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Drought has short-term effects on soil fungal communities leading to long-term effects on soil functions

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ABSTRACT

Climate change increases the magnitude and length of drought periods. Drought has direct and indirect effects on soil fungi and functions they provide. Here, we conducted a mesocosm experiment with four soil inocula representing gradient in levels of fungal biomass to study effects of drought on soil communities and functions. In a fully factorial design, half of the mesocosms were subjected to severe summer drought while half served as irrigated controls. Fungal biomass and community structure were monitored throughout first year after drought. Concomitantly, soil (multi)functionality was measured by plant yields, number of pests and other organisms, respiration, and decomposition. We show that drought has a direct negative effect on soil fungal biomass and diversity and that the magnitude of the effect depends on the initial community in soils. Furthermore, communities change in response to drought with observed decrease in network connectivity and changes in dominant taxa. While the effect of drought on soil fungal community and biomass gets smaller in time since drought, the functional legacy of the drought remains – potentially due to permanent changes in keystone fungal taxa. Particularly, the effects of drought legacy are apparent as reduction of crop yield in recovery period and slower decomposition rate 6 months after the drought. The effect on yield is however, soil inoculum dependent. Furthermore, the legacy effects of drought on fungal communities in bulk soil are smaller as compared to the effects on rhizosphere soil. We conclude that drought has unexpected long-term legacy effects on soil functions and that this effect is amplified in the rhizosphere. We further show that effects of drought depend on initial soil communities and that more diverse and fungal-rich communities recover faster from the drought. We conclude that watering of soils can alleviate the most acute drought stress affecting soil fungal communities and hence improve long-term functionality of the soil.

1. Introduction

Global climate change is one of the biggest human-induced problems in the world and affects almost every organism and ecosystem. One of the main consequences of global warming is an increased frequency and intensity of droughts (Ciais et al., 2005; Pörtner et al., 2022; Reichstein et al., 2013). The rising temperatures cause higher evaporative demands and more frequent and persistent dry spells, which suggests that drought conditions could worsen in many regions of the world (Jentsch et al., 2007; Trenberth et al., 2014). This can have enormous societal, economic, and environmental impacts across ecosystems (Naumann et al., 2018), via impacts on biodiversity and ecosystem services such as crop production or provisioning of clean water. As an already stressed ecosystems due to intensive use (Creamer et al., 2016; Tsiafouli et al., 2015), arable soils are especially vulnerable to drought and even short term lack of water (de Vries et al., 2012). Droughts may reduce functioning of these soils, e.g., carbon and nutrient cycling, water regulation and crop production, via impacting the communities living in these soils. However, we still have a limited understanding of how drought impacts soil (and particularly fungal) communities in arable soils, which are essential for many ecosystem functions (Frąc et al., 2022).

Soil microbial communities, the key drivers of essential soil processes such as carbon and nutrient cycling (Hannula and Morriën, 2022), are affected by drought both directly through changes in soil moisture and indirectly through changes in plant growth and plant community composition. Studies in grasslands suggest that indirect effects via plants can be long-lasting (Heinen et al., 2020) and even outweigh direct effects of drought on soil communities (Chomel et al., 2022; de Vries et al.,

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2019). This is because drought-induced changes in plant performance alter the quality and quantity of root exudates and plant litter inputs (Canarini and Dijkstra, 2015; Williams and de Vries, 2020) that provide carbon for the soil microbial community (Nannipieri et al., 2023; Prommer et al., 2020). Together, the limited availability of water and reduced carbon and nutrient inputs under drought have been shown to decrease microbial biomass, growth, and activity (Bapiri et al., 2010; Stark and Firestone, 1995; Steinweg et al., 2012). Furthermore, drought can alter the composition of soil microbial communities (de Vries et al., 2018), favoring drought-tolerant microorganisms and reducing the abundance of other microbial groups (Kaisermann et al., 2015). Finally, drought can induce stress responses in soil microorganisms, such as the production of stress proteins, and the activation of specific genes (Schimel et al., 2007). Such changes in the soil microbial communities will have important impacts on ecosystem functions such as decomposition rate of soil organic matter, which directly affects nutrient availability and soil fertility. Understanding consequences of drought for these ecosystem processes requires a detailed understanding of responses of microbial communities.

Earlier research has shown that during drought, fungi are less affected, i.e., more resistant, compared to bacteria (de Vries et al., 2018), but that their recovery from extreme drought events is slower, i.e., they are often less resilient (Barnard et al., 2013; de Vries et al., 2018; Kaisermann et al., 2015; Meisner et al., 2013). This is probably because single-cell organisms such as bacteria (but also yeasts) are more sensitive to changes in osmotic pressure and cannot escape the drought by extending over longer distances (Schimel et al., 2007), while fungi can use their hyphal networks to extend and explore water filled pores that are not accessible for plant roots and bacteria (Barnard et al., 2013; Meisner et al., 2018). Another reason why fungal community might be more resistant to drought is that they require less nutrients for growth (Fuchslueger et al., 2016) and in general, have better ability to form resting structures (Bridge and Spooner, 2001). There are, however, large differences in strategies between fungal species and some species might be more sensitive to drought than others (Hannula et al., 2020; Kaisermann et al., 2015; Meisner et al., 2018) depending on their traits (Crowther et al., 2014). Earlier studies comparing fungal and bacterial responses to drying and rewetting noted that it is important to measure different parameters such as growth and biomass development during drought (Bapiri et al., 2010). Furthermore, the responses of fungal communities and biomass are not consistent between studies and ecosystems (e.g. Acosta-Martínez et al., 2014; Barnes et al., 2018; Buscardo et al., 2021; Hartmann et al., 2017; Hawkes et al., 2017; Kaisermann et al., 2015; Meisner et al., 2018). Therefore, it is not fully understood how fungal communities respond to drought.

Most studies on drought impacts on soil fungal and bacterial communities, have focused on relatively short-term impacts and show that microbial parameters such as growth, respiration and biomass recover generally within weeks after re-wetting (Bapiri et al., 2010; de Vries et al., 2018). However, from studies in grasslands we also know that drought can have longer-lasting negative effects on microbial biomass (Yang et al., 2022) and even impact on microbial community composition across seasons. Furthermore, it has been recently shown that severe drought can cause permanent changes in soil microbial communities and functions in a grassland ecosystem (Cordero et al., 2023). However, there are still relatively few studies that have focused on the impact of long-term effects of drought (i.e. months after the event) on soil fungal communities, particularly in arable systems. Hence, it is important to understand these longer-term impacts of drought on fungal communities because they may drive longer-lasting impacts on ecosystem functions.

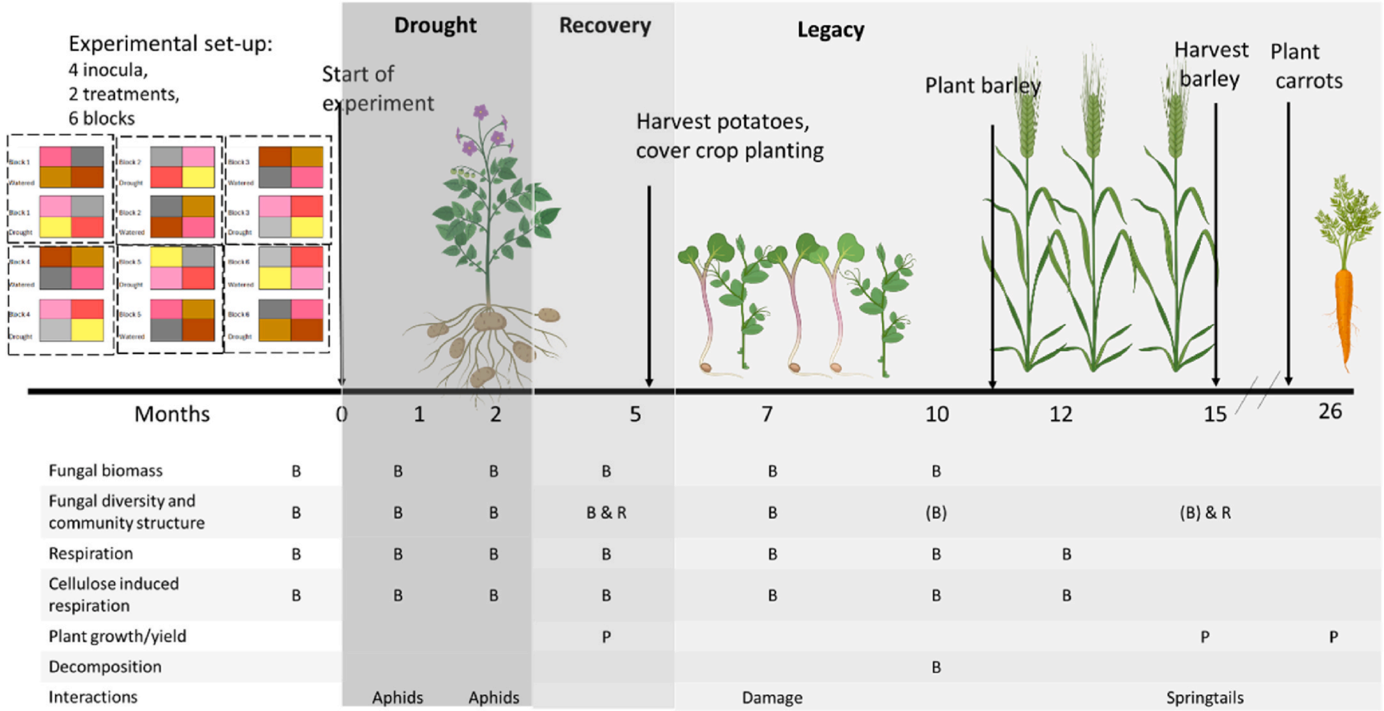
The aim of this study was to understand how fungal communities respond to and recover from drought and how does this affect soil functions in the long term. In our study, we used four different soils, which varied in fungal biomass and community composition. We added these soils as an inoculum to common sterilized soils creating similar abiotic conditions between the soils while manipulating the initial fungal biomass and community composition. This allowed us to test how initial variation in fungal communities affected the outcome of drought in standardized (abiotic) conditions. Our first hypothesis is that drought affects soil fungal community structure and composition and in general reduces fungal diversity and biomass. Although, this effect may diminish when time passes after end of the drought period, some parts of the community are expected to show long-term impacts. Our second hypothesis is that the alteration in soil fungal communities translates into changes in soil functions, such as litter decomposition and plant growth, on the longer term because part of the soil community may not be able to recover after drought. Our third hypothesis is that the effect of drought on fungal communities and soil functions is dependent on the initial fungal biomass and community composition in the soils, with soils with highest initial fungal biomass being most resilient and resistant towards drought. Lastly, we expect that the fungal community structure and diversity are linked to multiple soil functions and that this relationship is modulated by drought.

2. Material and methods

2.1. Soils and experimental set-up

Four soils were selected to be used as inocula based on similarity in soil type and gradient in fungal biomass. All soils were characterized as sandy soils and located in the Netherlands. Soil 1 was collected from a grassland abandoned from intensive agricultural management 2 years prior to the sampling and was located in Empe, Gelderland (N52.0830, E6.0639). Soil 2 was collected from a pasture used for conventional farming in Helvoirt, North-Brabant (N51.6423, E5.1998). Soil 3 was collected from neighboring farm to soil 2, also in Helvoirt, North-Brabant (N51.6460, E5.2172). This soil was managed as an organic pasture. Finally, soil 4 soil was collected at the experimental farm of Wageningen University and Research located at Vredepeel, Limburg (N51.3219, E5.5105). This soil had a relatively high organic matter content (6 %; Clocchiatti et al., 2020). To form a gradient, we confirmed that the soil 1 had the least fungi: it contained 0.13 mg ergosterol g^{-1} soil dw. Soil 2 had a fungal biomass of 0.51 mg ergosterol g^{-1} soil dry weight (dw), soil 3 contained 1.03 mg ergosterol g^{-1} soil dw, soil 4 had 1.44 mg ergosterol g^{-1} soil dw and hence most fungi (Fig. 2). From all soils separate bags per block were collected and kept separate in 4 °C until inoculated into sterile background soil. All soils were collected in April 2020 and inoculated in May 2020.The full set-up of the experiment and all measurements is presented in Fig. 1.

