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ARTICLE





Strong succession in arbuscular mycorrhizal fungal communities

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Abstract

The ecology of fungi lags behind that of plants and animals because most fungi are microscopic and hidden in their substrates. Here, we address the basic ecological process of fungal succession in nature using the microscopic, arbuscular mycorrhizal fungi (AMF) that form essential mutualisms with 70–90% of plants. We find a signal for temporal change in AMF community similarity that is 40-fold stronger than seen in the most recent studies, likely due to weekly samplings of roots, rhizosphere and soil throughout the 17 weeks from seedling to fruit maturity and the use of the fungal DNA barcode to recognize species in a simple, agricultural environment. We demonstrate the patterns of nestedness and turnover and the microbial equivalents of the processes of immigration and extinction, that is, appearance and disappearance. We also provide the first evidence that AMF species co-exist rather than simply co-occur by demonstrating negative, density-dependent population growth for multiple species. Our study shows the advantages of using fungi to test basic ecological hypotheses (e.g., nestedness v. turnover, immigration v. extinction, and coexistence theory) over periods as short as one season.

Introduction

Arbuscular mycorrhizal fungi (AMF) are among the most important fungi because they form obligate symbioses that provide phosphorus and nitrogen to 70 to 90% of plant species, including almost all agricultural crops [1, 2]. For more than two decades, the role of AMF as drivers of plant community structure, and vice versa, has been recognized

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[3–9]. However, owing to the resistance of AMF to cultivation, studies of their ecology have been hampered by controversies over their ability to reproduce sexually, the homogeneity of nuclei in a single individual, and the recognition of AMF species using rDNA regions of different evolutionary rate [10–16].

Detection of succession, the basic ecological process that describes the changes in community similarity over time [17], is one of the ecological investigations most sensitive to species recognition. The changes in communities over time, whether labelled succession or temporal dynamics, have been investigated extensively with modern approaches in plant communities [18-20], studied less extensively in microbial communities [21–26], and are just beginning to be examined with modern tools in AMF [reviewed by Bahram et al [27]; Table S1]. The three most thorough of these studies [reviewed by Bahram et al [27]; Table S1], include two studies that recognized fungal operational taxonomic units (OTUs) with the internal transcribed spacer (ITS) from samples taken either once in each of the four seasons [28], or three times in a single season [29], and a third that recognized AMF OTUs with small subunit (SSU or 18S) ribosomal rDNA from four sampling times from one season [30]. Using data from these three studies to analyze temporal change in fungal community composition, we found a low, albeit significant, rate of change; 0.001-0.006 units of Bray–Curtis dissimilarity per week (Fig. 1a–c).

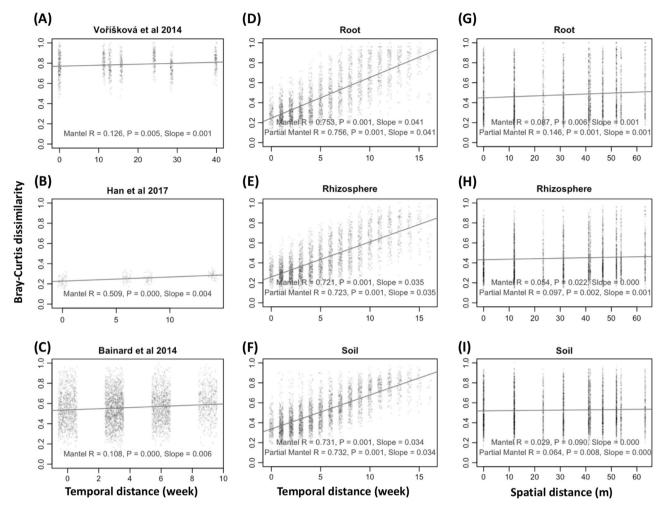


Fig. 1 Arbuscular mycorrhizal fungal community change correlated over time (temporal distance \mathbf{a} - \mathbf{f}) and space (spatial distance, \mathbf{g} - \mathbf{i}). Temporal distance (in weeks between sampling times) as correlated with Bray–Curtis community dissimilarity by Mantel testing in published data from (**a**) [28] [48 samples = 4 time points * 3 vertical layers * 4 plots (10 m² with c. 100 m border)], **b** [29] (21 samples = 3 time points * 7 treatments), and **c** [30] [96 samples = 4 time points * 3 crops * 2 sample type * 4 plots (6 * 2 m² plots with 6 m border)], and from new data presented in this study for (**d**) root (17 time points * 6 plots), **e** rhizosphere (17 time points * 6 plots) and **f** soil (18 time points*6 plots) with all plots having the dimensions 16 m * 8 m with at least a 3 m boarder. Spatial distance as correlated with Bray–Curtis

We hypothesized that the actual rate of change in AMF community composition should be higher than could be detected in these studies dues to two factors. First, temporal change is difficult to detect where variation in the components of the AMF system (plant symbionts, soil, hydration, and season) is large compared to the level of sampling (Table S1). Second, change in community composition is under reported when AMF species-level OTU recognition relies on the conserved SSU ribosomal rDNA [2, 10, 31, 32], rather than on the more variable ITS, which is the molecular "barcode" region used for OTU recognition in almost all other fungal studies [33–36].

