic fungi, have also been documented when trees are drought stressed and allocate less carbon belowground (Querejeta et al., 2021; Castaño et al., 2023; Jaeger et al., 2023).

Among ectomycorrhizal fungi, species vary in mycelial morphological traits, i.e. the exploration type and hydrophobicity of the extramatrical mycelia, which affects how they explore the surrounding soil and transport water and how they respond to drought (Agerer, 2001, 2006; Lilleskov et al., 2011). Species with long-distance exploration types and that form hydrophobic rhizomorphs, have been suggested to be more resistant to drought, since they can explore the soil for resources at larger spatial scales (Jalón et al., 2020; Boczoń et al., 2021; Castaño et al., 2023), but the opposite has also been found (Fernandez et al., 2017; Castaño et al., 2018; Querejeta et al., 2021). Furthermore, hydrophilic hyphae (often found in short-distance exploration types) lose water more easily through osmotic processes compared to hydrophobic hyphae (Fernandez et al., 2023). Among saprotrophic species, drought effects may vary across species that utilize different substrates (Manzoni et al., 2012), while at higher taxonomic levels, ascomycetes are generally better adapted to harsher and drier habitats and may be less sensitive to drought compared to basidiomycetes (Karst et al., 2014; Sterkenburg et al., 2015).

To gain a comprehensive understanding of how drought affects fungal communities, we evaluated the effects of an experimental rainfall exclusion during the summer on soil fungal biomass, species richness and community composition, as well as on sporocarp production. We experimentally intercepted all precipitation for 6 weeks at 25 sites across a boreal forest landscape in Sweden. We did this using 2 × 2 m sized rainout shelters that dried out the soil. At the end of the rainfall exclusion, we compared fungal biomass, community composition and sporocarp production under the rainout shelters and in paired control plots receiving ambient precipitation. Fungal biomass was assessed with phospholipid fatty acid (PLFA) analysis, and fungal species richness and taxonomical and functional community composition were evaluated based on sequencing fungal internal transcribed spacer 2 (ITS2) markers. Finally, we assessed sporocarp abundance and species richness during the autumn after the rainfall exclusion, to assess effects on the reproductive part of the fungal life cycle. We hypothesized that: 1) rainfall exclusion will reduce soil fungal biomass, alter species composition, and reduce sporocarp production, and 2) saprotrophic fungi will be more sensitive to rainfall exclusion than ectomycorrhizal fungi, basidiomycetes more sensitive than ascomycetes, and ectomycorrhizal fungi with hydrophilic, short-distance exploration mycelium will be more sensitive than fungi with hydrophobic, long-distance exploration mycelium.

2. Methods

2.1. Study area

The study was conducted in a boreal forest area of approximately 5 × 5 km in Västmanland county, Sweden (59.79521° N, Longitude: 15.90699° E, Fig. 1a). The area consists of natural and managed forests dominated by spruce (*Picea abies*), mixed with pine (*Pinus sylvestris*), birch (*Betula* spp.) and aspen (*Populus tremula*). The understory vegetation consists of bryophytes (mainly *Hylocomium splendens* and *Pleurozium schreberi*) and in some places Ericaceae shrubs (mostly *Vaccinium* spp.) or herbaceous vegetation. Boreal forests typically have Podzol soil (low pH-H_2O of 4.6 in the mineral soils with an organic layer on top, and extensive leaching of Fe), although some locations in this study have more mull-type soils, where there is a more mixed organic horizon.

The climate has distinct seasonality with a mean temperature of approximately 15 °C during summer and −4 °C during winter. The mean annual precipitation is around 700 mm of which 250 mm falls during the summer months June to August. Future projections for precipitation in this area remain uncertain, but higher temperatures will result in higher evaporation that will likely reduce soil water availability, especially in summer (Sjökvist et al., 2019; Grau-Andrés et al., 2022).

2.2. Experimental design

We experimentally excluded rainfall over a 45-day period during the summer of 2021 (2nd of June - 16th of July), by installing 25 rainout shelters. We used a paired design, with one treatment plot (rainfall exlusion) and one control plot (receiving ambient rainfall), at each site (Fig. 1). We replicated this across a boreal forest landscape, allowing for a substantial variation in local environmental conditions such as soil moisture and nutrient status (Fig. 1B–Table S1). We chose a duration of 45 days to imitate the extreme summer drought of 2018, which lasted 36 days in some parts of central Sweden (Koelemeijer et al., 2022). We chose a duration slightly longer than this observed drought to compensate for the fact that we were unable to experimentally simulate the heatwave and thus higher vapor pressure deficits experienced under the drought in 2018. The rainout shelters were 2 × 2 m surrounding the 1 × 1 m central plots, leaving a 0.5 m buffer zone around the sampling plots (Supplementary methods 1). The paired control plots received ambient rainfall, which was around 87 mm spread over 13 days during the 45-day study period (SMHI, 2022; Skinskatteberg weather station). In boreal forest soils, rainfall typically infiltrates quickly, resulting in minimal surface runoff and reducing the likelihood of runoff entering experimental plots.

2.3. Soil sampling

We collected three topsoil samples from randomly selected places within each of the plots at 0–10 cm depth with a 2 cm diameter auger on July 16, 2021, at the end of the 45-day rainfall exclusion. This depth, including both organic and mineral soil, was chosen to capture the layer that was likely to be most affected by the treatment and that harbored the highest fungal biomass and activity (Leckie, 2005; Fritze et al., 2000). We pooled and homogenized the three samples from each plot and sieved out larger roots and debris in the field over a 2 mm mesh. All sampling equipment was sterilized with water and 75 % ethanol between samples to minimize cross contamination. Soil samples were stored at −18 °C and freeze-dried before PLFA analyses and DNA extractions.