Background soil used was a sandy soil collected from agricultural field outside Wageningen (51°59′N, 5°40′E) the Netherlands. The background soil was sterilized using gamma-irridiation (>25 K Grey gamma irradiation, Isotron, Ede, Netherlands). 100-liter containers with holes in the bottom for water to exit were filled with a layer of clay pebbles (5 cm) and then 45 kg of the sterilized soil was added through a 10 mm sieve. This common sterilized soil was used to keep abiotic conditions as similar as possible between all containers while the soil biotic component (obtained through inoculation with live soil) varied between the soils (van de Voorde et al., 2012). Then 8 kg of live soil from one of the 4 inoculum types was added through a 10 mm sieve and mixed in with the sterile soil making the ratio of sterile to live soil 1:6.6. Any stones or visible plant remains were removed. All equipment was cleaned with ethanol and water in between samples. The experiment followed randomized block design so that each soil inoculum was present in each block and inocula were randomized within that block (Fig. 1). The experiment was conducted in the common garden at the Netherlands Institute of Ecology (NIOO-KNAW, Wageningen, The Netherlands; 51°59′N, 5°40′E) with total of 48 mesocosms (6 blocks of 8). Soil properties such as pH, organic matter content and N content were monitored regularly. Wet-sieving was performed to confirm that treatments had similar particle size distributions in the beginning of the



Months	0	1	2	5	7	10	12	15	26
Fungal biomass	B	B	B	B	B	B			
Fungal diversity and community structure	B	B	B	B & R	B	(B)		(B) & R	
Respiration	B	B	B	B	B	B	B		
Cellulose induced respiration	B	B	B	B	B	B	B		
Plant growth/yield				P				P	P
Decomposition						B			
Interactions		Aphids	Aphids		Damage			Springtails	

Fig. 1. Timeline and set-up of the experiment. The experiment was set-up with a gradient of soil fungal biomass inoculated into sterile soils (soil 1 lowest, soil 4 highest; Fig. 2A). The analyses performed for bulk soil ('B'), rhizosphere soils ('R') and plant ('P') are listed in the timeline per analyses. For interaction traits the measurement parameter is mentioned. The bulk soil samples for sequence based analyses that failed are marked with (B). For details on methods, see text.

experiment.

Potatoes (*Solanum tuberosum*; two tubers per container) were planted in the soils on May 21st' 2020 and harvested on August 21st' 2020. A cover crop mixture consisting of *Raphanus sativus, Phacelia tanacetifolia* and *Eruca sativa* was seeded in on September 3rd' 2020. Summer barley (*Hordeum vulgare*) was planted on May 17th' 2021 and harvested on July 22nd' 2021. Carrots (*Daucus carota* subsp. *sativus*) were planted on May 7th' 2022 and harvested on July 25th' 2022. These plants are very commonly cultivated plants and are part of wide crop rotation scheme in this region. Weeds were removed manually during crop growing periods. The plants present at different times is shown in Fig. 1.

2.2. Drought

Naturally occurring drought (spring 2020) was imposed on half of the mesocosms (n = 24; Fig. 1) while half of the mesocosms were released from drought by watering them daily. Soil moisture was continuously monitored during drought period, recovery period and legacy period using TOMST sensors. The volumetric water content was calculated using standard procedure for sandy soils. We further confirmed average moisture contents of soils monthly by drying soils in 105C oven for 24h. With these measurements we confirmed that the soil moisture was consistently lower in the containers under drought (Fig. S1). The reduction was highest during peak drought period in June 2020 when moisture percentage of the soils was 25–30 % in watered mesocosms and only 6–11 % in mesocosms undergoing drought. In July the differences were smaller due to rain (on average 28 % in drought vs 42 % in watered mesocosms). Overall, during the drought period (using hourly measurements) the drought decreased the soil volumetric content on average 1–2.5 %. The drought was released in the beginning of August by watering both treatments and differences in soil moisture disappeared between watered and drought conditions (Fig. S1), and all containers had an average soil moisture of 12–19 %. For our analyses, we considered the months of June and July 2020 as drought period, August 2020 as recovery period and all time points after that as legacy period. In years 2021 and 2022 all mesocosms were watered when needed to keep ambient moisture conditions (average 20–45 %; for differences between past drought and drought legacies see Fig. S1).

2.2.1. Samples and measurements

Soil samples from the mesocosms were collected over the course of over one year (June 2020 to August 2021) according to a scheme presented in Fig. 1. Each time, six soil samples were taken from each container at regular intervals (12 cm deep, 7 mm diameter), pooled and homogenized per time point. Part of the sample was immediately put into an Eppendorf tube and stored at −20 °C until molecular analyses, part stored in −20 °C in methanol KOH for analyses of fungal biomass with ergosterol and part was used to measure moisture, respiration and substate induced respiration immediately after sample was taken. Soil samples were collected two times during the drought period (June 3rd' 2020 and July 3rd' 2020), once in the recovery period (August 21st' 2020) and 4 times during the legacy period (October 6th' 2020, December 1st' 2020, March 8th' 2021, and May 5th' 2021). Rhizosphere soils were collected when harvesting the potatoes (August 21st' 2020) and during harvest of barley (July 22nd' 2021) by first shaking the plant to remove loosely adhering (bulk) soil and then brushing the roots with a tooth brush. All soil samples for molecular analyses were stored at −20 °C.

DNA was extracted from 0.50 g of soil using the PowerSoil DNA Pro Isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The fungal DNA was amplified using primers ITS3mix and ITS4ngs (Tedersoo et al., 2014) under conditions presented in Hannula et al. (2021). Purified amplicons were tagged using Nextera XT DNA library preparation kit sets A, B, and C (Illumina, San Diego, CA, USA), and equimolarly pooled. All samples were sequenced using Illumina MiSeq PE250 at McGill University and Genome Quebec Innovation Center. Extraction negatives and mock communities were sequenced along the samples and used to validate bioinformatic analyses. Raw data is archived in NCBI under accession number PRJNA1213495.

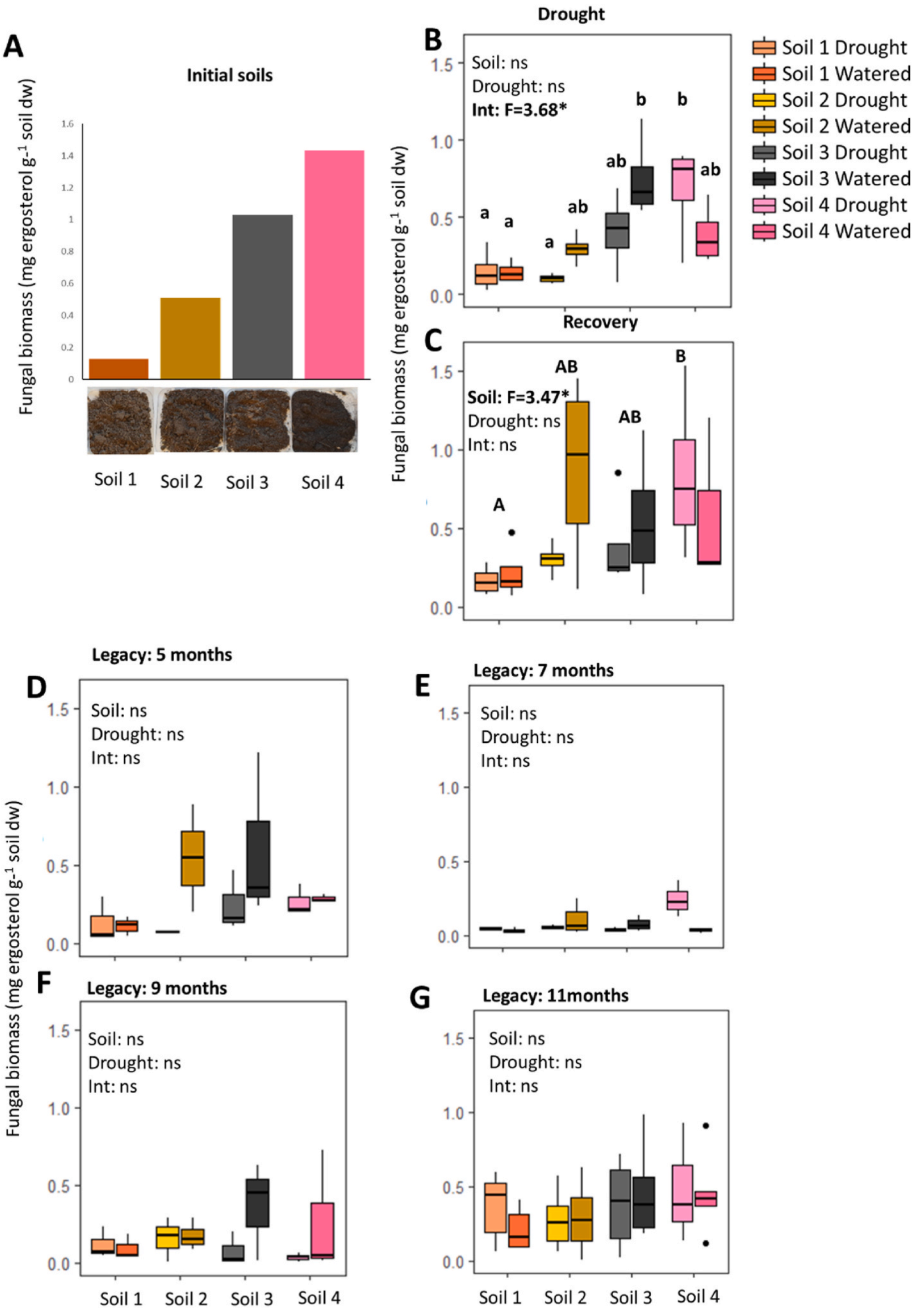


Fig. 2. Differences in initial fungal biomass between the soils used as inocula and the effect of drought on soil fungal biomass measured using ergosterol across timepoints. (A) represents initial soil inocula, (B) fungal biomass during drought period, (C) during recover period and (D–G) during legacy period (D = 5 months, E = 7 months, F = 9 months & G = 11 months). Colors of bars (A) and boxplots (B–G) denote the soils and drought treatments. In boxplots mean and 95 % confidence intervals are shown. The statistical model is given in the figure (* indicates statistical significance at level $p < 0.05$) and in case of significant full model, post-hoc is indicated with letters. Small letters indicate differences between interaction drought x soil inoculum and capital letters differences between soil inocula. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.3. *Ergosterol*

Ergosterol was used to quantify fungal biomass. This method is based on extraction of fungal cell-wall membrane ergosterol found across most prominent soil fungal phyla (yet, missing in Glomeromycota; Baldrian et al., 2013). Alkaline extraction of ergosterol was performed starting from 1 g soil samples, as described by (de Ridder-Duine et al., 2006). Briefly, samples were stored in 4 ml methanol 10 % KOH, processed by sonication (47 kHz, 15 min), followed by a heat treatment (70 °C for 90 min). Alkaline hydrolysis of esterified ergosterol carried out by the addition of water and n-hexane, combined with shaking. The hexane fraction was collected and the solvent was let to evaporate overnight. The pellet, containing ergosterol, was dissolved in HPLC-grade methanol. Finally, ergosterol concentrations were quantified by LC-MSMS (UHPLC 1290 Infinity II, Agilent Technologies and 6460 Triple Quad LC-MS, Santa Clara, California, United States). Ergosterol is expressed as mg of ergosterol per g soil (dry weight). Dry weight of the soil was determined by drying soil samples overnight at 105 °C to constant weight and calculating the difference in weight between fresh and dried soil.

