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Long-term agricultural management impacts arbuscular mycorrhizal fungi more than short-term experimental drought

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ABSTRACT

Agricultural management practices and extreme weather events associated with climate change can influence the diversity and abundance of arbuscular mycorrhizal fungi (AMF) with potential consequences for crop production. However, the importance of the interactive effects of long-term agricultural management and extreme weather events on AMF communities in agricultural soils is not yet fully explored. A short-term drought experiment with rainout-shelters was performed in winter wheat fields in a long-term agricultural trial with organic (biodynamic) and conventional management practices. During four months of the winter wheat growing period (March–June 2017), the rainout-shelters reduced the ambient precipitation by 65% on average. At two sampling dates, the AMF diversity and community composition were assessed using a single-molecule real-time (SMRT) DNA sequencing. A total of 955 amplicon sequence variants (ASVs), belonging to twelve genera were identified. The long-term farming systems and the short-term experimental drought did not affect AMF ASV diversity levels. The AMF community composition at the genus level differed between the organic and the conventional farming systems, but no distinctive communities were found in response to the experimental drought. The three most abundant genera *Acaulospora*, *Paraglomus* and *Funneliformis* were correlated to the two farming practices. Our study demonstrates that AMF communities in agricultural soils are responsive to long-term farming systems, and are resistant to one short-term summer drought event.

1. Introduction

Climate change and agricultural intensification are currently considered as the major threats to agricultural ecosystems that may cause losses in soil biodiversity (de Graaff et al., 2019; Geisen et al., 2019). Soil microorganisms are pivotal to the provisioning of essential ecosystem services, such as nutrient cycling, soil carbon sequestration and soil formation, which support agricultural production (Bardgett and van der Putten, 2014; van der Heijden et al., 2008). Climate models for Central and Southern Europe predict an increased number of extreme weather events, resulting in more frequent and extended drought periods, as well as rainfall extremes (Iglesias and Garrote, 2015; Spinoni et al., 2015a). These changes are expected to have a negative impact on agricultural production in Europe (Webber et al., 2018) and the diversity, abundance and functions of soil microorganisms (Cavicchioli et al., 2019; Jansson and Hofmockel, 2020). However, due to a lack of field experiments testing the effects of predicted extreme weather events under different farming practices, we still have an incomplete understanding of how microorganisms respond to the combination of intensive farming and extreme weather events (Bardgett and Caruso, 2020; Fierer, 2017; Schädler et al., 2019). It is therefore crucial to understand whether existing agricultural practices can be adapted to future extreme weather events in order to make agricultural ecosystems more resilient (Gornall et al., 2010; Spinoni et al., 2015b).

Management practices such as tillage, intensive fertilization and application of pesticides can reduce soil biodiversity (Tsiafouli et al.,

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2015). In combination with the predicted extreme weather events, this may impact soil microbial communities, their resistance and resilience to e.g. drought and limit their capacity to provide important services to society (de Vries et al., 2012; Giller et al., 1997). To reduce such negative effects on soil biodiversity, alternative farming practices as for example organic farming have been proposed (McLaughlin and Mineau, 1995). Organic farming aims at obtaining high-quality crop yields while maintaining soil biodiversity in the long-term (Birkhofer et al., 2016; Rundlöf et al., 2016). Numerous studies have shown positive effects of organic farming on soil fertility (Mäder et al., 2002), soil organic carbon (SOC) content (García-Palacios et al., 2018; Gattinger et al., 2012) as well as microbial biomass, activity and diversity (Esperschutz et al., 2007; Hartmann et al., 2015; Lori et al., 2017). However, the beneficial effects of organic farming on biodiversity often come at the cost of lower crop yield (Seufert et al., 2012).

To mitigate adverse effects of drought in soils, SOC levels can be promoted which improve soil properties such as water holding capacity, aggregate structure and water infiltration (Iizumi and Wagai, 2019; Lal, 2016; Lal et al., 2011). Moreover, higher SOC levels promote soil microbial biodiversity and activity (Birkhofer et al., 2012) and can buffer the negative effects of drought events on crop yields (Droste et al., 2020). Despite the fact that the appropriate agricultural management can promote SOC levels, which can increase the resistance of soil microorganisms to drought and lead to higher crop yields, the combined effects of long-term agricultural management and extreme weather events on soil microorganisms in agroecosystems with annual crops remain unexplored. Most studies that have addressed the question of how the interactive effects between management practices and drought affect soil microbes, were conducted in grasslands or forests (Bastida et al., 2019; de Vries et al., 2012; Karlowsky et al., 2018; Siebert et al., 2019). The focus on drought effects has mainly concerned fungal and bacterial communities, far less is known about the responses of arbuscular mycorrhizal fungi (AMF).

AMF form symbiotic relationships with plant roots, including most crop species, such as cereals (Schüßler et al., 2001; Smith and Read, 2010). The plants supply AMF with photosynthetic carbon and in return, AMF can provide benefits to the host plants (Smith and Read, 2008). AMF can improve the plant nutrient and water uptake (van der Heijden et al., 1998), protect plants from soil pathogens (Newsham et al., 1995), enhance soil stability and aggregation (Rillig and Mummey, 2006) and with this promote drought tolerance (Augé, 2001). Mycorrhizal hyphal networks can enter soil pores inaccessible to root hairs and increase host plants' root surface for water uptake (Marulanda et al., 2003; Ruiz-Lozano, 2003). While AMF can enhance the drought tolerance of their host plants, it is not fully understood how AMF communities respond to drought stress in agricultural ecosystems (Millar and Bennett, 2016). AMF can respond to water-deficits by changing community composition in grasslands (Deveautour et al., 2018; Deveautour et al., 2020). While, drought did not affect AMF communities in an agroecosystem in Canada (Furze et al., 2017) or Chinese subtropical secondary forests (Maitra et al., 2019). However, AMF biomass increased under experimental drought in grasslands (Karlowsky et al., 2018; Mackie et al., 2019) as well as in arable production systems with cereals (Kundel et al., 2020). Whether AMF communities' capacity to tolerate drought depends on soil abiotic factors, on AMF community diversity and composition or on associated plant community is unknown. Besides drought, agricultural management, in particular mechanical soil disturbance by tillage, affects AMF communities and leads to reduced abundance of spores, reduced root colonization, lower taxonomic diversity, reduced biomass and altered community composition of AMF (Helgason et al., 1998; Jansa et al., 2002; Schnoor et al., 2011). Farming practices such as organic farming can have positive (Birkhofer et al., 2012; Mäder et al., 2002; Verbruggen et al., 2010) or neutral effects on AMF communities (Williams and Hedlund, 2013). However, whether organic farming, aiming at buffering climate change effects through promoting SOC levels, also can enhance the drought tolerance of AMF communities remains unexplored. Therefore, investigating the responses of AMF communities to extreme drought in organic and conventional farming systems may improve our understanding of the functioning of arable crop production systems in a changing climate.