2.4. Site-level soil measurements: moisture and nutrients

In order to pick up variation in soil conditions among our study sites, we measured soil moisture and chemistry at each plot (rainfall exclusion and control plot) at each site. We measured soil moisture (up to 14 cm depth) in the middle of each control and treatment plot with TMS-4 (Temperature Moisture Sensor) loggers (TOMST, Wild et al., 2019), at a 15-min interval during May (the month before the experiment) and the whole experimental period. Soil moisture loggers were installed to quantify variability in soil moisture across sites, rather than to provide

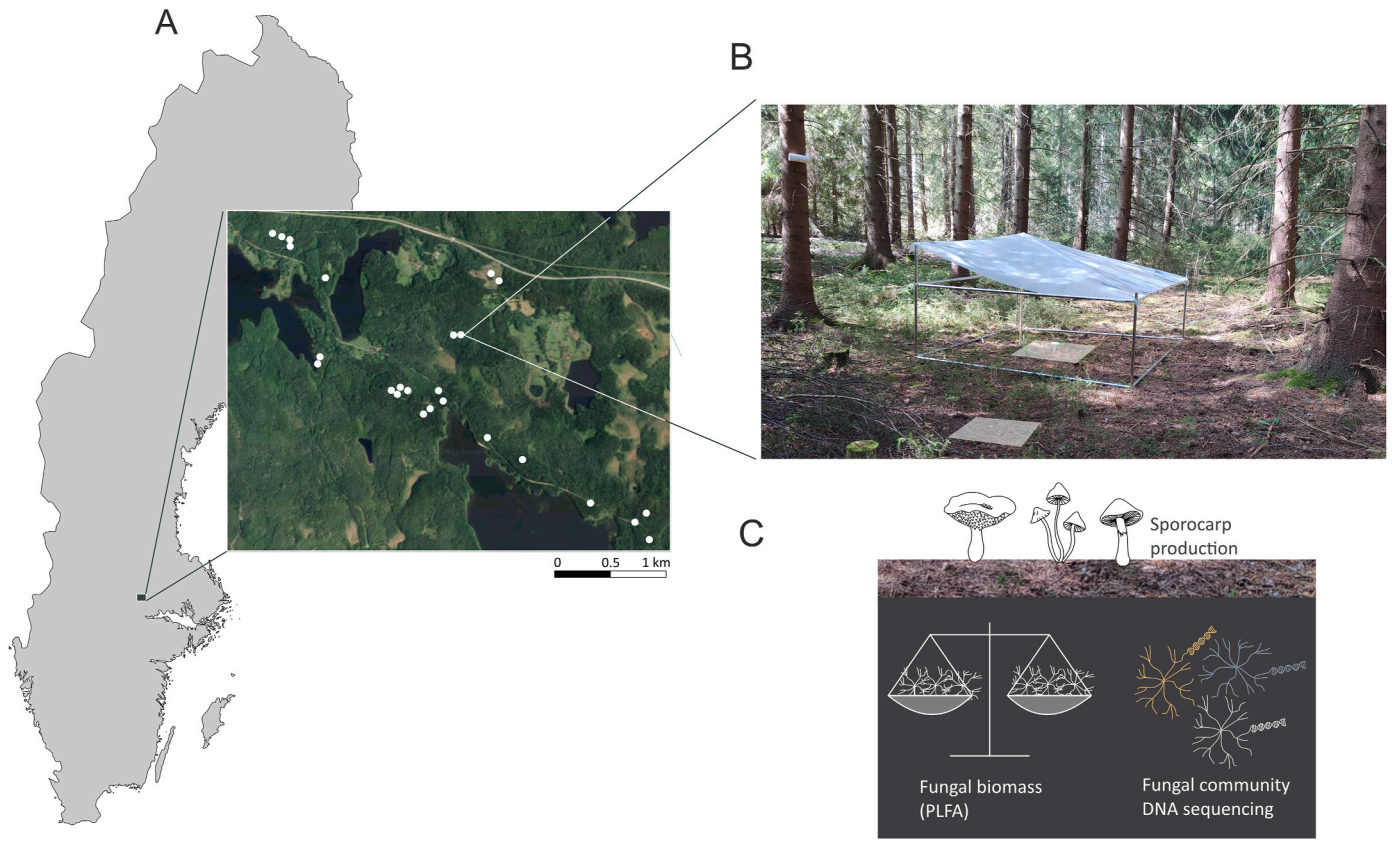

Fig. 1. The study set-up. (A) A map of Sweden, indicating the location of the study area with the 25 sites. (B) An example site with a rainout shelter and a paired control plot. (C) An overview of the measurements taken at each plot, which include PLFA analysis for fungal biomass, DNA sequencing for community composition, and sporocarp collection.

us with an absolute value of experimental soil moisture reduction. The soil moisture loggers have sensors penetrating into the lower soil layer (0–14 cm), while the drying effect was likely largest in the top-soil and litter layer (where there is also most microbial activity and biodiversity). The soil moisture values do therefore not fully represent the magnitude of drying in the study. Soil samples for nutrient analyses were taken from the treatment and control plots at the end of the experimental rainfall exclusion, sieved over a 2 mm mesh, oven-dried at 40 °C, and stored at room temperature. For total nitrogen and carbon, the samples were combusted at 1150 °C and the gases were measured by a thermal conductivity detector in a CNS elemental analyzer (vario Macro Cube, Elementar, Germany). Bioavailable phosphorus (P) was measured by extraction in $NaHCO_3$ (P-Olsen; according to ISO 11263:1994(E)) and colorimetric measurement according to the malachite green procedure (Lajtha et al., 1999). Exchangeable calcium (Ca), magnesium (Mg), and potassium (K) concentrations were measured by inductively coupled plasma optical emission spectroscopy (Thermo Scientific™ iCAP™ 7400 ICP-OES) after extraction in 0.1 M $BaCl_2$ (NEN 5738:1996).

2.5. PLFA analyses

Phospholipid fatty acids (PLFAs) were extracted from 1 g freeze-dried soil and identified with a modified method of Bligh and Dyer (1959) and White et al. (1979), at the SLU Biogeochemical Analyses Laboratory (BAL) in Umeå, Sweden. Of the 36 PLFA markers, four were indicative for fungi (including saprotrophic fungi and ectomycorrhizal fungi): 18:2ω6,9c, 18:1ω9c, 18:3ω3c, 18:3ω6c (an overview including references is shown in Table S2). The biomarker 18:1ω9c also occurs in bacteria (Table S2), so interpretation of this PLFA should be done with this in mind.