2.4. Respiration and cellulose induced respiration

The effects of drought on soil respiration and substrate induced respiration were considered as main functions related to microbial C release from the soils. We used cellulose as the substrate for respiration as it is more relevant measure of fungal decomposition in arable soils as compared to glucose or lignin (found more in natural soils) and has been shown to reflect the fungal community responses to drought (Liu et al., 2022). In addition, it is a more complex polymer representing more fungal dominated decomposition pathway. Approximately 5 g soil was weighed into 50-mL centrifuge tubes to determine soil microbial respiration. The lid of each tube was sealed gas-tight using an O-ring and a rubber septum in the middle. For basal respiration measurements, the tubes were capped and flushed with CO_2-free air to remove any CO_2 from the headspace. After 24 h of incubation at 20 °C, 12 mL of headspace was sampled using a gas-tight syringe (Steinauer et al., 2020). Substrate induced respiration was determined after addition of cellulose (Anderson and Domsch, 1978). In short, 2 mL of 75 mM cellulose solution was added to each soil sample and placed on a horizontal shaker for 1 h. Tubes were capped, flushed with CO_2-free air, and incubated for 24 h at 20 °C. As above, 12 mL of headspace was sampled. Measurements of the CO_2 concentrations were carried out on a Trace CG Ultra Gas Chromatograph (Thermo Fisher Scientific, Milan, Italy). The respiration and substrate induced respiration are expressed as C lost per gram of soil dry weight.

2.5. Plant biomass and yield

Potato plants were harvested on August 21st' 2020 (during recovery period). Aboveground biomass was clipped just above the surface of the soil, put in paper bags to be dried at 40 °C for at least 72 h and weighed. Belowground biomass was recorded as fresh weight, as that relates closely to yield. All potatoes produced per mesocosm were counted and their weight was recorded (making up large part of the belowground biomass). The weight of decent and uniform size and disease-free potatoes was further recorded under 'yield'. The growth of barley was measured on July 22nd' 2021 (legacy period) with height of the highest plant in a mesocosm measured from soil level in centimeters using measuring tape. The yield of carrots was measured on July 25th' 2022 by digging up carrots and measuring the total biomass (fresh weight).

2.6. Decomposition estimated with teabags

In order to assess how the soil conditioning treatments affected decomposition processes in the legacy phase, green (EAN 8710908903595) and rooibos (EAN 8722700188438) Lipton teabags were used in line with The Teabag Index as described in (Keuskamp et al., 2013). All teabags were oven-dried at 40 °C for 48 h before burying them in the soils, dry weights were recorded, and each teabag was given a unique code and placed into the mesocosms. The bottom of each teabag was placed approximately 8 cm beneath the soil surface, with the top of each teabag ending up approximately 5 cm beneath the soil surface. Each mesocosms received one of each type of tea. After 90 days in the soil, respectively, on April 12th, 2021, teabags were dug out of the soil, brought back to the lab and dried at 40 °C for 48 h. Each teabag was carefully cleaned, cut open and the dried tea was picked clean of all large roots before dry weights were recorded. The mass of the tea remaining was used to determine the mass loss. The litter stabilization factor (S) and decomposition rate (K) were calculated using formulas and data presented in Keuskamp et al. (2013). Briefly, the decomposition rate (K) is based on the mass lost from the tea bags during their time in the soil. The stabilization factor (S) stands for the transformation of components of the tea bags from fast-decomposing molecules into slow-decomposing molecules under the influence of environmental factors.

2.7. Herbivore feeding damage

Damage by (native) aphids was assessed on potato leaves by calculating the number of aphids on 3 leaves per potato plant on three occasions (June 24th' 2020, July 3rd' 2020 and July 14th' 2020). A cumulative aphid count per container was used for calculations. The damage by chewing herbivores (caterpillars) was evaluated on the cover crop mixture in October (October 16th' 2020) by visually estimating level of damaged leaves (ranging from 0 to 75 % of total leaves). This was done randomized without knowing the treatments and was confirmed from photos taken. At the same time the percentage of yellow leaves was estimated.

2.8. Springtails

During the harvest of barley soil samples were taken and extracted using Tullgren funnels and small mesh size to select for smaller organisms (for 24h). The number of springtails obtained per g of soil was estimated by microscopically counting all springtails in the sample and dividing the number with the amount of soil used for extraction.

2.9. Bioinformatic analyses

Fungal sequencing data was processed with ITSxpress for extracting the ITS2 region (Rivers et al., 2018). Then, the package DADA2 was used for quality filtering (maxEE = 2, truncQ = 2), joining pair-end reads, removing chimeric sequences, modelling sequencing errors and finally identifying amplicon sequence variants (ASVs; Callahan et al., 2016). Taxonomical assignment of SVs was performed by using the UNITE v2020 database (Abarenkov et al., 2010) and the RDP classifier.

All singletons and all reads from other than fungal origin (i.e. plant material and protists) were removed from the datasets and taxa was reassembled on genus level (as phylotypes). To account for differences in read numbers, all samples with less than 1000 reads or more than 60 000 reads were removed. This lead to removal of 4 samples. The data was further normalized using total sum scaling (TSS) as suggested by Weiss et al. (2017).

2.10. Statistical analyses

All data analyses were performed in R (v.4.3.3). Diversity was estimated using inverted Simpson index on non-normalized phylotype data and it was confirmed that read numbers did not correlated with estimated diversity ($R^2 < 0.1$, $p > 0.05$). NMDS ordination was performed using Bray-Curtis distances between fungal communities to visualize the effect of drought on community composition. The first two axis of PCoA calculated using Bray-Curtis distances was used as a proxy for fungal community composition across time points. PERMDISP (vegan) revealed a low homogeneity of dispersion in the dataset, therefore Hellinger transformation of data was used before running the Permutational multivariate analyses. Permutational multivariate analyses (PerMANOVA, vegan) was used for determining the effect of drought and soil inoculum over time on the fungal community composition (999 permutations). The permutations were controlled by block (as strata). A global model of PerMANOVA was used to evaluate the full model including time. Further analyses per time point and per period (i.e. drought, recovery, and legacy) were performed to reveal interaction between soil and drought when time was overriding effects. In order to show effects of different inocula on community structure samples that had undergone drought and that were watered originating from same block (Fig. 1.) were compared by Bray-Curtis dissimilarity between the pairs of data.

Co-occurrence networks of fungal species were constructed per soil inoculum type across time points for soils that experienced drought versus soils that were watered (Faust et al., 2015). Time dependent networks show which taxa are co-occurring across times. We used stringent filtering to improve accuracy (Röttjers and Faust, 2018) and removed all phylotypes present in less than 4 samples for each treatment combination. Co-occurrence networks were then calculated using the Fastspar in R relying on SparCC package in R. In brief, networks were inferred based on centered log-ratio transformed read counts and neighborhood selection. We removed spurious connections using the iDirect method (Xiao et al., 2022). The cut-off value for each network was calculated using random matrix theory using Poisson distribution at the level of $p < 0.001$. The networks were visualized in Cytoscape (Shannon et al., 2003) using both individual phylotypes and phylotypes aggregated at order level in which the average co-occurrences between orders were calculated from total possible co-occurrences.

We used general linear mixed effect models (lme in R) to determine how legacy of drought, soil inocula and sampling time affected the measured parameters (log transformed ergosterol content, respiration, substate induced respiration, yield of potatoes and carrots, height of barley, the abundance of pests, decomposition variables). For all parameters also the times (and periods) were tested under separate models in the same way (with drought and soil inocula as main factors). Block was used as a random factor. In case the residuals were not normally distributed, log transformation was used to ensure normality.

2.11. *Soil multifunctionality calculations*

All soil community and ecosystem function data from each sampling harvest period were standardized by z transformation (overall mean of 0 and SD of 1) and used in all subsequent calculations and analyses. This removed overall differences between soils and simultaneously equalized the variance among measures and sampling time points. Subsequently, the average of all standardized ecosystem functions (Fig. 1) was used as an index of ecosystem multifunctionality following the approach used by Maestre et al. (2012). Data for functions in which greater values reflect a more undesirable aspect of the ecosystem (aphid number and damage by chewing caterpillars) were multiplied by −1 (inverted around the 0 mean) to maintain directional change with other (positive) ecosystem functions. This way the general difference among soil community treatments in overall ecosystem functioning could be more easily assessed. Finally, soil multifunctionality was correlated using linear Pearson correlation with fungal diversity, biomass, and community composition measured with Pcoa1.

3. Results

3.1. *Effects of drought on soil fungal biomass*

Fungal biomass differed between the soil inocula ($F = 5.86$, $p < 0.001$) and time points ($F = 14.54$, $p < 0.001$) and there was a weak interaction between soil inoculum type and drought ($F = 2.86$, $p = 0.040$). During the drought period (months 1 and 2) we detected a significant interaction between drought and soil inoculum (soil x drought: $F = 3.678$, $p = 0.046$), showing that drought reduced fungal biomass in soils 2 and 3, while it enhanced it in soil with inoculum 4 and had no effect in soil 1 (Fig. 2B). The interactive effect of inoculum x drought on fungal biomass disappeared in time and the effect of drought got smaller as time since drought passed (Fig. 2D–G). In general, soil inocula 2, 3, and 4 had higher fungal biomass than soil inoculum 1 confirming the success of the design (post-hoc for comparisons between inocula 1 and 3: $p = 0.036$ and between 1 and 4: $p = 0.009$, comparison between soil inocula 1 and 2 was not significant). The effect of soil inocula were most apparent in the beginning in initial soils (Fig. 2A) and during recovery period (Fig. 2C) Furthermore, the fungal biomass was generally lower in (winter) months 7–10 than the other sampling moments (Fig. 2E and F), post-hoc all comparisons between times 1–3 and 12 with times 7–10: $p < 0.005$). Drought did not have a consistent effect across inocula on fungal biomass in any of the time points ($F = 1.26$, $p = 0.264$; Fig. 2).

3.2. *Effects of drought on soil fungal community composition and diversity*

Overall, time had the strongest impact on bulk soil fungal community composition (Permanova: $R^2 = 0.295$, pseudo $F = 13.648$, $p < 0.001$), followed by the effect of initial soil inoculum (Permanova: $R^2 = 0.034$, pseudo $F = 3.767$, $p < 0.001$) and finally drought (Permanova: $R^2 = 0.007$, pseudo $F = 2.497$, $p = 0.004$). There was also an interaction effect between time and drought ($R^2 = 0.023$, pseudo $F = 1.398$, $p = 0.006$). Similarly to fungal biomass, the effect of drought on soil fungal community composition was largest during the drought period and effects got smaller in time since drought (Fig. 3A). Moreover, during drought period, and shortly thereafter, the fungal community composition was affected interactively by drought and soil inoculum (Table 1). The calculated Bray-Curtis distances between soils that had undergone drought as compared to well-watered soils were significantly affected by time since drought ($F = 20.727$, $p < 0.0001$; Fig. 3A) and by an interaction between soil inoculum and time ($F = 2.533$, $p = 0.049$; Fig. 3A) indicating that the fungal communities in different soil incocula differed in their ability to recover from drought. During the drought period the community structure of fungi in soil inoculum 4 was affected most by drought while community structure in soil inoculum 1 was affected the least reflected as most similar communities between soils that were experiencing drought and watered soils (Fig. 3A). When looking at time points in the legacy period separately, we detected an effect of soil inoculum on drought response in month 7 (i.e., 5 months after ending the drought) showing that community composition in soils with inoculum 1 and 4 had recovered faster than communities with soil inoculums 2 and 3 (soil: $F = 6.709$, $p = 0.014$). For time point of 11 months this effect was gone. For rhizosphere soils sampled during harvest times, we found that the community structure of fungi in rhizosphere was strongly affected by drought (Fig. 3B and C) and this effect was stronger than the effect on bulk soils (Permanova for drought in rhizosphere: $F = 2.354$, $p < 0.005$, soil inoculum $F = 1.836$, $p < 0.014$; Permanova for bulk soil only interaction significant: $F = 18.063$, $p = 0.014$; Table 1). One year after the drought, effect of drought legacy was detected in the rhizosphere of the next crop (Permanova for drought: $F = 2.104$, $p < 0.005$, soil $F = 2.200$, $p < 0.005$, no interaction; Table 1, Fig. 3C) while no effect of drought was detected in the bulk soil in the legacy phase (Table 1).