In this study, we tested the effects of a short-term experimental drought on the diversity and community composition of AMF in soils from a 39-year old long-term agricultural experiment with organic (biodynamic) and conventional farming systems, the Swiss DOK trial (Krause et al., 2020; Mäder et al., 2002). To simulate a short-term drought, rainout-shelters (Kundel et al., 2018) were established in replicated winter wheat plots under the two farming systems over one growing season (Kundel et al., 2020). The AMF diversity and community composition were assessed using single-molecule real-time (SMRT) DNA sequencing. We addressed the following questions: (I) How do long-term organic and conventional farming practices and a short-term experimental drought and their interactive effects influence the diversity and community composition of AMF? (II) Which AMF taxa are particularly sensitive to drought and most abundant in a specific farming system? (III) What are the key soil properties influencing the AMF community composition and would SOC content mitigate drought effects?

2. Material and methods

2.1. Experimental site and field trial description

This study was a part of the ERA-Net Biodiversa project 'SOILCLIM' and conducted in the DOK long-term agricultural experiment (Therwil, Switzerland). The DOK trial (bio**d**ynamic, bio**o**rganic and conventional [**k**onventionell]) was established in 1978 and compares organic and conventional farming systems that differ in fertilization and plant protection practices, but follow the same seven-year crop rotation (Krause et al., 2020; Mäder et al., 2002). The trial site (47°30′09.3″N, 7°32′21.5″E, 300 m altitude) is situated on a Haplic Luvisol on deep deposits of alluvial loess (Fliessbach et al., 2007). The annual average temperature of 10.5 °C and the mean annual precipitation of 890 mm were measured over the last five years (Bodenmessnetz) (data retrieved on August 1, 2019). The rainfall amount is constant between different years, however in the past few years increased extreme weather events, either drought periods or even floods, were observed at the study site.

To examine the long-term effects of agricultural management practices and short-term effects of reduced precipitation on the diversity and community composition of AMF, a manipulative field experiment started in mid-March and ended in June 2017. Fixed location, partial rainout-shelters removing a major part of the ambient precipitation were established in four replicated winter wheat (*Triticum aestivum* L., cv. Wiwa) plots (5 m × 20 m) in two farming system (biodynamic (BIODYN) and conventional (CONMIN)). The BIODYN system is managed according to the guidelines for 'Demeter' food production (https://demeter.ch/) and receives organic fertilizers exclusively (farmyard manure and slurry). The CONMIN system receives mineral fertilizers and agrochemicals according to the Swiss guidelines (Richner et al., 2017), plant protection strategies are based on insecticides, herbicides and fungicides following principles of integrated production systems (IP-SUISSE). The SOC content in the BIODYN system (1.60%, 95% CIs: 1.36, 1.86) was higher than in the CONMIN system (1.27%, 95% CIs: 1.03, 1.53) (Kundel et al., 2020). Tillage in both systems was constant and conducted to a 20 cm depth, in October 2016. More details on the management practices during the growing season 2016/2017 and the experimental design of this experiment are provided in Kundel et al. (2020). In each of the four replicated plots (arranged in field blocks) per farming system three drought treatments (subplots) were established: I) a rainout-shelter aiming at a 65% precipitation reduction to simulate drought conditions (Roof treatment), II) a control treatment to quantify potential rainout-shelter artefacts where the rainout-shelter was present but did not actively reduce the precipitation, see further description below (Roof-Control treatment), and III) an unmanipulated control

without a rainout-shelter (Control treatment). The Roof treatment consisted of twelve V-shaped acrylic glass profiles which intercepted part of the ambient precipitation, whereas in the Roof-Control treatment the V-shaped acrylic glass profiles were turned over allowing the precipitation to fall onto the subplot. The selected acrylic glass is permeable for the full range of photosynthetically active radiation and transparent for most UV-A and -B radiation (transmission: 380–780 nm ≥90%, 315 nm ≥80%). The rainout-shelter covered an area of 2.5 m × 2.5 m. A detailed description of the rainout-shelters, and their effects on air and soil temperature is provided in Kundel et al. (2018).

2.2. Soil sampling

Soil samples were collected at two dates: 4 (mid-April 2017) and 13 weeks (mid-June 2017) after the rainout-shelter establishment. A total of 48 soil samples (2 farming systems (BIODYN, CONMIN) × 4 plots × 2 sampling times (4 and 13 weeks) × 3 drought treatments (Roof, Roof-Control, Control)) were collected from a sampling area of 0.1 m^2 inside the subplot. Available sampling area inside the subplot was defined by the maximum edge effect assessed under the Roof-treatment as described in Kundel et al. (2018). From each sampling area, multiple soil cores of bulk soil (~1 kg of soil, 3 cm in diameter, 20 cm depth) were collected between the wheat rows (~5 cm away from wheat) and pooled together. The soil samples were transported in cooling boxes, sieved (2 mm) and stored at −20 °C until DNA extraction.

2.3. Soil and plant properties

Data on soil physical and chemical properties (soil pH, total soil carbon, phosphorus, bulk density, water holding capacity), vegetation data (crop biomass, weed cover) and neutral lipid fatty acid (NLFA) 16:1ω5 to estimate AMF biomass used in the analyses was measured in the field experiment and obtained from Kundel et al. (2020). Carbon (C) and nitrogen (N) concentrations in the roots were measured on air-dried and ball-milled samples (10 mg) using an elemental analyser (Vario EL III, Elementar Analysensysteme GmbH, Langenselbold, Germany). Data of C and N concentrations in the root material is available in Table S1.

2.4. DNA extraction and PCR cycling

DNA was extracted from 500 mg of soil with the NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. The quality and the quantity of the extracted DNA were determined using NanoDrop 2000 (NanoDrop Technologies, Wilmington, Delaware, USA).