2.6. Soil fungal DNA extraction and amplification

We extracted DNA from 0.1 g freeze-dried and milled soil using the NucleoSpin Soil Genomic DNA isolation kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. We diluted the templates to the concentration 1 ng/μL. We PCR amplified the ITS2 region in a 2720 Thermal Cycler (Life Technologies, Carlsbad, CA, USA), using the forward primer gITS7 (Ihrmark et al., 2012), and the reverse primers ITS4/ITS4arch (White et al., 1990; Sterkenburg et al., 2018), that were elongated with unique tags of 8 bases for each individual sample (Clemmensen et al., 2023). The 50 μl PCR reactions included 25 ng DNA template, 1 × buffer, 200 μM of each nucleotide, 0.75 mM $MgCl_2$, 0.025 U μl^{-1} polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA), and 0.5, 0.3 and 0.1 mM of gITS7, ITS4 and ITS4a primers, respectively. The PCR cycles were 5 min at 95 °C, followed by 28–30 cycles of 30 s at 95 °C (optimized for each sample based on visual inspection on 1 % agarose electrophoresis gels), 30 s at 56 °C, 30 s at 72 °C and 7 min at 72 °C (Castaño et al., 2020). We amplified samples in duplicates, including two negative extraction controls and PCR controls. We purified the PCR products with the AMPure kit (Beckman Coulter Inc. Brea, CA, USA), measured DNA concentration with a Qubit fluorometer (Life Technologies, Carlsbad, CA, USA) and pooled equal amounts (20 ng) of DNA from each PCR product. We purified the pooled sample with the ENZA Cycle Pure kit (Omega Bio-Tek, Norcross, GA, USA), and conducted final quality controls using Qubit and the Bio-Analyzer DNA chip (Agilent Technologies, Santa Clara, CA). The sample was sequenced on a Pacific Biosciences Sequel 2 SMRT cell after ligation of sequencing adaptors (Uppsala genome center, SciLifeLab).

2.7. Bioinformatics and rarefaction

We used the SCATA pipeline (https://scata.mykopat.slu.se/) for quality control and clustering of the obtained sequencing reads. We discarded reads that contained bases with a quality score lower than 10, full reads with an average quality score less than 20, and reads shorter than 200 bases. We reduced homopolymers to three bases and then clustered reads with a minimum similarity of 98.5 % to the closest neighbor, into species-level clusters (from here designated "species").

We identified the most common genotype of each fungal species by aligning it to the UNITE (version 9) and INSD databases using massBLASTer, with a threshold of 98.5 % similarity (Kõljalg et al., 2013; Nilsson et al., 2019), and filtered out non-fungal sequences. To cross-validate our identifications, we compared results with SH (species hypothesis) matching in UNITE and the automatic taxonomic assignments generated during clustering in SCATA. In cases of discrepancies between sources, we used the lowest available taxonomic level shared by all (e.g., genus instead of species). We rarified our dataset to 12,499 reads per sample, which was the smallest number in any sample. This was done using the rrarefy(.) function from the *vegan* package, resulting in a resampled species-by-site matrix. We manually curated the taxonomic identifications of 1899 out of 4220 species-level clusters in total, covering 98 % of all reads.

We identified 93 % of the species to phylum, 45 % to family, 39 % to genus, and 21 % to species. Of the reads, 96 % were identified to phylum, 75 % to family, 71 % to genus, and 43 % to species. We categorized species that were at least identified to genus level, into mycorrhizal or saprotrophic guilds using the FungalTraits database (Põlme et al., 2020). The saprotrophic fungi were distinguished by substrate affiliation (soil, litter, wood) when possible. Saprotrophic soil fungi include some taxa, e.g. *Oidiodendron*, *Hyaloscypha* and *Archaeorhizomyces,* that can switch between saprotrophic and endophytic lifestyle (Terhonen, 2021). For ectomycorrhizal fungi we assigned extramatrical mycelium exploration type (pooled into short, medium and long-distance) and hydrophobicity (hydrophilic and hydrophobic) following Agerer (2006, 2001), Lilleskov et al. (2011) and Jörgensen et al. (2023) (Table S3). When there were discrepancies between studies or within genera, we assigned the focal taxa to the exploration type of the most abundant species.

From this dataset, we calculated the species richness and relative number of reads for the separate fungal subgroups in each sample. The number of reads and species included in each subgroup, and the proportion they represent of the total dataset, can be found in Table S4.

2.8. Sporocarp production

To provide a snapshot of fungal fruiting activity, we collected all visible sporocarps in the 1 × 1 m treatment and control plots on one occasion during peak autumn mushroom production (15–18 September 2021), approximately two months after the rainfall-exclusion treatment had ended. Sporocarps were identified to species and were categorized into mycorrhizal or saprotrophic guilds. We calculated the abundance and species richness of each group for each plot. Four percent of the specimen were too old for identification (n = 18 out of 447).

2.9. Statistical analyses

All statistical analyses were conducted in the software R (version 4.1.3, R core Team, 2022).

2.9.1. Site-level soil moisture and nutrients

We tested the effects of the rainfall exclusion treatment on soil moisture, and soil (trace)-nutrient levels and pH, using linear mixed effect models using the lme4 package (Bates et al., 2015). To analyze the effects of the treatment on soil moisture at the level of the loggers (down to 14 cm below ground), we modeled soil moisture levels in each plot (rainfall exclusion treatment and control plot) as a function of the treatment, with site included as a random factor to account for our paired design. To account for pre-existing spatial variation in soil moisture prior to the experiment, we included soil moisture in May (the month prior to the experiment) as a co-variate. In order to assess how the drought treatment affected the soil chemistry, we modeled the different soil chemistry variables (%N, %C, pH, Phosphorous, Calcium, Potassium and Magnesium) as a function of the rainfall exclusion treatment, assuming a gaussian distribution and including site as a random effect.

Fungal community responses to rainfall exclusion may depend on site characteristics. To account for site-level variation in soil conditions on effects of the drought treatment, we used estimates of soil moisture and soil nutrient levels in all statistical models. To capture differences in soil moisture across our 25 sites, as well as initial differences between control and treatment plots, we used measurements of soil moisture in May (the month before the experiment) from both treatment and control plots at each site. In order to capture differences in soil properties over our 25 sites, we conducted a principal component analysis (PCA) on the soil nutrient and chemistry properties from the control plots. Since the rainfall exclusion experiment likely altered soil properties in the treatment plots, we used only control plots to establish a baseline for soil nutrients and chemistry at each site. To address potential correlation among soil variables and limited statistical power, we used a PCA to reduce dimensionality while retaining the overall information of all soil variables. We extracted the PCA axis 1 as a site-level variable for soil nutrient levels that we used as a predictor in subsequent statistical modeling, where higher PCA values indicated higher nutrient levels (in terms of C and N, Fig. S2). PCA axis 1 explained 46 % of the variation.

2.9.2. Rainfall exclusion effects on fungal biomass (PLFA)

In order to test if fungal biomass was lower in the rainfall exclusion plots compared to the control, we modeled the fungal biomass (concentration of fungal PLFAs in nmol/g) as a function of the treatment, in interaction with the ambient soil moisture and nutrient variables. We used linear mixed effect models assuming a gaussian distribution and included site as a random effect. We repeated these models for the different fungal markers.