The diversity of fungi in the bulk soil, expressed as Simpson index calculated from phylotypes, was mainly affected by time ($F = 3.54$, $p = 0.003$; Fig. 4) and interaction between soil inocula and time ($F = 2.04$, $p = 0.015$; Fig. 4 & Fig. S2). Generally, diversity across soils was lowest during drought and legacy periods (post-hoc between drought period and legacy period: $p < 0.05$; Fig. S2). Drought did not affect fungal diversity in bulk soils in a predictable manner (Fig. 4A–E) yet during drought and recovery periods effects of drought were evident in soils with certain inocula (Fig. 4A and B). The effect of drought on fungal diversity was most evident in soils of inoculum 1 during recovery period (Fig. 4B). No effect of drought was detected during legacy period (Fig. 4D–F). Difference in diversity between soils with different inocula were evident in the beginning of the experiment but disappeared later: soil inoculum 1 resulted in lowest diversity during drought period (Fig. 4B and C).

In rhizosphere soils in the recovery phase higher diversity of fungi was detected in soils that had been watered as compared to soils recovering from the drought ($F = 15.66$, $p < 0.001$; Fig. 4C) and there was an interaction between drought and soil inoculum ($F = 2.95$, $p < 0.05$; Fig. 4C). The effect of drought was largest in rhizosphere soils with inoculum 1; the lowest diversity was detected in rhizosphere soils with inoculum 1 under drought and highest diversity in soils from inoculum 4 under watered conditions. The effect of drought on fungal diversity in the rhizosphere was not evident anymore a year after the drought in the rhizosphere of the following crop (Fig. 4G).

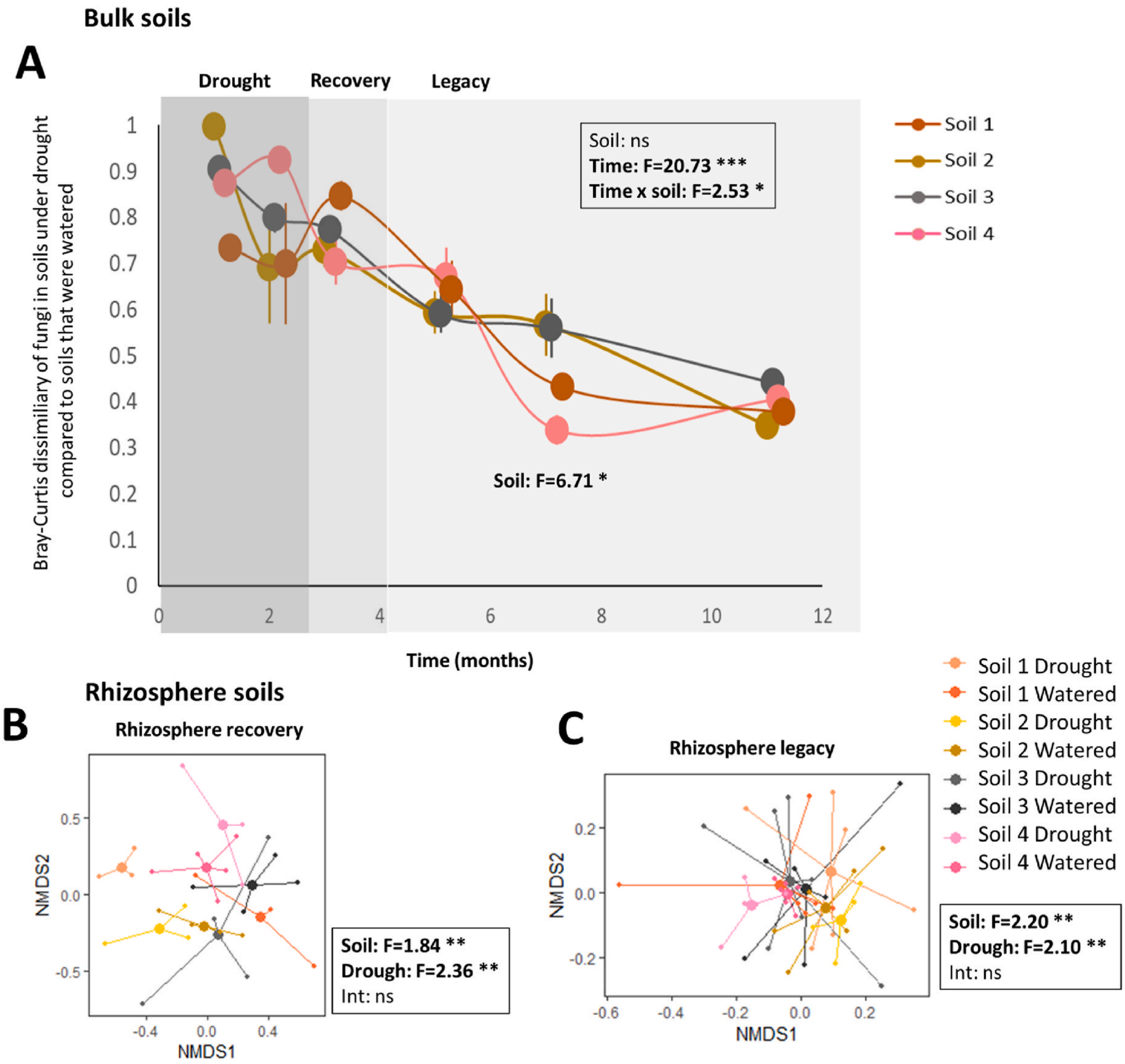


Fig. 3. Effect of drought on bulk soil (A) and rhizosphere (B–C) fungal community composition measured in Bray-Curtis dissimilarity (A) and with NMDS (B–C). For bulk soils (A) difference in community structure between soils that were watered and soils that experienced drought is shown for each paired soil across time points. Here the average and standard deviation are shown for each time point and soil inoculum. For rhizosphere soils (B–C), NMDS plots showing the centroids and variation among replicates is shown for samples collected at recovery phase (B: August 2020; month 3) and a year later in legacy phase (C: July 2021; month 15). Different colors denote different soils (A) and soil-drought combinations (B&C). Statistical significance is given in the figure. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Effect of drought, soil inoculum and their interaction on soil and rhizosphere fungal communities measure with PERMANOVA. Significant values are marked in bold. Differences in rhizosphere fungal communities (months 3 and 15) are highlighted in Fig. 3B and C.

	Drought			Soil			Drought x soil		
	Pseudo-F	R2	p	Pseudo-F	R2	p	Pseudo-F	R2	p
Drought period (soil)	0.8	0.027	0.570	143.2	0.152	0.132	**238.3**	**0.253**	**0.009**
Recovery period (soil)	14.2	0.042	0.162	12.9	0.115	0.143	**18.1**	**0.161**	**0.014**
Recovery period (rhizosphere month 3)	**23.5**	**0.072**	**0.003**	**18.4**	**0.168**	**0.002**	13.2	0.121	0.081
Legacy period (soil month 5)	108.6	0.045	0.287	**145.7**	**0.181**	**0.026**	0.9	0.113	0.613
Legacy period (soil month 7)	110.9	0.053	0.300	134.5	0.193	0.084	0.9	0.131	0.612
Legacy period (rhizosphere month 15)	**210.4**	**0.045**	**0.004**	**151.7**	**0.097**	**0.004**	0.8	0.049	0.974

3.3. *Effects of drought on soil fungal network connectivity and specific fungal taxa*

We further constructed co-occurrence networks of each inocula x treatment combination across time. Essentially the time-dependent networks show how stable the communities are in time (i.e. how many species co-occur with each other in same samples). We observed that while there were more fungi co-occurring in drought (legacy) treatments as evidenced by higher numbers of nodes in the networks (Fig. 5A), both at the species level (Fig. 5A) and at the order level