A 1.5-kb long fragment of the nuclear ribosomal RNA gene, comprising the entire internal transcribed spacer (ITS), parts of small (SSU) and large (LSU) subunit (Kruger et al., 2009), was amplified according to the protocol of Schlaeppi et al. (2016), with minor modifications. For the first polymerase chain reaction (PCR) round, SSUmCf/LSUmBr (Kruger et al., 2009) primers consisting of oligonucleotide mixtures prepared by equal molar mixing of individual oligonucleotides, were used. The PCR amplifications were done in triplicates, to minimize the stochastic PCR effects of individual reactions, where each 20 µl reaction mixture contained 0.5 µM of each of the primers pair, 0.6 µM bovine serum albumin (Thermo Fischer Scientific, Carlsbad, CA, USA), Phusion High-Fidelity PCR Master Mix (Thermo Fischer Scientific, Carlsbad, CA, USA) and 2 µl of template DNA. Negative (water) and positive controls (DNA extracted from grasslands) were included in each PCR round. Thermal cycling was performed on a Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The cycling conditions for the first PCR round were as follows: initial denaturation at 98 °C for 2 min, followed by 35 cycles of 98 °C for 10 s, 63 °C for 30 s and 72 °C for 1 min, with a final extension for 10 min at 72 °C. The PCR products were visually inspected on 1% agarose gel. Replicates were pooled, purified with PCR Clean-Up Kit (Thermo Fischer Scientific, Carlsbad, CA, USA) and used as a template (2 µl) for the second PCR. In this round, wobble primers (wSSUmCf/wLSUmBr (Schlaeppi et al., 2016)), containing 5-nucleotide-long padding sequence and sample-specific barcodes for sorting after sequencing were used. These primers were synthesised at Thermo Fischer Scientific (HPLC purified grade). For the second PCR round conditions were as follows: initial denaturation at 98 °C for 2 min, followed by 7 cycles of 98 °C for 10 s, 60 °C for 30 s and 72 °C for 1 min, with a final elongation for 10 min at 72 °C. As before, triplicate reactions were pooled, purified and visually inspected on an agarose gel. Purified PCR products were quantified (Quant-it PicoGreen Assay (Invitrogen, Carlsbad, CA, USA)) and equal molar amounts of the samples were pooled together.

2.5. Library preparation and sequencing

The SMRT methodology for AMF communities was chosen, because it allows sequencing of longer fragments, in this case 1.5-kb long fragment covering parts of the SSU, ITS and LSU regions. That offers improved specificity and higher resolution compared to Illumina sequencing of shorter AMF amplicons, where different DNA markers within this region are used for AMF taxa description. The library preparation and SMRT DNA sequencing were conducted at the Uppsala Genome Center. The sequencing libraries were prepared using the SMRTbell™ Template Prep Kit 1.0-SPv3 (Pacific Biosciences, Menlo Park, CA, USA) according to the manufacturer's instructions. The final library was sequenced on four Sequel SMRT cells using a PacBio Sequel I instrument. The raw sequencing data is stored and publicly available at the EMBL-ENA database (http://www.ebi.ac.uk) under the project ID PRJEB38629.

2.6. Sequence data processing

The SMRT portal (v8.0.0) was used for the generation of circular consensus sequences (CCS) from the raw sequencing reads. Overall, 481,165 CCS reads (range: 2,255 to 19,427 reads per sample including multiple passes of the nuclear ribosomal region) were generated. Read quality was checked through FastQC (Andrews, 2010) and demultiplexed using the PacBio barcode demultiplexer, Lima (https://github.com/pacificbiosciences/barcoding), where primers and sample-specific barcodes were removed. CCS reads were clustered into amplicon sequence variants (ASVs) using DADA2, v1.14 (Callahan et al., 2016). Singleton reads were not filtered initially, but ASVs that have a single read after error correction were then removed from the analysis. A total of 185,825 sequencing reads (length: min. 404, max. 11,426, mean 3871.4) from 48 samples clustered into 962 ASVs. The number of sequences retained after each bioinformatics filtering step in the DADA2 is available in Table S2. The taxonomic classification of the unique ASVs was done using the UNITE database, v8.2 (Abarenkov et al., 2020), with the dynamic clustering threshold and reverse strand matching, using the naïve Bayesian classifier (Wang et al., 2007). Fungal sequences other than Glomeromycota (1% that belonged to Asco- and Basidiomycota) were discarded and the final number of 955 ASVs (comprising 185,364 sequences) was obtained.

2.7. Statistical analyses

All analyses and statistical tests were computed in R, v3.6.1 (R Core Team, 2019) within RStudio (RStudio Team, 2018). The graphical visualization was performed using the GGPLOT2 package (Wickham, 2016).

A rarefaction analysis was performed using the function 'vegan::rarecurve' (Oksanen et al., 2019) and indicated that the sampling intensity was sufficient to detect the majority of sequence types (Fig. S1). Due to differences in sequencing depth, samples with less than 500 sequencing reads were discarded and the dataset was normalized by a total sum scaling (TSS) using the PHYLOSEQ package (McMurdie and

Holmes, 2013). The analyses related to AMF diversity were determined at the ASV level.

Two alpha diversity indices (observed richness, and the Shannon index (Shannon, 1948)) were computed from the ASV data, using the function 'phyloseq::estimate_richness'. The effect of the farming systems and the experimental drought on the AMF diversity, soil moisture, C and N concentrations in roots, were assessed with Bayesian linear mixed models using Stan's probabilistic programming language (Carpenter et al., 2017) for full Bayesian inference through the BRMS package (Bürkner, 2017). The farming systems, the drought treatments, the sampling times and their interactions were defined as fixed, and the plots nested in the field blocks as random effect. A negative binomial probability distribution was used to model the effect of observed richness, and the Gaussian distribution to model the Shannon index, soil moisture, C and N concentrations in roots. The sampling quality and model fit was confirmed using the web application available via the SHINYSTAN package (Gabry, 2018). Median values, 95% credible intervals (CIs), lower (2.5%) and upper (97.5%) limits of the CIs were calculated from the full posterior distributions.

To analyse the responses of the AMF community composition to the farming system, the drought treatment, the sampling time, we first performed principal coordinates analysis (PCoA) at the ASV level, using Bray-Curtis dissimilarity (Fig. S2). As next, permutational multivariate analysis of variance (PERMANOVA, function 'vegan::adonis2') with 999 permutations was used (Table S8). However, due to a high intraspecific variation, the community composition was finally assessed at the genus level as there were more ASVs assigned to genera than to species. The effects of the farming system, the drought treatment, the sampling time and all two-way interactions on AMF community composition were examined by PERMANOVA. A TSS normalized ASV table agglomerated on the genus level was used as the input data, and permutations ($n = 999$) restricted on the field blocks. The Bray-Curtis dissimilarity was used as a distance measure. Because the PERMANOVA test is sensitive to differences in multivariate dispersion, the homogeneity of variance was assessed using the 'vegan::betadisper' function. The factors with significant effects on the AMF community composition determined with the PERMANOVA test were then used as constraining term in a distance-based redundancy analysis (db-RDA ordination) (Legendre and Anderson, 1999), using the function 'vegan::dbrda'. The relationship between soil and plant properties and the AMF communities were assessed using the 'vegan::envfit' function. The significant variables ($p < 0.05$) were fitted onto the ordination as vectors (represented by arrows), to visualize their relationship with the AMF community structure (AMF taxa represented by dots). In addition, db-RDA ordination was performed within each of the two sampling times (4 or 13 weeks), to assess relation between AMF genera and the drought treatments (Roof, Roof-Control, Control) in each of the two farming systems (CONMIN or BIODYN).