2.9.3. Rainfall exclusion effects on fungal species richness and community composition

In order to investigate effects of the rainfall exclusion on the fungal community composition, we conducted analyses of the overall community composition, as well as the species richness and relative abundances of different fungal groups. A community can be expected to first respond to rainfall exclusion by shifting relative abundances of existing species. Over time, or in cases of extreme drought, this might result in species losses.

We first tested if the overall community composition differed between the treatment and control plots by conducting a multivariate Permanova (Permutational Multivariate Analysis of Variance) on a Bray-Curtis dissimilarity. We included the soil variables as covariates. We used function *adonis2()* from the *vegan* package (Oksanen et al., 2013), set at 999 permutations and included site as random effect using the strata argument.

We then tested if the rarefied number of species differed between the rainfall exclusion plots and the control plots, by modeling species richness as a function of treatment, in interaction with the soil variables. We performed generalized linear mixed effect models (*glmer* function from the *lme4* package) assuming a negative binomial or Poisson distribution, after verifying the best fit based on model diagnostics and AIC comparisons. We included site as a random effect, and repeated these models for the different fungal subgroups.

To investigate whether the rainfall exclusion shifted the relative species richness and abundances of different groups, we modeled the counts of each group using the *cbind*() function in a binomial generalized mixed effect model (*glmer* function from the *lme4* package), with

treatment and soil variables as fixed effects, and site as a random effect. In cases of overdispersion, we added a row-level random effect to account for excess residual variation (Hartig, 2022). We contrasted the following groups: ascomycetes vs. basidiomycetes, saprotrophs vs. ectomycorrhizal fungi, subgroups of saprotrophs (ascomycetes vs. basidiomycetes and different substrate groups), and ectomycorrhizal mycelial traits (extramatrical mycelial exploration types and hydrophobicity).

In order to investigate which genera were more or less affected by the rainfall exclusion, we aggregated species into genera and conducted a CLAM test (multinomial species classification method) on a relative abundance genus-by-site matrix, using the *vegan* package. This method employs a multinominal classification framework to classify whether taxa are disproportionately associated with a categorical environmental factor (in our case rainfall exclusion versus control plots) (Chazdon et al., 2011; Fernandez et al., 2017). We only considered the results for genera that were present in at least 15 sites and assigned specialist-group with a $p < 0.005$, in order to adjust for multiple comparisons and to reduce the influence of sampling error on the results.

2.9.4. Rainfall exclusion effects on sporocarp production

In order to test the effects of the rainfall exclusion on sporocarp production, we modeled sporocarp abundance and species richness as a function of the rainfall exclusion treatment, in interaction with the soil moisture and nutrient variables. We used generalized mixed effect models with site as a random effect and assuming a negative binomial or Poisson distribution. We conducted these models for the total community, and for saprotrophic and ectomycorrhizal fungi separately.

Model diagnostics were checked using the packages DHARMa and Performance, including tests for residual normality, heteroscedasticity, and overdispersion, to ensure adequate model fit (Hartig, 2022; Lüdecke et al., 2021).

3. Results

3.1. Effects of experimental rainfall exclusion on soil moisture and nutrients

Soil moisture (measured up to a depth of 14 cm) showed a clear difference in dynamics between control and rainfall exclusion plots, indicating that the rainout shelters effectively intercepted rainfall and created relatively drier conditions. The control plots showed clear moisture increases after precipitation events, while these distinct soil moisture peaks following rainfall events were absent in the treatment plots (Fig, S3). Average soil moisture was lower under the rainout shelters (34 %) compared to the control plots (39 %) during the experimental period ($p = 0.006$). Prior to the experiment, soil moisture did not differ between treatment and control plots ($p = 0.15$). However, we lacked measurements from the topsoil and litter layer, which appeared to be the driest based on personal observations.

The rainfall exclusion plots had significantly lower pH, plant-available calcium, potassium and magnesium, but rainfall exclusion did not affect plant-available phosphorus nor C/N content (Table S5).

3.2. Effects of rainfall exclusion on fungal biomass, community composition and sporocarp production

We found no significant effects of the rainfall exclusion on the fungal biomass (fungal PLFA markers) in the soil (Table S6).

Belowground, we detected 4220 fungal species in 285 families and 524 genera. The majority of the species belonged to the Ascomycota (35 %) or Basidiomycota (36 %) (Table S4). For the species that we could identify to genus and to which we could assign to guild, 24 % of the species were ectomycorrhizal and 59 % saprotrophic. The fraction of species belonging to other phyla and functional guilds can be found in Table S4. The rainfall exclusion significantly affected the fungal species-level community composition (Permanova, $p = 0.01$, $R^2 = 0.017$, Table S8).

Across all plots, we found 447 sporocarps representing 61 species. The most commonly observed taxa were *Cortinarius* subgenus *Telamonia*, *Strobilurus* sp., *Marasmiellus perforans*, *Galerina* sp. and *Laccaria laccata*. Sporocarp abundance was significantly lower in the rainfall exclusion plots compared to the control plots for both saprotrophic and ectomycorrhizal species (Fig. 2A, Table S10). We did not find effects of the rainfall exclusion on the species richness of sporocarps (Fig. 2A). Sporocarp reductions were particularly profound in places in the landscape with already low soil moisture levels prior to the treatment (Fig. S4B), especially for ectomycorrhizal fungi (Table S10).

3.3. Effecs of rainfall exclusion differ among taxonomical and functional groups

Species richness of belowground fungal communities was lower in the rainfall exclusion plots than in the control plots for several groups (Fig. 3, Table S8). Basidiomycete species richness was lowered by rainfall exclusion, while ascomycete richness was unaffected, leading to a relatively higher contribution of ascomycete species to total and saprotrophic species richness (Table 1). We found an overall lower species richness of both ectomycorrhizal and saprotrophic guilds in the rainfall exclusion plots (Fig. 3). Within the ectomycorrhizal guild, species with long and medium extramatrical mycelial exploration types, and that were hydrophobic, were negatively affected by rainfall exclusion, whereas species with short exploration types and hydrophilic mycelia were not significantly affected (Fig. 3). This led to a relative increase in ectomycorrhizal fungal species with short exploration types, compared to longer exploration types (Table 1). Finally, the relative abundance of saprotrophic species associated with litter increased relative to those associated with soil and wood (Table 1). Effects of the rainfall exclusion on saprotrophic basidiomycete species richness and on soil saprotrophic species richness were lower in sites with higher pre-treatment soil moisture (Table S8, Fig. S4A). Furthermore, differences in species richness between treatment and control plots were larger in sites with higher soil nutrient levels, mainly for saprotrophic fungi, but this effect seemed to be mainly driven by two outlier sites (Table S8, Fig. S4B).