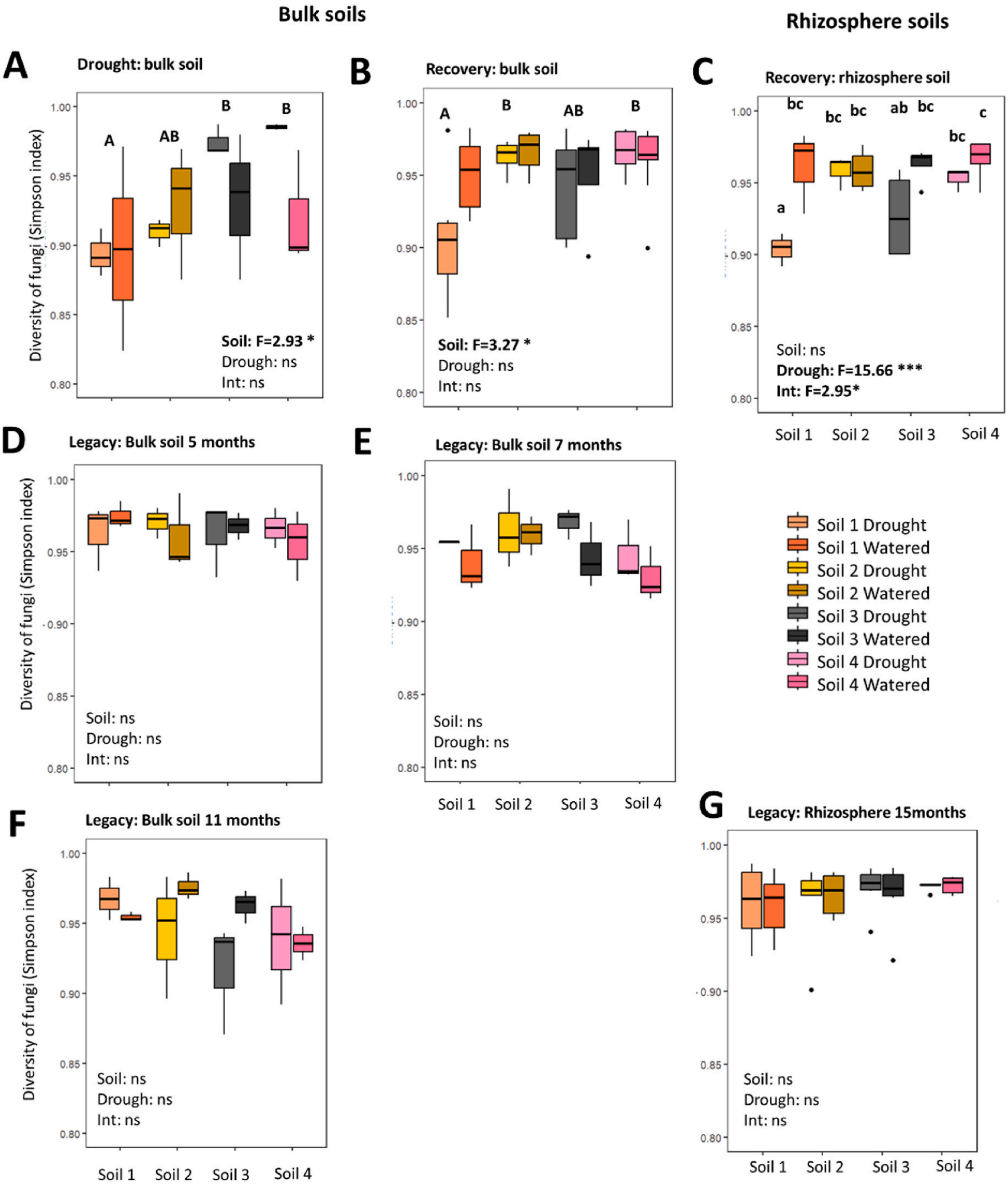


Fig. 4. Effect of drought on fungal diversity measured with Simpson diversity index in the bulk soils (A-B, D-E & F) and in rhizosphere soils (C & G) throughout the experiment. (A) shows the effect of drought on bulk soil fungal diversity during the drought period, (B) shows the effect during recovery period and (D–F) during legacy period (D = 5 months, E = 7 months and F = 11 months, respectively). (C) shows the effect of drought on potato rhizosphere fungal diversity during recovery period and (F) on barley rhizosphere fungal community during legacy period. Different colors denote different soils, and boxplots with mean and 95 % confidence intervals are shown. Statistical significance is given in the figures. Small letters indicate differences between interaction drought x soil inoculum and capital letters differences between soil inocula. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 5B) there were more co-occurrences of fungi (estimated with multiple network parameters including the average clustering coefficient) in the treatments with (a legacy of) watering. This was evident across three out of four of the soil inocula (Fig. 5A). Only in soil 4 (with highest fungal biomass), the soil that had experienced drought had slightly more connections at the species level (average clustering coefficient 0.189 vs. 0.209). When looking at most connected taxa we noted that watering increased connections between Pleosporales and other fungi ($F = 2.51$, $p = 0.044$) and Capnodiales and other fungi ($F = 3.59$, $p = 0.036$) across the soils, while connections between Glomerellales and other fungal groups ($F = 3.51$, $p = 0.020$) increased in soils that had experienced drought (Fig. S3; Fig. 5B).

At the level of fungal orders, we found that the abundances of most fungal orders fluctuated in time (Table 2). From dominant orders of Ascomycota, we found that Pezizales were affected by soil inoculum and drought interactively (interaction drought x soil: $F = 6.574$, $p < 0.001$; Fig. 6; Fig. S4), namely there were more Pezizales in soil inocula 1 and 2 and less in soil inoculum 3 after the soils that had experienced drought.

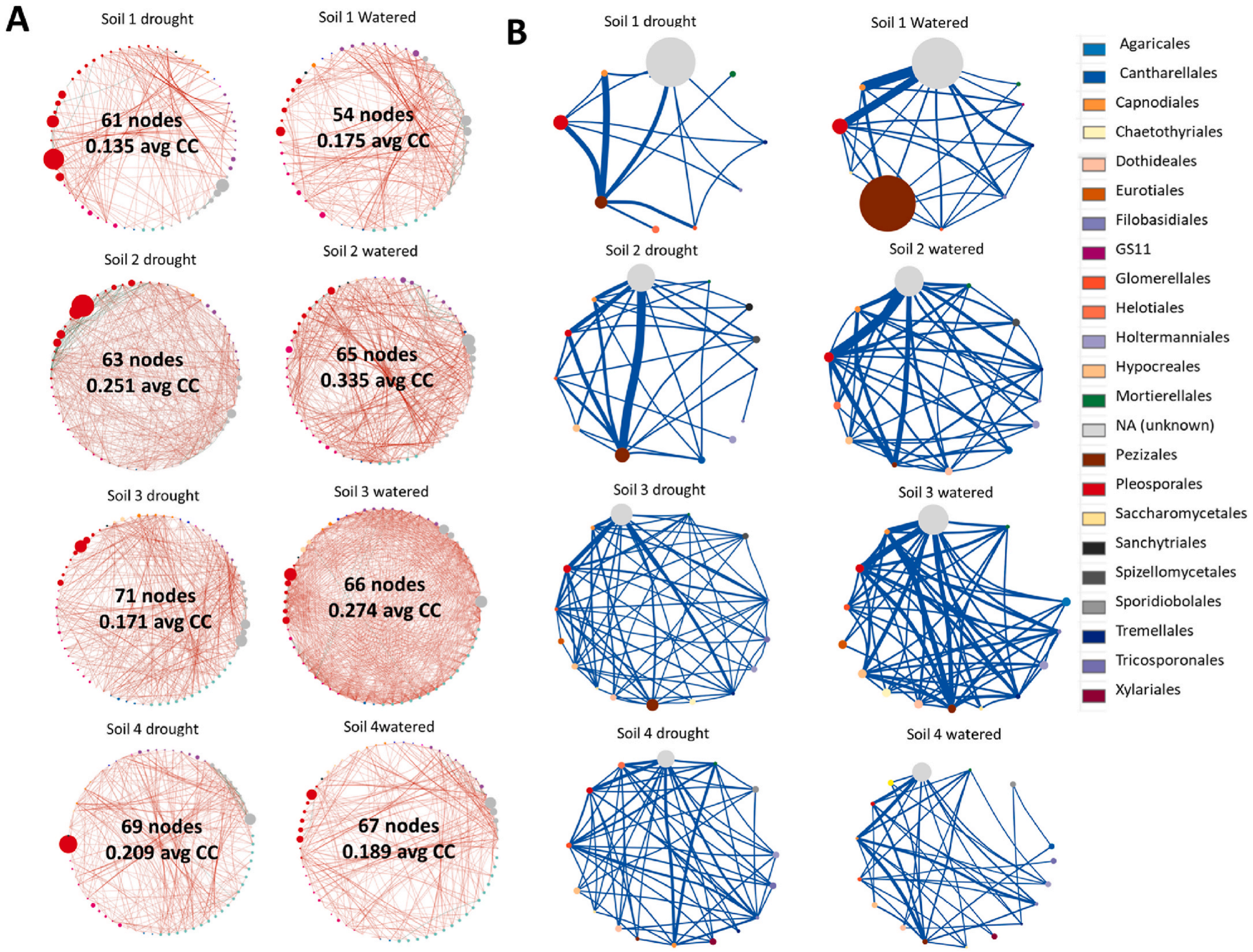


Fig. 5. Co-occurrence networks of fungal species in time in the soils and in differently watered treatments on (A) species level and (B) level of major fungal orders. The number of nodes and average clustering coefficient are given for each network. Basidiomycota taxa are blueish colors, Ascomycota in reddish colors, Mortiriellomycotina in green and others in grey colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Effect of time, drought, soil inoculum and their interaction on relative abundances of major fungal classes.

		Time		Drought		Soil		Time x Drought		Drought x soil		Drought x soil x time	
		F	p	F	p	F	p	F	p	F	p	F	p
Ascomycota	Pleosporales	**22.58**	**<0.001**	1.09	0.299	**4.69**	**0.003**	1073	0.381	1.17	0.324	0.83	0.646
	Pezizales	**9.17**	**<0.001**	3.81	0.053	2.39	0.071	1032	0.406	**6.57**	**<0.001**	0.97	0.219
	Capnodiales	**16.63**	**<0.001**	2.77	0.098	0.66	0.577	**3.06**	**0.007**	0.53	0.657	0.46	0.961
	Saccharomycetes	**4.09**	**<0.001**	0.49	0.484	1.38	0.25	1.03	0.409	0.75	0.529	1.12	0.343
	Helotiales	**13.72**	**<0.001**	0.88	0.351	**4.65**	**0.003**	0.87	0.521	**3.21**	**0.025**	0.53	0.093
	Hypocreales	**2.51**	**0.024**	0.01	0.917	**10.87**	**<0.001**	1.56	0.161	0.74	0.332	0.63	0.854
	Glomerellales	**9.01**	**<0.001**	0.32	0.57	**15.49**	**<0.001**	1.66	0.135	1.84	0.14	1.11	0.353
Basidiomycota	Tremellales	**6.18**	**<0.001**	3.09	0.080	**4.27**	**0.006**	**2.41**	**0.030**	2.59	0.054	**1.90**	**0.024**
	Filobasidiales	**3.51**	**<0.001**	0.56	0.456	**7.38**	**<0.001**	1.53	0.172	0.32	0.817	1.19	0.279
	Agaricales	**3.6**	**0.002**	**11.08**	**0.001**	**3.57**	**0.016**	0.92	0.479	2.74	0.05	1.100	0.356
	Cantharellales	**3.34**	**0.004**	**3.99**	**0.04**	**4.24**	**0.006**	1.03	0.404	0.82	0.483	1.120	0.343
Mortiriellomycota	Mortiriellales	**17.07**	**<0.001**	0.56	0.456	**6.01**	**<0.001**	2.08	0.06	**2.72**	**0.04**	1.18	0.288
Chytridiomycota	Spizellomycetales	**11.90**	**<0.001**	**4.57**	**0.035**	0.67	0.569	**2.4**	**0.030**	0.49	0.687	0.72	0.780