To further test whether individual ASVs were specifically associated with the farming systems (CONMIN or BIODYN), one of the drought treatments (Roof, Roof-Control, Control) or the sampling time (4 or 13 weeks), an indicator species analysis based on the ASV count data was conducted. In addition, we screened for the drought-sensitive indicator ASVs separately within each farming system and the sampling time. The analysis was done using the function 'indicspecies::multipatt' (De Caceres and Legendre, 2009). Indicator values (IndVal) range from 0 to 1, where the highest values are associated with the strongest indicators of an environment. ASVs with IndVal > 0.3 and $p < 0.05$ were considered as good indicators. In addition, to each ASV identified as significant indicator taxa of the farming systems, the drought treatments or the sampling time, taxonomy (from Class to Species) was assigned.

The full ASV table, ASV sequences, full taxonomic classification (from Kingdom to Species level) and detailed documentation of sequencing data processing, statistical analyses and associated commands are publicly available on the GitHub (https://github.com/KatjaKo/PacBio_AMF/tree/v1.2).

3. Results

3.1. Effects of the experimental drought on soil water content

The Roof treatment compared to the Control treatment provided a short-term drought effect across the winter wheat growing season, by reducing the soil moisture levels in the experimental plots of both farming systems, here shown as a proportion of the water holding capacity (Fig. 1). The soil was generally drier at week 13 than at week 4. Averaged over the drought treatment levels, the soil moisture content in the BIODYN system at week 4 was higher than in the CONMIN system, however at week 13 no farming system effect was observed (Table S3).

3.2. Effects of the farming systems and the experimental drought on AMF diversity

From the sequence analyses, 955 ASVs were determined and taxonomically assigned to the Glomeromycota. These were classified into three classes: Glomeromycetes, Paraglomeromycetes and Archaeosporomycetes, which were further divided into five orders, eight families and twelve genera. A total of 16 species were identified, *Acaulospora cavernata*, *Acaulospora ignota*, *Acaulospora nivalis*, *Ambispora fennica*, *Archaeospora trappei*, *Claroideoglomus hanlinii*, *Claroideoglomus luteum*, *Diversispora celata*, *Diversispora epigaea*, *Dominikia iranica*, *Funneliformis caledonium*, *Funneliformis mosseae*, *Gigaspora margarita*, *Glomus macrocarpum*, *Palaeospora spainiae* and *Septoglomus constrictum*. More than half of the ASVs (63.5%) could not be assigned to a species level, and 42.2% could not be assigned to a genus level. A few ASVs were unassigned on a family (2.2%), order and class level (1.9%). The sequences that were assigned at the species level displayed a high intraspecific variation (Table S4). The diversity of ASVs, according to the two diversity indices used, did not show any general response to the farming systems, the drought treatments or the sampling time (Fig. 2, Table S5a). Among all comparisons, significant differences were only detected between the Roof and Control treatment in the CONMIN farming systems in week 13, with increased observed richness under the Roof in comparison to the Control treatment (Table S5b).

3.3. AMF indicator taxa

Taxa associated with the different farming systems, the drought treatments and the sampling time were identified with an indicator analysis at the ASV level. Four ASVs were identified as indicators for the CONMIN farming system, whereas no indicators were found for the BIODYN system. Three ASVs were linked to the drought treatment, one to the Roof treatment and two to the Control treatment (Fig. 3, Table S6a). We only detected drought treatment responses from ASVs in the BIODYN system where one indicator ASV was associated with the Control treatment at week 4, and one ASV with the Roof treatment at week 13 (Table S6b). For the sampling time, three indicator ASVs were identified for week 4 and one for week 13 (Fig. 3, Table S6a).

3.4. Effects of the farming systems and the experimental drought on AMF community composition

In total, 58% of the ASVs were assigned to twelve AMF genera (Fig. 4). The AMF community composition at the genus level as assessed with the PERMANOVA test (based on the Bray-Curtis dissimilarity matrix calculated at the genus level) was significantly affected by the sampling time and the farming systems, explaining 11.6% and 6.5% of the variation (Table 1). An analysis of multivariate dispersion suggested group homogeneity for all main effects except for the sampling time (Table S7). This result suggests that the significant effect of the sampling time may be caused by both, the genera abundances and compositional heterogeneity. In a db-RDA ordination, 26.2% of the variation in AMF communities was explained by the first two constrained axes (Fig. 5a, b).

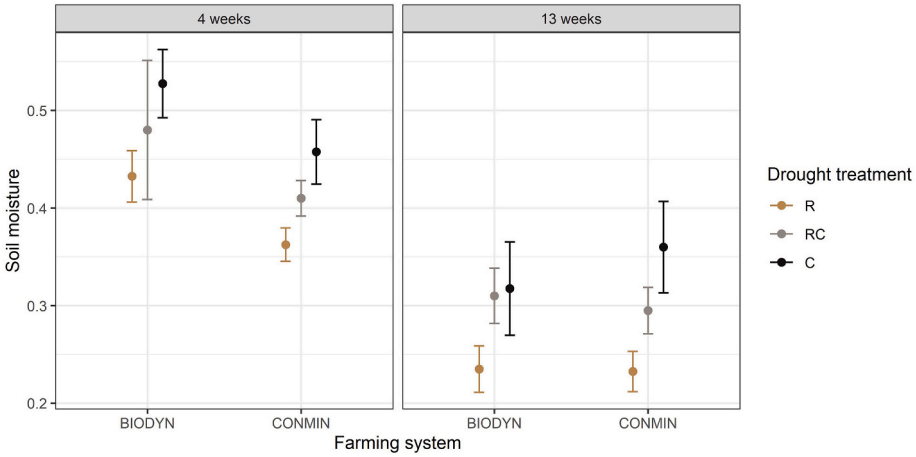

Fig. 1. Soil moisture (shown as the proportion of the maximum water holding capacity) in the experimental plots in the two farming systems (BIODYN, CONMIN) and three drought treatments (R - Roof, RC - Roof-Control, C - Control) across the growing season of winter wheat (4 and 13 weeks after the rainout-shelter establishment). Data are medians of the posterior distribution with 95% credible intervals (CIs) using the 2.5 and 97.5% quantiles as upper and lower limits.