Rainfall exclusion affected neither the relative abundance of the ectomycorrhizal nor the saprotrophic guild (Table 1). However, we found variation among genera within guilds in response to drought. Using the CLAM test, we identified several genera that had higher vulnerability to rainfall exclusion (lower relative abundance in the treatment plots) or that were tolerant (higher relative abundance in the drought plots). Within the ectomycorrhizal guild, the genera *Inocybe, Leccinum, and Hebeloma* were identified as tolerant, and *Suillus, Hygrophorus, Alpova* as vulnerable (Fig. 4A, Table S9). Within the saprotrophic guild, the CLAM test identified the genera C*ladosporium, Hyaloscypha, Lachnum, Clitopilus, Hormonema, Pseudopenidiella* (almost all ascomycetes) as tolerant, and *Geminibasidium, Cryptococcus, Hypholoma, Dissophora, Galerina, Agaricus, Absidia, Leucosporidium, Acremonium, Ampulloclitocybe, Chloridium, Curvibasdium, Cystoderma, Goffeauzyma, Hypochnicium* (most of them basidiomycetes) as vulnerable (Fig. 4B–Table S9).

4. Discussion

Our study shows that small-scale rainfall exclusion on the forest floor altered the community composition of soil fungi. The rainfall exclusion resulted in a reduction in species richness and relative abundances of several taxonomical and functional fungal groups, even if total fungal biomass in the soil was sustained. Rainfall exclusion also reduced aboveground sporocarp formation. Given the small-scale of the rainout shelters (2×2 m) and modest effects on soil moisture levels in the lower soil layer down, these observed effects are likely conservative in relation to effects after real summer drought events.

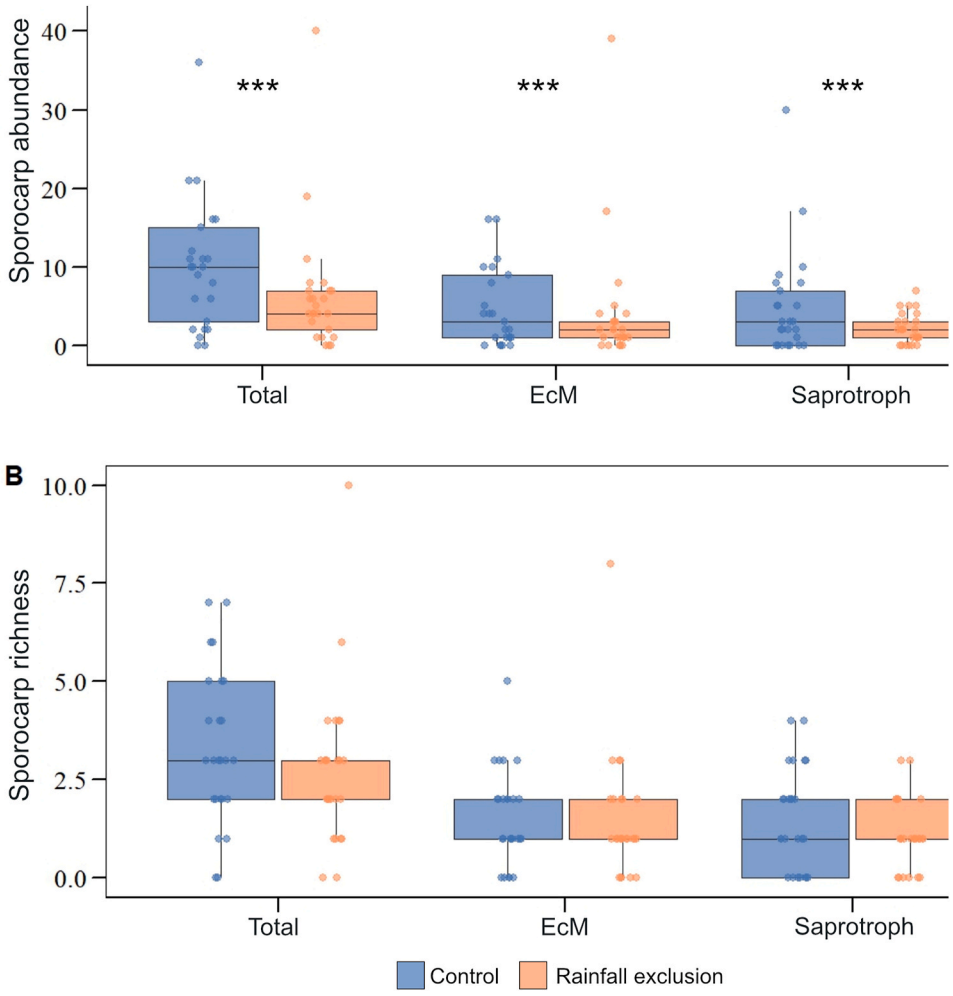

Fig. 2. Effects of the rainfall exclusion on (A) sporocarp abundance and (B) species richness in total, on ectomycorrhizal fungi and saprotrophic fungi. Statistical analysis was performed using mixed effect linear models for abundance and generalized mixed-effects models for richness, assuming a negative binomial or Poisson distribution. Significance is indicated as follows: ***p < 0.001. Test statistics can be found in Supplementary information, Table S10.