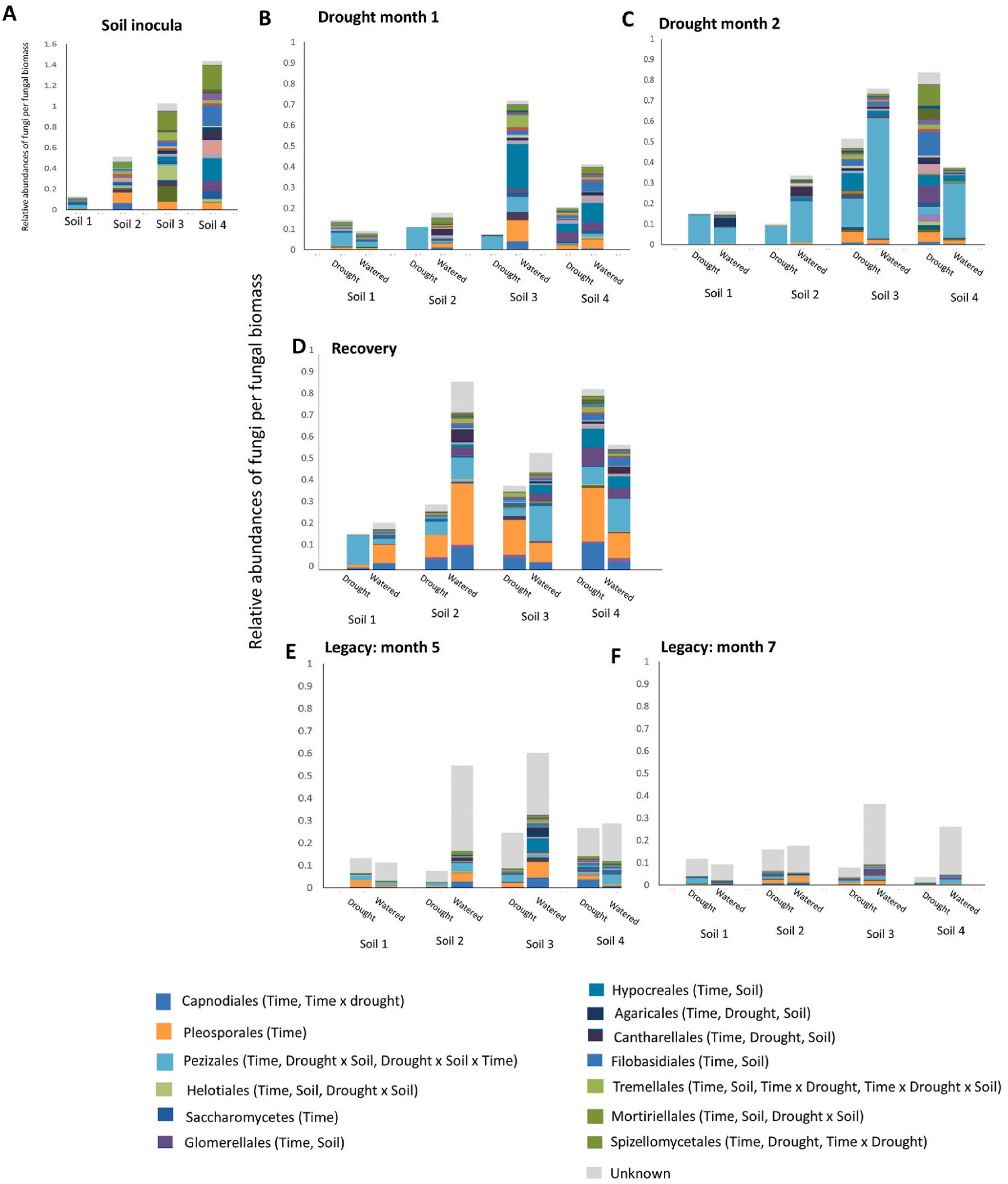


Fig. 6. Fungal community composition in order level expressed per fungal biomass in each treatment measured with ergosterol. (A) represents initial soil inocula, (B–C) fungal communities during drought period, (C) during recovery period and (E–F) during legacy period (E = 5 months & F = 7 months). The statistical significance is given for each major order, for details see Table 2 and Table S1. Data as relative abundance before scaling is shown in Fig. S4.

Capnodiales (major species: *Mycosphaerella tassiana*) were affected by interaction between time and drought ($F = 3.060$, $p = 0.007$), indicating that during drought period and shortly thereafter there were relatively more Capnodiales in soils that experienced drought but later in the legacy period there were relatively more Capnodiales in soils that had legacy of watering. Furthermore, there were relatively more Capnodiales in the rhizosphere of the crop grown in soils in the next season that were watered than in soils that had experienced a drought in the previous year ($F = 8.4516$, $p = 0.006$; Table 2). These effects were consistent across the four soil inocula. Glomerellales (major species: *Plectosphaerella cucumerina*), Saccharomycetes and Pleosporales were not affected by drought overall (Table 2) but in recovery phase in the rhizosphere of crops they were all more abundant in the soils that were well watered (Table S1; Fig. 6). From Basidiomycota orders, Tremellales were affected by drought in a time-dependent manner (interaction time x drought: $F = 2.41$, $p = 0.03$; Table 2). The strongest effect of drought on Tremellales was observed in recovery phase in soils when we detected more Tremellales in soils that have been previously subjected to drought ($F = 6.84$, $p = 0.014$; Table S1). Another group of Tremellomycetes, Filobasidiales, were affected by initial soil inoculum as well as time but there were also significantly more of them in the recovery phase in the rhizosphere soils that had experienced drought (F = 7.66, p

< 0.001; Table 2). There were more Agaricomycetes in soils that had been watered ($F = 11.08$, $p = 0.001$) and this was consistent in time and across soil inocula (no interaction time x drought or soil x drought). This effect of drought on abundance of Agaricomycetes was strongest in recovery period and noted for both rhizosphere and soils (Table S1). Spizellomycetales (dominant order of Chytridiomycota) were more abundant in soils that had been watered ($F = 4.57$, $p = 0.035$; Fig. 6) and the effect was dependent on time (interaction drought x time: $F = 2.40$, $p = 0.030$). This effect was most evident in the rhizosphere in recovery period ($F = 46.76$, $p < 0.001$; Table S1).

3.4. *Effects of drought on plant growth and soil functions*

Significant effect of the drought on growth of the plants was detected in the recovery period. The aboveground (soil inoculum: $F = 12.04$, $p < 0.001$, drought: $F = 6.61$, $p = 0.028$) and belowground biomass (soil inoculum: $F = 7.59$, $p < 0.001$, drought: $F = 6.39$, $p = 0.031$) of potatoes were affected by initial soil inoculum and the drought but not their interaction. The yield of the potatoes was affected by the soil inoculum ($F = 6.52$, $p = 0.0012$) and the interaction of the drought and the soil inoculum ($F = 3.90$, $p = 0.019$; Fig. 7A) but not drought alone. In general, plants growing in soil inoculum 4 produced most aboveground and belowground biomass and biggest yield, yet drought severely reduced the yield also in this soil (post hoc $p < 0.05$; Fig. 7A). Drought had a slight negative effect in soils with inocula 1 and 2, but this was not significant (post hoc $p > 0.05$; Fig. 7A). In contrast to all other inocula, the yield in soils with inoculum 3 was slightly higher after the drought (Fig. 7A). One year after the drought when growth of barley was measured, we found that the height of the barley was not affected by the legacy of drought, but still differed between soils with different inocula, with the tallest plants in soil inocula 2 and 3 and the smallest in soil inoculum 1 (soil: $F = 4.55$, $p < 0.022$: Fig. S5). Two years after drought, no effects of legacy of drought or soil inocula on growth of carrots were detected (Fig. S5).

For the decomposition measured with tea-bag index, we found that both the stabilization factor (s) and the decomposition rat (K) e were affected by a legacy of drought, but there was no effect of soil inocula or an interaction between drought and soil inoculum. The stabilization factor was higher in soils that had legacy of drought ($F = 9.80$, $p = 0.003$; Fig. 7B) while the decomposition rate was lower in soils with legacy of drought ($F = 11.00$, $p = 0.002$; Fig. 7C).

Across the entire experimental period, soil respiration was mainly affected by time ($F = 6.82$, $p < 0.001$), with respiration in general being highest during summertime (Fig. 8). Soil respiration was not consistently affected by soil inocula, drought or interactions of factors. Similarly, cellulose-induced respiration was overall affected most by time ($F = 80.44$, $p < 0.001$), with highest substrate induced respiration in October (month 5; Fig. S7). In addition, cellulose-induced respiration was also affected by interaction of time and drought (drought x time: $F = 5.1682$, $F < 0.001$) and the effect ranged from legacy of drought having positive effect on respiration in December (month 7) to having slightly negative effect on respiration in June (drought period), August (recovery period) and May (month 12; Fig. S7). During the drought period respiration differed between soils with different inocula ($F = 7.44$, $p = 0.013$), drought treatment ($F = 6.441$, $p = 0.020$), and the interaction of the two ($F = 4.39$, $p = 0.050$; Fig. 8B). More specifically, more CO_2 was released from the soils experiencing drought and the pattern was strongest in soils with inocula 2 and 3 (Fig. 8B). During the recovery period, no effect of soil inocula or drought on respiration was detected although in soil 4 respiration was higher in soils under drought

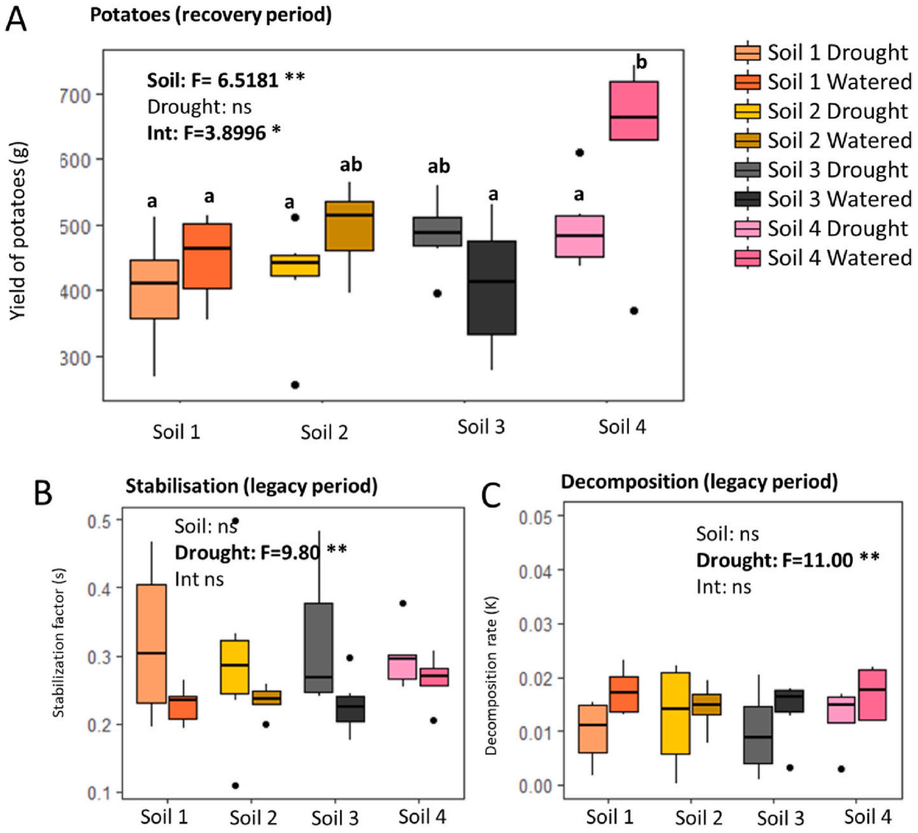


Fig. 7. Effect of soil inoculum and drought legacy on plant growth and decomposition of tea bags during recovery period. The yield of potatoes (A) was measured after 3 months of growth at the beginning of recovery period and (B) stabilization factor (s) and (C) decomposition rate (K) measured with tea-bags after 6–9 months in legacy period. Different colors denote different soils, and boxplots with mean and 95 % confidence intervals are shown. Statistics are shown in figure. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