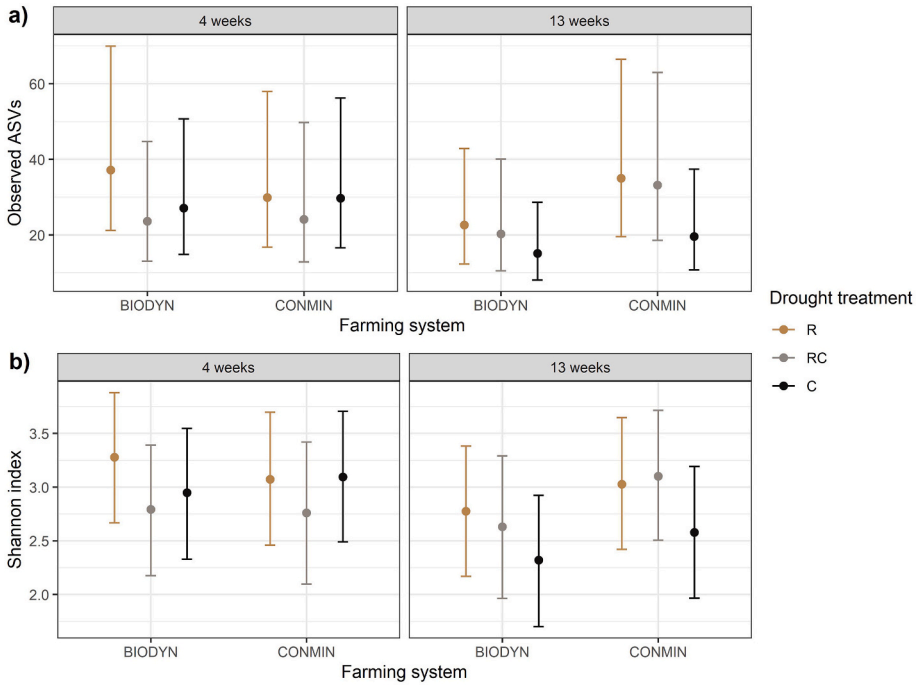

Fig. 2. Alpha diversity indices including (a) observed ASV richness and (b) Shannon index in the experimental plots in the two farming systems (BIODYN, CONMIN) and three drought treatments (R - Roof, RC - Roof-Control, C - Control) across the growing season of winter wheat (4 and 13 weeks after the rainout-shelter establishment). Data are medians of the posterior distribution with 95% credible intervals (CIs) using the 2.5 and 97.5% quantiles as upper and lower limits. Abbreviation: ASV, amplicon sequence variant.

Acaulospora was most abundant at both times of sampling and characteristic for the BIODYN system, followed by *Funneliformis* being more abundant in week 4 and *Palaeospora* being more abundant in week 13 (Figs. 4, 5a). In-depth analyses within each of the sampling times separately (Fig. S3a, b), revealed that in the BIODYN system the abundance of *Acaulospora* was affected by the Roof-treatment, in particular in week 13 (Fig. S3b). On the other hand, compared to the Control-treatment, the abundance of *Palaeospora* was enhanced in the Roof-treatment in the BIODYN system in week 13.

3.5. Effect of soil and plant properties on AMF community structure

Soil properties, such as total soil carbon (C), phosphorus (P), pH and bulk density (BD) were significantly related to the AMF community composition at the genus level. Plant properties such as C and N content of roots, crop biomass and weed cover were also significantly related to the AMF community structure (Table 2, Fig. 5b). Furthermore, soil C content and soil pH were significantly correlated to the BIODYN system and the genus *Acaulospora*. Total soil P and bulk density were significantly correlated to the CONMIN system and the genus *Funneliformis* (Fig. 5a, b).

4. Discussion

4.1. Farming system effects

Based on previous work in the DOK trial (Oehl et al., 2004), the biodynamic system was expected to promote the diversity of AMF in comparison to the conventional system, but we did not observe such an effect in this study. However, previous findings were based on fungal spore morphology from the soils and trap cultures, therefore a direct comparison with our results, based on the molecular diversity of AMF in soils, is thus difficult. On the other hand, studies on other experimental sites using molecular approaches showed that organic farming resulted

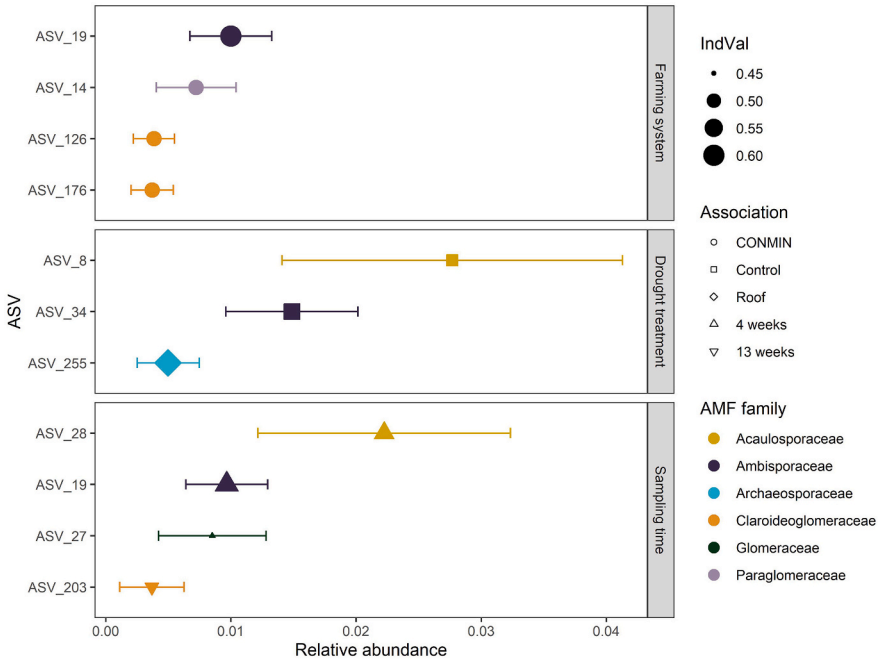

Fig. 3. The indicator ASVs identified in the farming systems, the drought treatments or at the sampling time, here shown as the relative abundance. The different shapes represent the association either with one of the farming systems (BIODYN, CONMIN), the drought treatments (R - Roof, RC - Roof-Control, C - Control) or the sampling time (4 and 13 weeks after the rainout-shelter establishment). The size of the shapes represents an indicator value (IndVal) and colours the AMF families. Indicator ASVs with IndVal > 0.3 and p < 0.05 are shown. Error bars represent standard error of the mean value. Abbreviation: ASV, amplicon sequence variant.