4.1. The effects of rainfall exclusion on fungal biomass, community composition and sporocarp production

Fungal biomass was sustained after the rainfall exclusion, in contrary to our first hypothesis. This indicates that fungal communities are relatively tolerant to drought, potentially due to their often filamentous life form, thick cell walls and because they can accumulate osmolytes in their cells without impairing metabolism (Schimel et al., 2007; Manzoni et al., 2012; de Vries et al., 2018; Osburn et al., 2022; Jaeger et al., 2023). Furthermore, soil moisture levels may have been sufficient for many fungal groups, and species differ in their ability to exploit this moisture. However, in accordance with our first hypothesis, we observed a shift in belowground community composition in the treatment compared to the control plots. Some species seem to have disappeared or at least declined to undetectable levels, whereas others increased in relative abundance, possibly due to reduced competition (see paragraph 4.2). Sporocarp abundance of both saprotrophic and ectomycorrhizal fungi was reduced as well, indicating that summer drought is an important driver of the reproductive part of the fungal lifecycle, in line with our first hypothesis and previous studies (Ogaya and Peñuelas, 2005; Boddy et al., 2013; Jarvis et al., 2017; Karavani et al., 2018). Fungal fruiting is an energy expensive process and requires accumulation of resources over a period of time (Boddy et al., 2013). When water is limited, there may be a trade-off between maintaining mycelial biomass and reproduction, which may explain the contrasting effects on belowground biomass and sporocarp abundance in our study. Such trade-offs remain poorly understood in fungi and opposite patterns, i.e. higher allocation to sporocarp production at the expense of belowground fungal biomass after environmental stress, have also been found (Collado et al., 2019). Finally, is plausible that small-scale rainfall exclusion affected sporocarp production more directly than belowground DNA and biomass, which may reflect more persistent or dormant components of the fungal community.

Our results suggest that negative effects of rainfall exclusion on fungal species richness and sporocarp production were buffered in generally wetter sites. In generally drier sites, there were larger negative effects of the rainfall exclusion on fungal species richness and sporocarp abundance, while in wetter sites there were minimal effects, indicating that moisture levels remained sufficient to support fungal performance.

4.2. Variation in responses to the rainfall exclusion is associated with mycelial traits, substrate affiliation and taxonomy

We found a lower species richness of both saprotrophic and ectomycorrhizal fungi in the rainfall exclusion plots, contrary to our second hypothesis that saprotrophic fungi would be more vulnerable due to their dominance in the upper soil layer and their dependence on water for decomposing organic matter (Pérez-Izquierdo et al., 2021). As such, there was no shift in the ratio saprotrophic vs. ectomycorrhizal fungal species and abundances, which is surprising as these two functional

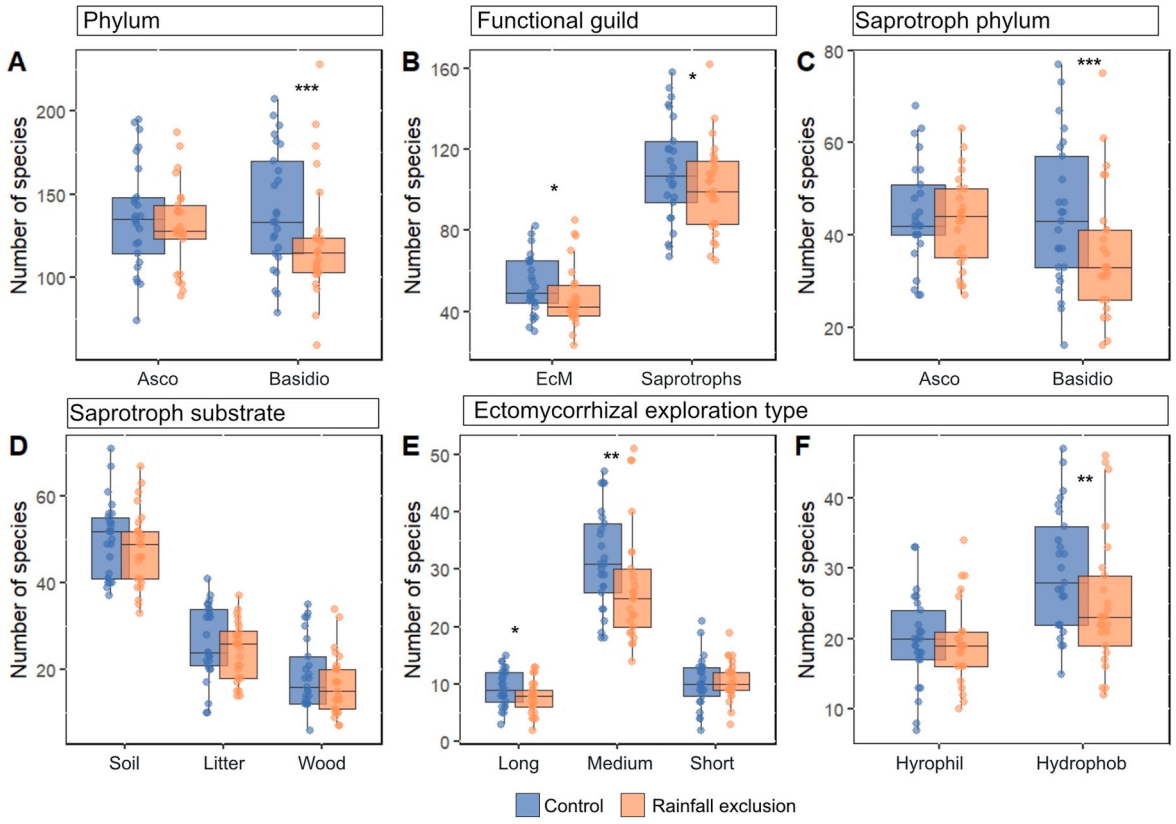

Fig. 3. Effects of the rainfall exclusion on the rarefied species richness (top row, a-f, blue = control plots, orange = treatment plots) Statistical analysis was performed using generalized mixed-effects models, assuming a negative binomial or Poisson distribution, after verifying which was the best fit based on the model diagnostics and AIC. Significance is indicated as follows: ***p < 0.001, **p < 0.01, *p < 0.05. Test statistics and R^2 can be found in Supplementary information, Table S8. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Effects of the rainfall exclusion and soil characteristics on the ratios of different fungal groups in both number of species and number of reads. The effect sizes are given as incidence rate ratios, where below 1 indicates a negative effect and above 1 a positive effect. The soil moisture variable picks up the variability in moisture across sites, rather than the treatment effects. Statistical analysis was performed using generalized mixed-effects models, where the response variable was the counts of two groups (using the *cbind*(.) function), including site as a random effect and specifying a binomial distribution. The marginal R^2 (i.e. of only fixed effects) is given.