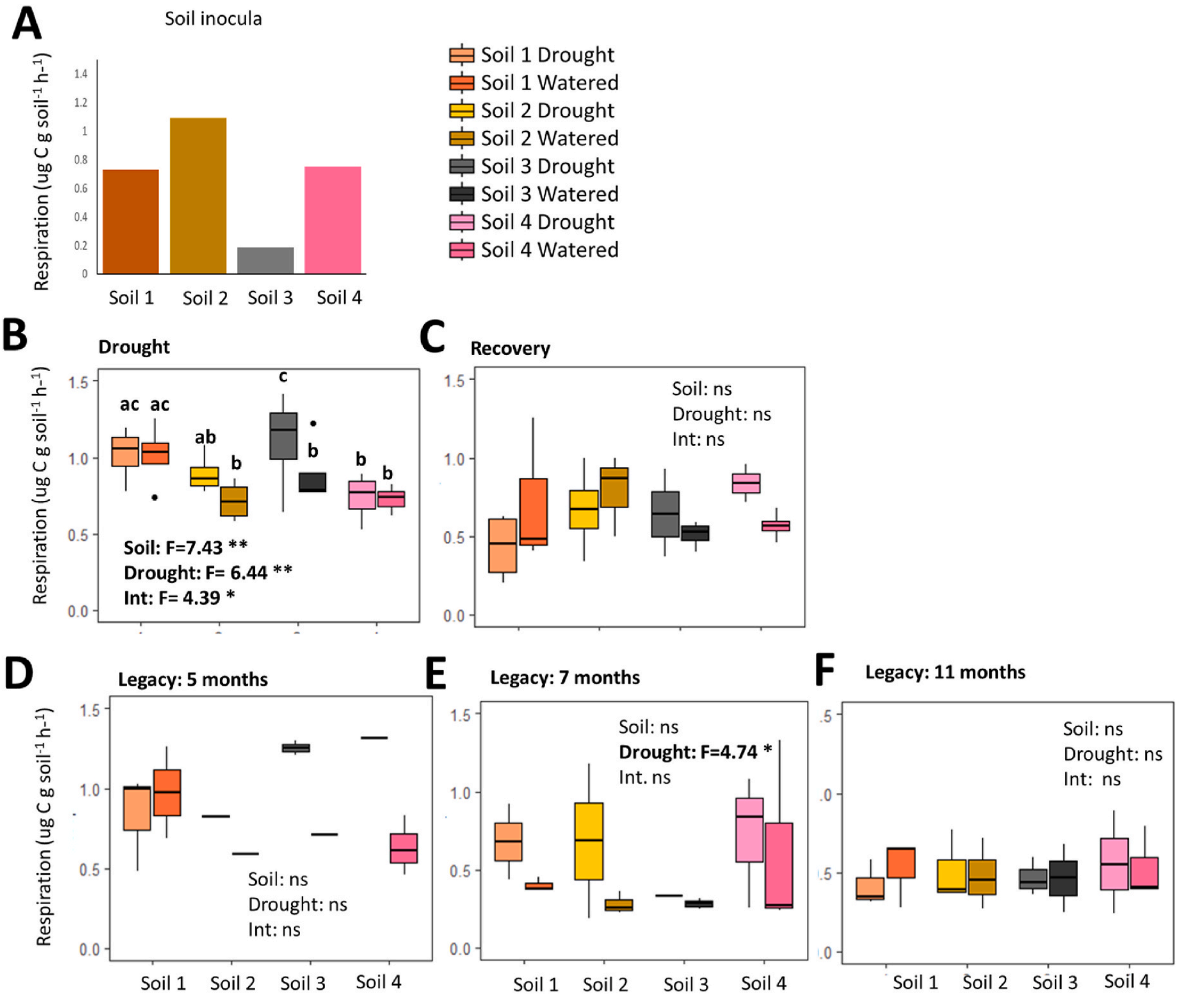


Fig. 8. The initial differences between soils used as inocula in soil respiration (A) and the effect of drought on soil respiration (B–F). Differences in responses between soils to drought and its legacy are shown for (B) period of drought, (C) recovery period and (D–F) legacy period (when D = 5 months, E = 7 months & F = 11 months). Different colors denote different soils (A) and soil-drought combinations (B–F) and and boxplots with mean and 95 % confidence intervals are shown. Statistical significances are shown in figure. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

as compared to soils that were watered (Fig. 8C). During legacy period (Fig. 8D–F), in general, there was more CO_2 released from soils with legacy of drought and this effect was significant in month 7 ($F = 4.7411$, $p = 0.042$; Fig. 8E). Cumulative respiration (calculated as sum of respiration at different times) was higher in soils that had experienced drought, but this was not significant ($F = 0.03$, $p = 0.87$; Fig. S6a). Cellulose-induced respiration was not affected by drought during drought. Only in the legacy phase (month 7) the substrate induced respiration was higher in soils that had experienced drought compared to watered soils ($F = 7.82$, $p < 0.005$; Fig. S7). Cumulative cellulose induced respiration was not affected by drought ($F = 0.005$, $p = 0.95$; Fig. S6b)

During the drought period, we evaluated the number of (naturally occurring) aphids on potato leaves at three time points. We did not detect significant effects of drought, soil inoculum or their interaction on number of aphids (Fig. S8A). In the legacy period we evaluated damage on leaves of cover crops caused by chewing caterpillars and no effect of legacy of drought was detected on the damage (Fig. S8B). In contrast, over a year after the drought the number of soil springtails was affected by an interaction between the legacy of drought and soil inoculum (drought x soil: $F = 8.68$, $p = 0.005$; Fig. S8C). In all other soil inocula but in soil inoculum 2 the drought caused an increase in the number of collembola extracted from the soil (Fig. S8C).

3.5. *Multifunctionality*

We summarized functional data into one multifunctionality index using Z-values of each functional measurement. The Z value itself was significantly affected by soil inoculum type ($F = 9.73$, $p = 0.004$) but not by drought or interaction between drought and soil inoculum ($p > 0.05$ for all; Fig. S11). Soils with inoculum 4 had highest (and most positive) score while all other soil inocula had similar (and mostly negative) Z-values. We correlated the Z-value with fungal diversity overall and measured at different time points and noted that overall diversity was significantly correlated with the multifunctionality ($R^2 = 0.086$, $p < 0.001$; Fig. 9A; Table 3). We further observed that the fungal diversity during the drought ($R^2 = 0.251$, $p < 0.005$; Fig. 9B; Table 3; Fig. S10A) and during recovery period in bulk soil ($R^2 = 0.23$, $p < 0.001$; Fig. 9B; Table 3; Fig. S10A) but not in the rhizosphere ($R^2 = 0.09$, $p = 0.167$; Fig. 9B; Table 3; Fig. S10A) was correlated with overall multifunctionality of the soils. The association between diversity and multifunctionality disappeared in the legacy period. When looking in more detail, all measured functional parameters also themselves responded positively to fungal diversity but none were significant alone (Fig. 9B). The correlation between fungal diversity and multifunctionality was stronger in soils that had experienced drought and this was strongest during the direct drought period (Fig. 9C; Fig. S11A). Also the community structure of fungi measured with PcoA axis based on Bray-Curtis

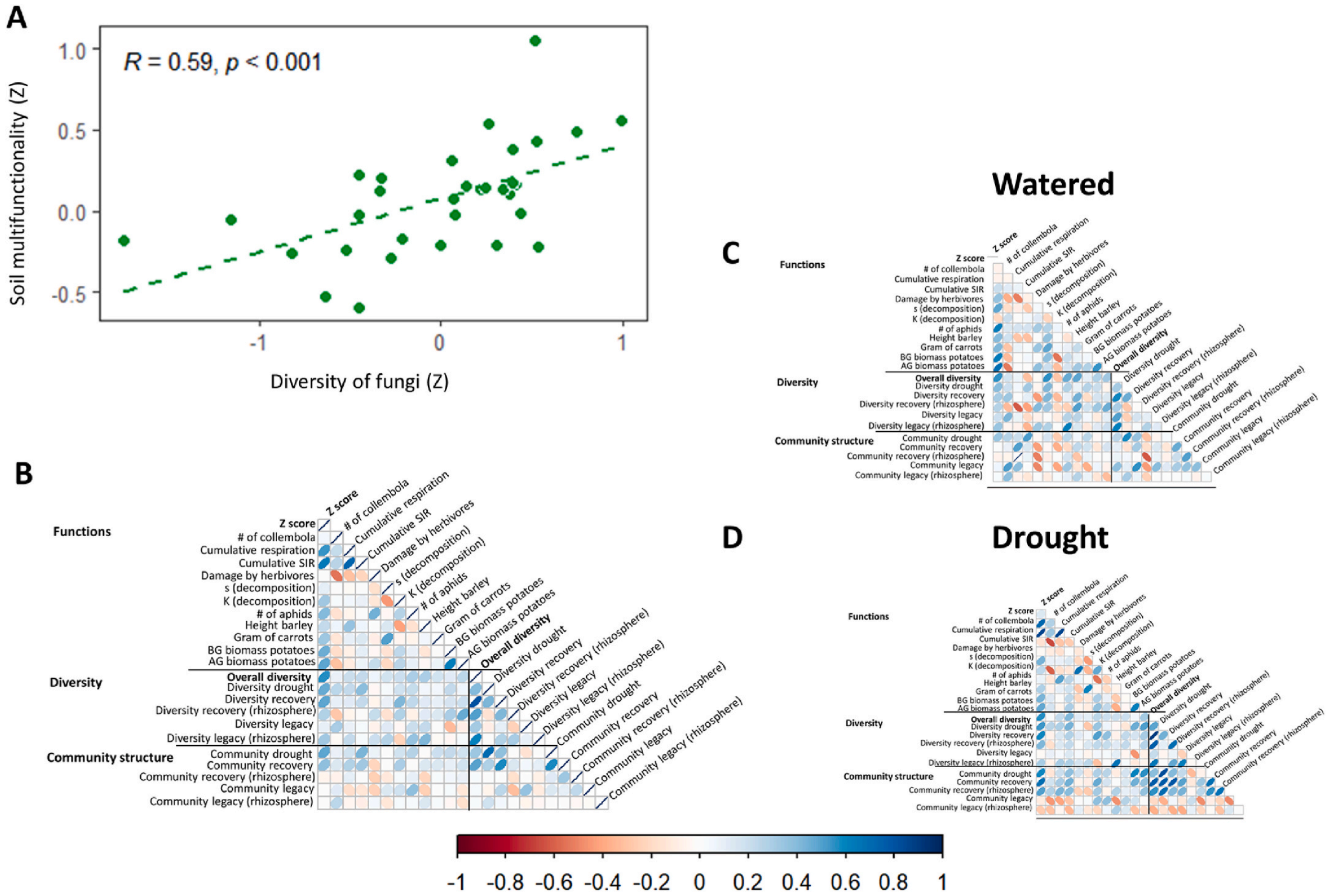


Fig. 9. The relationship between multifunctionality and fungal diversity across time points (A) and correlations between individual functions, fungal diversity and community structure at different times after drought overall (B) and separated between watered soils (C) and soils that experienced drought (D).

Table 3
The correlations between multifunctionality (Z) with biomass, diversity and community structure of fungi. Significant correlations are marked in bold and R^2 values are given. Diversity was measured with Simpson diversity index and standardized using z-transformation and community structure analysed using two first PcoA axis explaining 17.8 % and 13.5 % of variance in community structure. Ergosterol was not measured for rhizosphere samples and hence no data on fungal biomass for rhizosphere soils are available.

	Simpson diversity (R2)	Community structure: Pcoa1 (17.8 %) (R2)	Community structure: Pcoa2 (13.5 %) (R2)	Fungal biomass (R2)
Total	**0.086**			0.022
Drough period (soil)	**0.266**	**0.264**	0.000	0.000
Recovery period (soil)	**0.229**	**0.154**	0.010	0.001
Recovery period (rhizosphere)	0.089	**0.225**	0.021	nd
Legacy period 1 (soil)	0.000	0.022	0.074	0.047
Legacy period 2 (soil)	0.008	0.004	0.040	0.023
Legacy period (rhizosphere)	0.013	0.015	0.001	nd

dissimilarity was significantly correlated with multifunctionality during drought (R^2 = 0.263, p = 0.001 Fig. 9B–Table 3; Fig. S10B) and immediately in the recovery phase both in the bulk soil (R^2 = 0.154, p = 0.019, Fig. 9B–Table 3; Fig. S10B) and in the rhizosphere (R^2 = 0.225, p = 0.007, Fig. 9B–Table 3; Fig. S10B). Similarly to the measurements of diversity, the relationship between multifunctionality and community structure got weaker in time after drought. Finally, we evaluated the relationship between multifunctionality and fungal biomass and found that these parameters were not linked (Table 3).

4. Discussion

We show that drought affected the bulk soil fungal community structure and biomass during the drought period and immediately thereafter. Even though the effects on fungal community structure and biomass got smaller after time passed since drought (i.e., during the recovery and legacy period), legacy effects of the drought were strong enough to affect fundamental soil functions such as decomposition and respiration indicating of long-term impacts of drought. Importantly, the responses to drought depended on the initial soil microbiome, represented here by the four different inocula selected based on biomass of fungi but varying also in terms of community composition of fungi and other organisms. This indicates that the initial soil microbiome drives the impacts the drought has on soils.