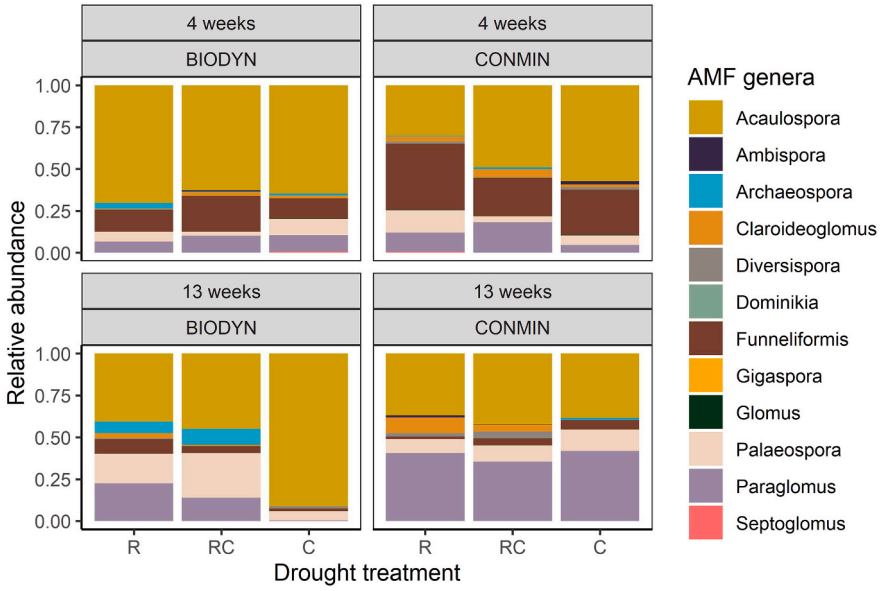

Fig. 4. Mean relative abundances of the twelve identified AMF genera in soil samples from the two farming systems (BIODYN, CONMIN) and three drought treatments (R - Roof, RC - Roof-Control, C - Control) across the growing season of winter wheat (4 and 13 weeks after the rainout-shelter establishment).

Table 1
Results from a PERMANOVA assessing effects of the drought treatment, the sampling time, and the farming system as well as their two-way interactions on the AMF community composition at the genus level. Significant p-values are indicated in bold (*p < 0.05, **p < 0.01).

Effect	R^2	F-value	p-value
Drought treatment	0.039	0.956	0.416
Sampling time	0.116	5.756	**0.005****
Farming system	0.065	3.223	**0.042***
Drought treatment × farming system	0.012	0.303	0.918
Drought treatment × sampling time	0.032	0.800	0.537
Sampling time × farming system	0.028	1.371	0.264

in greater AMF diversity compared to the conventionally managed soils (Lumini et al., 2011; Manoharan et al., 2017; Verbruggen et al., 2010). In the DOK trial, both farming systems have similar management practices, such as crop rotation and soil tillage, and thus differ mainly in fertilizer types and plant protection practices (Fliessbach et al., 2000). In the same drought experiment, Kundel et al. (2020) assessed the impacts of organic and conventional farming practices on fungal (non-AMF) and bacterial diversity. In line with our results, there were no differences in fungal diversity (non-AMF) in the two farming systems. The results from both studies indicate that differences in fertilization strategies and crop protection regimes compared to the common soil origin and identical crop rotation are not enough to promote or reduce non- AMF and neither AMF diversity in one of the farming systems. In addition to similar management characteristics of farming systems, an important factor

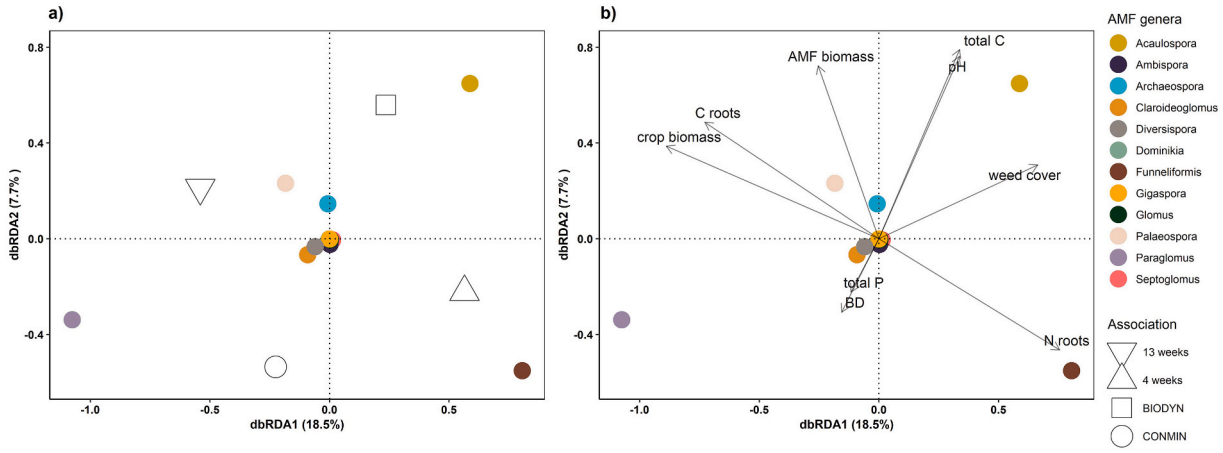

Fig. 5. Distance-based redundancy analysis (db-RDA) of arbuscular mycorrhizal fungal (AMF) communities at the genus level, constrained for the farming systems and the sampling time. (a) The AMF community composition and its relation to the farming systems (BIODYN, CONMIN) and the sampling times (4 and 13 weeks after the rainout-shelter establishment). The different shapes represent the association with one of the farming systems (BIODYN, CONMIN), and the sampling times (4 and 13 weeks after the rainout-shelter establishment) and colours represent the AMF genera. (b) The relationship between the AMF community composition and the significant plant properties, biological, chemical and physical soil properties. Arrows denote the magnitudes and directions of the significant effects. Abbreviation: BD, bulk density; total C, total soil carbon; total P, total soil phosphorus; N, nitrogen.