Group	Proportional	Rainfall exclusion	Soil moisture	Soil chemistry	Rainfall exclusion* Soil moisture	Rainfall exclusion* Soil nutrients	R^2
Ascomycetes:	Species	**1.10****	0.97	0.94	0.94	1.05	0.004
Basidiomycetes	Reads	1.09	0.88	1.15	1.10	1.21	0.016
Saprotrophs:	Species	1.02	**0.89***	1.05	1.02	0.94	0.003
Ectomycorrhizal	Reads	1.10	**0.56***	1.20	1.16	0.94	0.045
Ectomycorrhizal							
Hydrophilic: Hydrophobic	Species	1.16	1.03	1.02	0.94	1.01	0.002
	Reads	1.18	1.27	0.89	0.91	1.23	0.010
Short: Medium/Long	Species	**1.23***	1.10	0.95	0.88	1.06	0.004
	Reads	1.11	1.53	1.34	0.74	1.08	0.056
Saprotrophic fungi							
Ascomycetes:	Species	**1.17** *	0.91	0.92	0.89	1.15	0.011
Basidiomycetes	Reads	1.13	0.86	1.00	1.20	1.15	0.006
Litter: soil	Species	0.97	1.00	1.04	0.97	0.99	0.000
	Reads	**1.87****	0.89	1.35	0.95	1.04	0.037
Litter: wood	Species	1.04	0.98	0.87	0.92	1.07	0.008
	Reads	**2.66** ***	0.62	0.76	1.12	1.33	0.101
Wood: soil	Species	0.93	1.01	1.21	1.05	0.93	0.010
	Reads	0.70	1.38	1.78	0.83	0.80	0.079

Significance is indicated as follows: ***p < 0.001, **p < 0.01, *p < 0.05. n = 50.

guilds are known to directly interact and compete (Fernandez and Kennedy, 2016). For example, ectomycorrhizal fungi can in some cases outcompete saprotrophic fungi (Lindahl et al., 1999; Lindahl and Olsson, 2004). Ectomycorrhizal fungi might have an advantage over saprotrophs during drought because they obtain carbon and potentially water from their host trees (Castaño et al., 2018; Querejeta et al., 2021), depending on the drought intensity and duration.

While species richness of both saprotrophs and ectomycorrhizal fungi was reduced in the rainfall exclusion plots, there were diverging responses within these guilds depending on traits related to mycelial morphology and substrate affiliation. Specifically, extramatrical mycelial exploration type and hydrophobicity of ectomycorrhizal fungi were

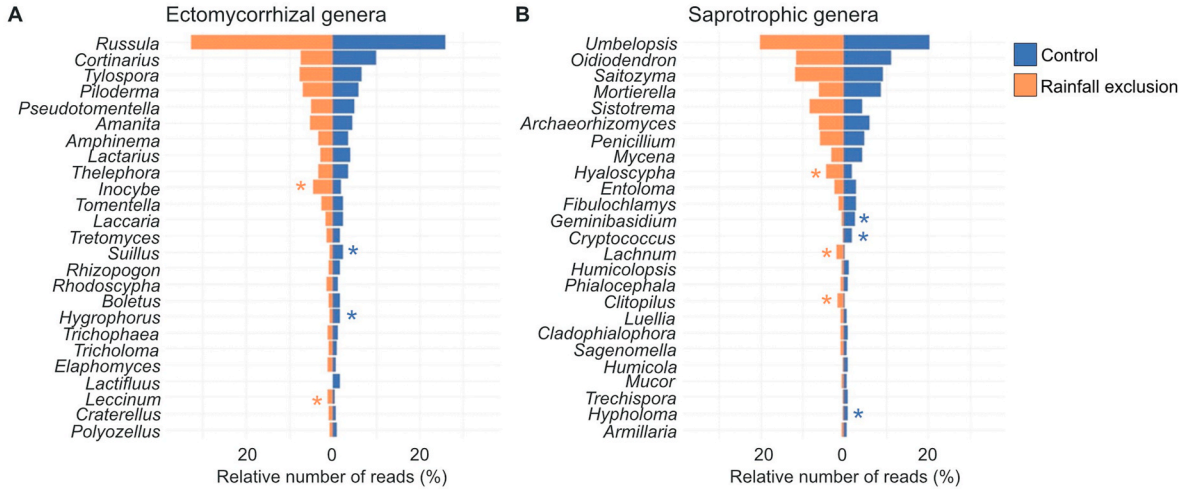

Fig. 4. The 25 most frequent genera within the ectomycorrhizal (A) and saprotrophic (B) guilds and their relative read abundance in rainfall exclusion (orange) and control plots (blue). We indicated genera that were identified by the clam test as tolerant or vulnerable with a star (*) (orange and, blue respectively). Several less abundant genera were also identified as tolerant or vulnerable (see Table S9). Some ascomycete genera included in the saprotrophic guild can include putative or facultative root associated species, including *Oidiodendron*, *Hyaloscypha* and *Archaeorhizomyces* (11 %, 3 % and 6 % of the reads respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

related to how they responded to the drought treatment. Against our hypothesis, the number of species with more extensive exploration types (long-distance and medium distance extramatrical mycelia) was lower in the rainfall exclusion plots. Far-ranging extramatrical mycelia with hydrophobic rhizomorphs can spatially explore resources and reallocate this across significant distances (Agerer, 2001; Jalón et al., 2020; Boczoń et al., 2021; Castaño et al., 2023), which has been suggested to make them relatively drought resistant (Pérez-Izquierdo et al., 2021). At the same time, this allows them to reallocate biomass, as well as fruiting bodies, outside of the treatment plots, where water was available (Boddy and Watkinson, 1995; Lindahl and Olsson, 2004; Hawkes et al., 2011), and our study suggests that ectomycorrhizal fungi with long-distance exploration types may be able to re-distribute their mycelial biomass during drought for optimal exploitation of spatially patchy soil moisture. Mycorrhizal fungi with short distance exploration types did not decrease in species richness after rainfall exclusion. This could be because short distance exploration types may have more access to water, since they have their biomass in close proximity to roots where water may be more readily available through hydraulic lift, compared to long-distance exploration types that may reside partly in the bulk soil (Fernandez et al., 2017; Castaño et al., 2018; Preece, 2019; Querejeta et al., 2021). Besides the direct effects of rainfall exclusion on mycorrhizal fungi that we assessed in this study, indirect effects through drought stressed host trees may be important during natural drought events. Constrained photosynthetic activity during drought reduces carbon allocation belowground to mycorrhizal fungi (Hagedorn et al., 2016; Gao et al., 2021). Short or contact exploration types pose a lower carbon cost on host trees and may be favored relative to species forming ample mycelium during drought when carbohydrates are scarce (Hagenbo et al., 2021; Fernandez et al., 2023). However, higher carbon allocation to long-distance exploration types during drought has also been found for arbuscular mycorrhizal fungi and for the some ectomycorrhizal genera (Wang et al., 2021; Forczek et al., 2022; Fernandez et al., 2023). In our case, the local effect of the treatment (i.e. rainfall exclusion applied to a small soil patch rather than the entire tree) may have primarily hampered root tip production and carbon allocation to roots within the upper soil layer of the rainfall exclusion plot. In this case, fungal species with potentially higher carbon demands (i.e. fungal species with long exploration types) could have been the most harmed.