In this study we show that, in line with our first hypothesis, fungal communities changed during drought and while the effect of drought legacy on fungal community structure got smaller in time, some fundamental change had happened that profoundly affected ecosystem functions in longer term. This was reflected in the clear relationship we detected between fungal diversity and soil multifunctionality that was evident during the drought and in recovery period. Earlier studies have shown that in short term, drought and following re-wetting leads to enhancement of microbial activity (so called Birch effect; Birch, 1958) yet here we were well beyond these short term effects and show long-term net negative effects of drought and consequent re-wetting on many of the soil microbial functions. Although fungi are thought to be more resistant but less resilient to drought than bacteria (Bapiri et al., 2010; Meisner et al., 2013; Barnard et al., 2013; de Vries et al., 2018; Canarini et al., 2024), the observed fundamental shift in structure and functions is in line with recent evidence showing that abrupt events such as severe drought can lead to irreparable changes in soil microbial communities and further impair major soil functions (Barnard et al., 2013; Cordero et al., 2023). Although, we here focused on drought impacts on fungi in our current study, it could still be that the changes in ecosystem functions were partly driven by impacts of drought on bacteria and other soil organisms. Furthermore, it could be that ratios between organisms (for example fungi to bacteria ratio) are affected by drought and hence affecting soil functions (Ullah et al., 2021). Previous studies have shown that soil bacteria, although more sensitive to drought (de Vries et al., 2018), have rapid turnover rates as compared to fungi (Hannula et al., 2019). Furthermore, it is known that the effects of soil legacies on fungal communities last longer than those for bacteria (Buscardo et al., 2021; Hannula et al., 2021) and hence we speculate that the long-term effect of drought in soil functions are largely mediated by changes in fungal taxa.

We expected changes in fungal communities over time, irrespective of drought (Hannula et al., 2019) and indeed, detected that both time and season, independent of drought, affected fungal community biomass, diversity, community structure and activity (measured with respiration). Generally, lower biomass, and activity was observed during the cold season. In addition, and in line with earlier work, these temporal changes in fungal communities were also modified by drought. Particularly, the temporal stability of networks (Röttjers and Faust, 2018) was lower in all soils with legacy of drought. This indicates that communities are potentially decoupled as a result of drought (Ochoa-Hueso et al., 2021). Previous studies did not find short-term effects on fungal networks as a result of drought (de Vries et al., 2018) yet, long term effects of drought on stability are virtually un-explored. We noted that certain fungal orders such as Capnodiales (especially *Mycosphaerella tassiana)* and Pleosporales were more co-occurring across time points in soils under legacy of watering as compared to soils under drought while Glomerellales were more connected with legacy of drought. This indicates that different species become core species in a community (Banerjee et al., 2018) following a drought event. Within Glomerellales, the dominant species and the species with biggest increase in connections and stability following drought, was *Plectosphaerella cucumerina*, a known pathogen of many plants and well known endophyte of potatoes however, without known pathogenicity towards potato (Scholte et al., 1985), one of the crops used in this study. This increase in centrality of potentially pathogenic species could further be linked to reduced crop performance and soil functions.

It is known that drought can increase the ratios of fungal functional groups and change the key species within the system (Buscardo et al., 2021). In line with this, we detected a major shift in dominance of fungal taxa in soils during the drought. Most notably, Pezizales made up almost the entire fungal community in the soils with low initial fungal biomass when these soils were experiencing drought. Members of this order of fungi are known to be drought resistant and have been observed to even increase in abundance after and during drought events (Maisnam et al., 2023). Interestingly, in soils with higher fungal biomass and diversity, the increase in Pezizales was more modest. There were more Capnodiales in soils experiencing drought during drought but during legacy period the effect reversed and there were more Capnodiales (and specifically known endophyte species *Mycosphaerella tassiana* (Sharma-Poudyal et al., 2017; Sun et al., 2023)) in soils with a legacy of watering. This effect extended to the rhizosphere of barley one year after the drought event. This would indicate that Capnodiales are resistant to drought but when situation reverses after watering is continued (and new species establish), they lose their dominant position in the community. Furthermore, there were less Basidiomycetes and specifically Agaricomycetes and Tremellomycetes in both rhizosphere and bulk soils with a drought legacy in recovery period. It seems that drought conditions led to loss of certain species from the system or shift in relative abundance of organisms which could potentially be linked to changes in ecosystem functions such as decomposition.

Overall, it is interesting that the effects of a past drought were stronger in rhizosphere and extend to the rhizosphere of the next crop. This could be due to specific selection of plants for their rhizosphere microbiome that is not a random subset of the bulk community (Hannula et al., 2021) or due to effects of reduced plant fitness during drought period and hence change in rhizodeposits or signaling chemicals (Canarini and Dijkstra, 2015; Williams and de Vries, 2020). It could be the plant-soil interactions that are more affected by drought than either soil fungi or plant alone (de Vries et al., 2023) which would be seen as stronger effect on rhizosphere microbes. In the rhizosphere of the crop that experienced drought, we detected overall decreased diversity and reduced abundance of Glomerellales, Saccharomycetes, Pleosporales and Spicellomycetes. As these are not same orders affected by drought in the bulk soil, we speculate that these fungi are affected more indirectly by drought via interactions with the plant.

In line with our second hypothesis, we found long-lasting shifts in soil functioning, e.g. decomposition and crop yield. It has been shown that full recovery of an ecosystem after severe drought can take from months to years (Anderegg et al., 2015; Vilonen et al., 2022). Earlier studies have shown that in short term, drought and following re-wetting leads to enhancement of microbial activity (Birch, 1958) yet here we were well beyond these short term effects and show long-term net negative effects of drought and consequent re-wetting on many of the soil microbial functions. While there are less studies on soil ecosystems and mycobiomes, recent evidence on grassland shows that shifts in microbiomes during drought can have long lasting effects on soil enzymatic activities (Cordero et al., 2023). Here we show that the change in fungal community connectivity and structure as a result of drought coincided with changes in soil multifunctionality. Our findings align with earlier work suggesting that arable soils may be more sensitive to drought disturbances (de Vries et al., 2012) as they are subject to multiple disturbances acting at the same time, increasing pressure on these soils (Creamer et al., 2016; Tsiafouli et al., 2015). In addition, we know that when fungal communities are subject to multiple stressors, shifts in community composition and diversity and result in a loss of functions (Rillig et al., 2019). We show that in short term, drought had an effect on the key function in arable systems, namely crop yield. Although drought reduced overall yield, the magnitude of the effects differed strongly between the initial microbiome present, in line with predicted changes in plant-microbe interactions following drought (de Vries et al., 2018, 2023; Kaisermann et al., 2017; Meisner et al., 2013). In longer term, the legacy of drought affected important soil functions such as decomposition rate (affected negatively by legacy of drought), soil respiration (increased in soils with legacy of drought) and cellulose-induced respiration. These findings concur with earlier work showing that drought legacy affected fungal cellulose decomposition and the amount of C released from the system (Liu et al., 2022), carbon use efficiency (Ullah et al., 2021) and decomposition in both mid-term (Manrubia et al., 2019) and long-term (Martiny et al., 2017). Furthermore, we detected that the drought legacy led to higher stabilization factor for the tea bags, which could indicate an inhibition of litter decomposition after drought (Keuskamp et al., 2013). We speculate that long-term effects of drought on soil functions can further have fundamental effects on fungal mediated soil ecosystem functions related to carbon and nutrient cycling (Arnone III et al., 2008; Canarini et al., 2021) and eventually have big effects on soil carbon storage (Chomel et al., 2022; Müller and Bahn, 2022; Reichstein et al., 2013) either direct or due to reduce carbon inputs through from plants (Ciais et al., 2005; Frank et al., 2015). As soils are among largest sinks of carbon and linked to plant communities, it is crucial to understand the long-term responses of soil organisms to drought and from the perspective of climate mitigation it is of utmost importance to understand especially the fungal responses to drought (Hannula and Morriën, 2022).

We partially confirm our third hypotheses that soils with highest initial fungal biomass would be more resilient and resistant towards drought. Earlier studies have investigated the effects of drought on microbiomes and related functions across land-use gradients (Chomel et al., 2022; de Vries et al., 2012; Glass et al., 2023) but few have used inoculation approach to standardize the soil abiotic conditions and exactly same other conditions in experimental gardens. The strength of our study is its ability to detect consistent responses to drought across fungal biomass levels and communities. We show across all inocula that soil fungi and associated functions were affected by drought but the magnitude of the effect varied depending on initial community. Intriguingly, some patterns like more stable species co-occurrence networks in soils that were well watered and consistent effects on decomposition were detected across most soil inocula while other patterns like respiration and effects on collembolan feeding were affected differently by drought depending on the initial microbiome. The reduction of network complexity was most notable in the soils with low fungal biomass at the beginning yet the observed negative effects of drought on immediate yield were largest in soils with highest fungal biomass. Furthermore, community composition changed more in soils with initially lower diversity and fungal biomass. While the effect on plant growth disappeared in time and was not measured for following plants, we showed that that richness of collembola was affected in a soil specific manner a year after the drought which is in line with long term effects on microbial feeders detected earlier (Siebert et al., 2019). Our findings on more negative effects of drought on community structure and network complexity in soils with lower fungal biomass are in line with recent results showing that increasing intensity of soil management, through disruption of food-web complexity, amplifies the severity of effects of drought on soil organisms (Chomel et al., 2022). However, we note that effects of drought on plant yield were more context dependent and the inoculum with most fungi led to most yield but also biggest reduction in yield due to drought.

All the soils and consequently fungal communities tested here were probably pretty naïve for long-lasting drought events as they have been all until recently been in agricultural use and hence watered. It is to be speculated that the novel fungal community composition resulting from previous drought(s) would be better adapted to future drought events (Bonebrake and Mastrandrea, 2010; Bouskill et al., 2016; Canarini et al., 2021; Coleman and Chisholm, 2010; Narayanan et al., 2021) and this could make the effects of future droughts on the functions of these ecosystems less severe (Fuchslueger et al., 2016). It has been proposed by Kaisermann et al. (2015) that within same fungal population, different sub-populations occupying different moisture niches would co-exist and higher plasticity would make them good indicators. We show that there were differences in how the different soil inocula containing different fungal biomass and communities responded to drought and consequently how it affected functions.

Lastly, using both time series and different soil inocula we detected that the soil multifunctionality was positively correlated with diversity of fungi. This is in line with findings from field studies (Delgado-Baquerizo et al., 2016) that soils with more diversity also provide more functions. However, we detected that the soil functions were not more resilient to drought in more diverse soils indicating that they are potentially more vulnerable to external factors questioning the so called 'insurance-hypothesis'. We could further show that the relationship between multifunctionality and fungal community structure and diversity was strongest during the drought period and immediate recovery in the bulk soil potentially indicating that functions were more coupled to fungal communities during disturbance (Ochoa-Hueso et al., 2021). Moreover, the diversity and community structure of fungi affected the functionality of soils stronger when it is experiencing drought compared to well-watered conditions.

5. Conclusions

In summary, drought, which is predicted to increase in frequency with climate change, has short term effects on soil fungal communities that lead to long term changes in soil functions mediated by soil communities. We show that severity of drought legacy is dependent on initial soil microbiome. From a management perspective, the effect of watering should be considered as an option to manage long-term soil functions. Furthermore, we call for more investigations of longer term (over a year) effects of drought on soil biodiversity and functions related to especially carbon cycling.

CRediT authorship contribution statement

S.E. Hannula: Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Data curation, Conceptualization. **G.F. Veen:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Data availability

Sequencing data is available in NCBI nucleotide archive under accession number PRJNA1213495 and all soil functional data is archived in Dataverse.nl https://doi.org/10.34894/N4XNPL.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Emilia Hannula reports financial support was provided by Maj & Tor Nessling foundation. Emilia Hannula reports financial support was provided by NWO. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.109893.

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