Table 2
Correlations between plant properties, biological, chemical and physical soil properties and the db-RDA ordination of the AMF community composition based on the function 'vegan::envfit' (999 permutations). The goodness-of-fit statistic is the squared correlation coefficient (R^2). Significant p-values are indicated in bold (*p < 0.05, **p < 0.01, ***p < 0.001).

Soil and plant properties	R^2	p-value
pH	0.694	**0.001*****
Bulk density (g/cm^3)	0.118	**0.005****
Total soil C (%)	0.738	**0.001****
Total soil P (mg/g soil)	0.063	**0.018***
Crop biomass (t/ha)	0.939	**0.001*****
Weed cover (%)	0.536	**0.001*****
N roots (%)	0.787	**0.001*****
C roots (%)	0.765	**0.001*****
AMF biomass (nmol NLFA 16:1ω5/g soil)	0.586	**0.001*****

Abbreviation: C, carbon; N, nitrogen; P, phosphorus.

influencing AMF diversity might be the proximity of the experimental plots within the two farming systems. Farming systems were 6 m distance from each other, and it is possible that the BIODYN system provided a set of AMF species to the CONMIN systems or vice-versa and as a consequence no pronounced differences in the AMF diversity between the farming systems were detected (Brown et al., 2016). Despite no observed changes in the AMF diversity, different farming practices can impact root colonization rates, in terms of arbuscule formation and hyphal length density, or spore density (Bending et al., 2004; Dai et al., 2013; Xiang et al., 2014). For instance, organic farming systems were shown to promote AMF root colonization (Mäder et al., 2000) or spore abundance (Oehl et al., 2004).

The AMF community composition differed significantly between the farming systems and across the growing season of the winter wheat. These results are consistent with other studies showing that different farming systems or land-use types are important drivers shaping the AMF community composition (Birkhofer et al., 2012; Manoharan et al., 2017; Xiang et al., 2014). Our study highlights the effects of the farming systems on the community composition of AMF in contrast to AMF diversity, and suggests that the same taxa are present across the farming systems with shifts in abundance rather than AMF diversity changes. In the same field experiment, Kundel et al. (2020) showed that the fungal (non-AMF) and bacterial community composition differed under organic and conventional farming. Previous studies have shown that different tillage practices are one of the main factors shaping AMF communities (Jansa et al., 2003; Säle et al., 2015; Verbruggen and Kiers, 2010), our findings now show that long-term organic and conventional practices with contrasting fertilizer applications affected the AMF community composition.

4.2. *Effects of the experimental drought*

The short-term experimental drought did not affect the fungal (non-AMF) and bacterial diversity (Kundel et al., 2020), and based on our results, neither the AMF diversity. These results are in accordance with previous studies conducted in grasslands and subtropical secondary forest, where AMF diversity did not respond to drought (Deveautour et al., 2018; Li et al., 2015; Maitra et al., 2019). However, an increase in the AMF diversity was found in the drought treatment in week 13 in the CONMIN systems, what can be explained by different factors, such as competition, i.e. occupation of ecological niches in the soils exposed to drought by opportunistic or highly beneficial AMF species and replacement of native AMF, or exclusion of specific AMF species due to their lower ability to obtain necessary nutrients for their growth or their inability to survive in dry environments (Brown et al., 2016).

Our results show that despite an ambient precipitation reduction of 65% across the winter wheat growing season produced by the drought treatment, the achieved reduction in soil moisture was not enough to cause drought effects on the AMF community composition. Possibly, even more extreme drought would be needed to influence the AMF community composition. As climate models forecast more intense drought periods, potentially leading to greater losses in crop production (Lesk et al., 2016), highly reduced soil moisture levels over more extended periods should be considered in future studies. A few studies have observed changes in the AMF community composition due to reduced rainfall but only after a lag time of more than two years (Deveautour et al., 2018; Deveautour et al., 2020). Besides, the nature of AMF, by forming filamentous structures and building complex hyphal networks that can exceed several meters in diameter, allows them to survive in environments with low soil moisture levels (Allen, 2007; Manzoni et al., 2012). This is in line with Kundel et al. (2020), showing that the AMF biomass (as indicated by NLFA 16:1ω5) in soils responded to drought in the experimental plots. Compared to the CONMIN system, the BIODYN system under drought conditions strongly promoted AMF biomass in bulk soils across the main growing season, and resulted in overall higher abundance. However, a short-term AMF biomass response

with increased AMF abundance cannot be linked to AMF genetic diversity.

A high SOC content can act as a buffer to the drought as it increases water holding capacity and maintains soil moisture at levels that enable crops and microorganisms to withstand shorter drought periods (Droste et al., 2020). The BIODYN system had a higher SOC concentration than the CONMIN system, along with higher soil moisture levels in the BIODYN at week 4 compared to the CONMIN. Later in the season, a general lack of precipitation leveled out the effects of the farming systems, though the drought application still had an effect on the soil moisture levels. Despite the observed effects of SOC content on soil moisture levels (Rawls et al., 2003), the benefits of enhanced SOC content in soils are complex and can also be dependent on management practices within farming system or chemical and physical soil properties that affect the SOC content (Jiao et al., 2020). Testing the SOC factor more specifically is difficult, as the DOK trial compares holistic farming systems in small plots, rather than controlling the variation of each individual component, such as SOC, therefore SOC content within different farming systems cannot be taken out, i.e. controlled independently. Consequently, it is impossible to conclude if high SOC levels have a direct influence on the resistance of AMF community composition and if AMF have the ability to buffer the effects of the extreme drought at different stages of the growing season on the crop yields. In order to understand if SOC content has positive effects on AMF communities exposed to drought events, factors influencing SOC dynamics in soils should be studied separately. This raises interesting opportunities for further work, and highlights the need to better understand changes in SOC content and soil physical properties influenced by different farming systems. Understanding these relationships may help us to determine favorable agricultural management to mitigate the effects of extreme drought periods on AMF communities and crop yields.

Furthermore, our results show that the AMF community composition was significantly affected by the sampling time, indicating that the fluctuations in soil moisture content across the growing season had a stronger influence on the AMF communities than the experimental drought. The resistance of AMF communities to the experimental drought may be overshadowed by the natural drought effect in the experimental year, but this cannot be clearly separated from the drought treatment effect. Therefore, future studies could more precisely explore drought effects on soil microorganisms or plant traits by watering control plots during times of natural drought or when water availability is close to or below the critical threshold (Hoover et al., 2018). Previous studies demonstrated that the reduction in soil moisture resulted in effects on the AMF communities in roots (Deepika and Kothamasi, 2015; Li et al., 2015). These changes in AMF diversity and community may influence plant productivity and plant community composition, therefore, investigation of AMF communities in the roots besides the bulk soils should be considered in further studies (Deveautour et al., 2018). This will allow to better understand symbiotic associations between AMF and plants, how these symbiotic relationships are affected by drought and how this impact growing plants and crop yields.