Within the saprotrophic guild, vulnerability to rainfall exclusion was related to substrate affiliation of the fungi. We detected an increase in the relative abundance of litter saprotrophs compared to the soil and wood saprotrophs in the rainfall exclusion plots. Soil fungi have been suggested more vulnerable to drought, since their biological activity is halted when solute flow in the soil is constrained. In contrast, litter fungi inactivity has been associated with direct dehydration rather than solute diffusion (Manzoni et al., 2012). Wood-decaying fungi have previously been shown to be among the most drought-sensitive fungal groups (Manzoni et al., 2012), but since debris larger than 2 mm was excluded from the soil samples, these were only few in our samples.

We identified vulnerable and tolerant genera within both the ectomycorrhizal and saprotrophic guild, some consistent with previous studies, while others diverging. Within the ectomycorrhizal guild, *Hebeloma* and *Inocybe,* both contact-short exploration types, increased in relative abundance in the rainfall exclusion plots compared to control plots. Both of these taxa have previously been shown to be tolerant to drought (Fernandez et al., 2023; Wilhelm et al., 2023). We also identified *Leccinum* (long-distance exploration type) as tolerant. On the other hand, *Hygrophorus, Alpova* and *Suillus* were found to be vulnerable in our study. *Hygrophorus* species generally need high forest continuity and species within this genus have also been shown sensitive to warming at an alpine tree line (Solly et al., 2017). The vulnerability of *Suillus* was surprising, since species in this genus have been shown drought-tolerant due to their efficient water use (Coleman et al., 1989; Castaño et al., 2023). Generalization of drought vulnerability at genus level might be too coarse, given substantial variation among species (Treseder et al., 2018). This underscores the need for further research to discern and understand variation among species.

Our results indicate that basidiomycetes were more vulnerable to rainfall exclusion than ascomycetes. The saprotrophic genera that we identified as tolerant were mainly ascomycetes, while the vulnerable genera were mainly basidiomycetes. These results are in line with previous studies showing that the dominant ascomycetes in boreal forests are generally less sensitive to drought (Karst et al., 2014; Fernandez et al., 2023) and warming (Allison and Treseder, 2008), potentially due to melanin deposition and thick cell walls (Lindahl and Clemmensen, 2016; Egidi et al., 2019; Maisnam et al., 2023).

The larger responses to rainfall exclusion in species richness compared to relative abundances, combined with the absence of effects on total fungal biomass, possibly indicate that uncommon species were lost from the treatment plots. Rare species are intrinsically more vulnerable to local extinctions due to their low abundance and often

lower competitive ability (Macarthur and Wilson, 1967; Hawkes et al., 2011). The opposite has also been found, i.e. that rare microbes increase in abundances due to drought (Meisner et al., 2018), likely in cases where drought stress moderate competition among taxa (Hawkes et al., 2011).

4.3. Methodological considerations

Drought experiments can provide essential insights into the impacts of extreme climate on soil communities in a natural setting. However, they do come with limitations and can underestimate drought effects. In our study, we only intercepted natural precipitation and induced top soil drying. We could not imitate atmospheric drought, i.e. hot temperatures and vapor pressure deficits, which are important aspects of natural droughts that can have significant impacts both in isolation and in interaction with other abiotic stressors. For example, the combination of warming and drought will likely have larger effects than either factor alone, at least on ectomycorrhizal communities (Gehring et al., 2020). The rainfall exclusion likely impacted primarily the top-soil, whereas the soil moisture loggers measured soil moisture deeper into the soil. Our estimates of soil moisture reduction very likely underestimated what fungal communities in the top soil experienced. In addition, the absence of rainfall may have also reduced nutrient inputs from the moss and litter layers. We could only simulate rainfall exclusion on small spatial scales and did not directly stress the surrounding host-trees. Future studies, where also host trees experience drought stress, could give more comprehensive insights into how fungal communities are likely to change during future extreme drought events, particularly if mature trees are stressed (e.g. Wilhelm et al., 2023). Currently most of the research has been done on seedlings or saplings for practical reasons (Castaño et al., 2023; Fernandez et al., 2023; Jaeger et al., 2023), but carbon assimilation and below-ground allocation may differ between mature trees and seedlings (Gao et al., 2021). Furthermore, we may have underestimated effects of the rainfall exclusion on soil fungal biomass using phospholipid fatty acids as biomarkers, since they can take some time to degrade (Frostegård et al., 2011), particularly under dry conditions when decomposition is reduced (Jones et al., 2022). Finally, our analyses on fungal fruiting reflects only a snapshot in sporocarps. While we recognize that conducting sporocarp inventories on multiple occasions would have resulted in a more comprehensive assessment, e.g. about potential drought effects on fruiting timing, we believe that a single time-point sampling still provides qualitative information about the kind of changes that can be caused by drought events.

4.4. Concluding remarks

Our results suggest that belowground fungal communities react dynamically to even small-scale rainfall exclusion on the forest floor. Some species or groups may be more disfavored during drought events, depending to their ecological guild, phylum or mycelial exploration type, although also within ecological and functional groups there is variation among genera and species in their response to drought. Interestingly, we observed shifts already after a small-scale rainfall exclusion, which indicates that longer-term or larger-scale droughts may have more profound effects on fungal communities, despite the common view that fungi are relatively drought tolerant. Community shifts induced by droughts may be long-lasting and persist even after moisture conditions have been restored (Gehring et al., 2020), but such longer-term effects and recovery remain to be investigated.

CRediT authorship contribution statement

Irena A. Koelemeijer: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Carles Castaño:** Writing – review & editing, Validation, Supervision, Methodology. **Karina E. Clemmensen:** Writing – review & editing, Supervision, Methodology. **Johan Ehrlén:** Writing – review & editing, Supervision, Conceptualization. **Pieter De Frenne:** Writing – review & editing, Methodology. **Mari Jönsson:** Writing – review & editing, Funding acquisition. **Kristoffer Hylander:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Data availability

Data is available on the Dryad Digital Repository (https://doi.org/10.5061/dryad.w0vt4b94v) and sequence data is published in NCBI SRA with projectnumber: PRJNA1268892.

Declaration of competing interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2025.101452.

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