A set of identified indicator ASVs characterized the conventional farming system with ASVs belonging to families of Claroideoglomeraceae, Paraglomeraceae and Ambisporaceae. The indicator taxa for the drought treatments were represented by ASVs belonging to Acaulosporaceae, Ambisporaceae and Archaeosporaceae. When searching for drought-sensitive ASVs in the two farming systems separately, one ASV found in the organic farming system from the Archaeosporaceae was linked to the drought treatment. Only a few studies have focused on AMF responses to drought conditions. In a study in Australian grasslands, Deveautour et al. (2018) found that the majority of indicator taxa associated with drought belonged to *Glomus*, while others have found that *Glomus* species were poorly adapted to drought and replaced by drought-tolerant species, such as *Diversispora* (Yang et al., 2010; Zhang et al., 2016). We found Archaeosporaceae to be associated with the drought treatment, but based on our findings we cannot conclude if *Glomus* species are linked to drought or not, as in our study *Glomus* was only present in low abundance. Whether fungi from the family Archaeosporaceae are particularly resistant to drought conditions remains to be explored in other agricultural systems and under an extended range of environmental conditions (both soil properties and climate).

The methodological advances, particularly the development of high-throughput molecular tools, such as SMRT methodology linked with the identification of ASV allow us to study AMF communities at a higher resolution. Compared to Illumina sequencing of shorter AMF amplicons, SMRT methodology is appropriate to sequence longer fragments, such as in this study 1.5-kb long fragment. SMRT methodology offers higher species resolution and requires lower number of sequencing reads to cover AMF diversity compared to short-read sequencing approaches (Dirks and Jackson, 2020; Kolaríková et al., 2021; Schlaeppi et al., 2016). Currently the identification of AMF communities at a fine level of taxonomic resolution, at the ASV level, displays some shortcomings. The use of ASVs and accessing the AMF community composition at the ASV level is challenging due to the intraspecific genetic variation within a species and even within a single spore, moreover it is not fully resolved to what extent this might be reflected in the interspecific genetic variation (Dirks and Jackson, 2020; Lee et al., 2013). However, the limitations of assigning ASVs to a genus or species level might be better addressed in future studies by expanding the databases used to assign the taxonomy, as each identified indicator ASV represents a unique DNA sequence (Callahan et al., 2017; Glassman and Martiny, 2018). Current taxonomic databases do not allow to assess the information stored in these sequences, but in the future, knowledge of intraspecific variation in AMF will allow better delineation of AMF and improve the understanding of functional characteristics of AMF and their ecological importance in agroecosystems.

A set of soil properties was significantly correlated to the AMF community composition, among them soil pH, which is one of the key factors affecting AMF communities (Oehl et al., 2017; Van Geel et al., 2018). Besides soil pH, soil P was recognized as important soil property for shifts in AMF community composition. These results are in accordance with the studies by Zhang et al. (2016) and Maitra et al. (2019). Among all, *Acaulospora* was the most abundant genera and present in the organic farming system, whereas *Paraglomus* was related to the conventional system. The high abundance of *Acaulospora* in organically managed plots has been recorded earlier in the DOK trial (Hijri et al., 2006; Oehl et al., 2004) and is in accordance with our findings. Moreover, *Acaulospora* was related to weeds, that are important in organic crop production and may support the abundance and diversity of beneficial AMF species. Previous studies highlighted the relationship between AMF and host weeds, in particular the weeds importance for the AMF development in soils and how this symbiosis can promote crop growth and yields (Kubota et al., 2015; Vatovec et al., 2005). Similar to our results, the high abundance of *Paraglomus* has been predominantly found in conventional production systems (Dai et al., 2014; Harkes et al., 2019). In contrast, some studies found *Paraglomus* to be more common in organically managed systems (Douds et al., 1995; Gosling et al., 2014). *Funneliformis* has been previously described as a common AMF taxa in agricultural fields (Öpik et al., 2006; Rosendahl et al., 2009) and is considered to be important for the formation of mycorrhizal networks (Walder et al., 2012). The species *Funneliformis mossae* is a common species that can promote N uptake under agricultural conditions (Wang et al., 2008). In our experiment, *Funneliformis* was more common in week 4 than in week 13 and was significantly related to the N content in roots. This can imply that the genus *Funneliformis* is important in earlier stages of crop development. However, AMF generally show high intraspecific variation in traits, such as *F. mossae* displaying variable uptake of P and N (Mensah et al., 2015; Munkvold et al., 2004). The presence of intraspecific differences highlights the needs to discover the links between the intraspecific variation and the functional differences within AMF taxa, to enhance our knowledge of AMF and their plant interactions

at different farming systems under changing climate.

5. Conclusions

We found that AMF community composition was shaped by long-term organic and conventional farming systems but not generally influenced by a short-term experimental drought event. Although there were no effects of the experimental drought on AMF communities in general, some taxa were identified as indicators for the drought conditions. Furthermore, no significant interaction effect was found between different long-term agricultural management practices and short-term experimental drought on the diversity and community composition of AMF. Our study shows that the AMF community seems capable of coping with a short-term drought, however, the responses of AMF to prolonged and more intense drought periods need to be explored further. Our results highlight that more attention shall be paid to agricultural management practices that can potentially mitigate drought effects and simultaneously enhance the resistance of AMF to drought. This knowledge is needed to better understand the functioning of AMF in arable production systems and how current agricultural practices should be adapted to maintain crop production levels under the increasing frequency of drought periods.

CRediT authorship contribution statement

KB developed the idea for the rainout-shelter experiment in the framework of this project. DK, AF, KH contributed to the conceptualization of the rainout-shelter and the development of the field study in the DOK trial. KK, PAO, DA and KH designed the AMF study. KK conducted the molecular laboratory work, the bioinformatical and statistical analyses, and led the writing of the manuscript. DK conducted fieldwork, provided plant- and soil-related data and contributed to the statistical analyses. SKK contributed to the bioinformatical analysis. PAO, DA and KH provided overall guidance throughout the work. All authors contributed critically to the drafts and gave the final approval for the publication.

Declaration of competing interest

The authors 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

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