community dissimilarity by Mantel testing from new data present in this study for (g) root, **h** rhizosphere, and **i** soil. Note the much stronger association of community dissimilarity and temporal distance reflected by *R* and slope for root, rhizosphere and soil in this study than [28, 29] and [30], and the near absence of association of community dissimilarity and spatial distance in this study. *The [29] result is based on a total fungal community dataset rather than AMF community, due to the low recovery of AMF in that study. Analyses in (**d**–**f**) treat sequence data as counts rarefied among AMF fungi and are nearly identical to analyses treating data as counts rarefied among all fungi or treating data as compositional (Fig. S5)

Here we revisit the basic ecological process of succession by (i) using a system with low environmental heterogeneity comprising only one soil type, one irrigation scheme, two cultivars of the agricultural host plant, sorghum [Sorghum bicolor (L.) Moench], and weekly, triplicate sampling of soil, rhizosphere, and roots throughout the 17 weeks from seedling emergence through grain maturation, and (ii) using OTUs characterized with ITS2 by a recently published approach [37, 38] (Database S1-S2). Our data show a signal of succession in AMF communities that is more than an order of magnitude larger than previously reported. To understand the basis for this signal, we explore

patterns of the nestedness and turnover, the processes of appearance and disappearance (proxies for the processes of immigration and extinction that are appropriate for microbial, HTS datasets), and ask if the processes are deterministic or stochastic, and positively or negatively dependent on initial population size.

Methods

Sampling and sequencing

This experiment was conducted at the semiarid Kearney Agricultural Research and Extension (KARE) Center in Parlier, CA, USA (36.6008° N, 119.5109° W). Two sorghum [Sorghum bicolor (L.) Moench] cultivars with similar flowering times, RTx430 and BTx642, were planted in three, separate, $16 \times 8 \text{ m}^2$ plots (each with ten rows) with 3 m borders between plots (Fig. S1), and were watered using drip irrigation with 80% of calculated evapotranspiration on a weekly basis [39]. The trial was planted on 27th May 2016 and plants emergence was recorded on 1st June. Weekly samples of root, rhizosphere and soil were taken in 2016 on June 8, 15, 22, 29; July 6, 13, 20, 27; August 3, 10, 17, 24, 31; and September 7, 14, 21, 28. At each sampling time, ten or more individual sorghum plants were removed from randomly chosen locations within one of the central eight rows in each plot and combined to generate one root sample and one rhizosphere sample. At the same time, ten soil cores were taken from random locations in each plot and combined to generate one soil sample. Thus, a total of 312 samples were taken, which comprise 17 weekly samples of the two cultivars, and three compartments (root, rhizosphere, and soil), all with three replicates, plus six soil samples collected prior to planting. DNAs of root, rhizosphere, and soil samples were extracted using the MoBio PowerSoil DNA kit (MoBio, Carlsbad, CA, USA). The fungal internal transcribed spacer 2 (ITS2) region was amplified using forward and reverse primers designed to contain a 29 (forward) or 25 (reverse) base linker, a 12 base barcode, a 29 (forward) or 34 (reverse) base pad, a 0-8 base heterogeneity spacer [40], and either the fungal ITS2 specific 21 base 5.8SFun primer (forward) or 27 base ITS4Fun primer (reverse) [38] (Table S2). We used Lee Taylor's ITS2 primers [38] because the 5.8SFun and ITS4Fun matched well with all Glomeromycotina lineages when we matched the primers with published SSU-ITS-LSU alignments [41] (Database S1–S2). All the raw sequences are deposited in Sequence Read Archive with the accession codes: Bioproject PRJNA412410 Biosamples SAMN07711256 - SAMN07711567. Detailed information about site description, experiment design, and molecular analysis can be found in the supplementary methods.

Bioinformatics

Overall sequencing quality was evaluated using FastQC v0.11.5 [42]. Forward and reverse reads were merged using the fastq mergepairs command (-fastq maxdiffpct 3) in USEARCH v8.0 [43]. Primers were removed using cutadapt v1.9.1 [44]. Quality control was carried out using the fastq filter command (-fastq maxee 1.0 -fastq minlen 200) in USEARCH [43]. High quality sequences were subjected to de-replication and de-singleton, and then clustered into OTUs using the cluster otus command in USEARCH [43]. The OTUs were searched against the raw reads using the usearch global command (-id 0.97) in USEARCH [43]. This step generated a table of 312 samples×1293 OTUs (10,770,762 reads). The representative sequence of each OTU was identified by a BLAST search against the curated, fungal specific UNITE database [45] and the NCBI database. Fifty-two OTUs (167,749 reads) were identified as AMF (Table S3), whereas 1026 OTUs were non-AMF (10,341,780 reads) and 215 were non-fungal (261,233 reads). To use phylogenetics to equate AMF OTUs with known species, sequences representing the 52 AMF OTUs were combined with vouchered sequences downloaded from NCBI and UNITE and all sequences were aligned using MAFFT v 7.310 [46] placed in a neighbor-joining tree using MEGA v8.0 [47]. Representative sequences of AMF OTUs were deposited in GenBank with the accession codes: MG008508 - MG008559. Owing to the possibility of multiple ITS2 sequences within an individual AMF [16], we searched for OTUs with identical read abundance by analysis of variance (ANOVA). For OTUs with no difference in abundance, a series of pairwise correlations was then carried out and those OTUs with equal abundance and strong positive correlation were combined to avoid the issue of multiplicity of ITS2 sequences within individual AMF.

Statistical methods

Recent recognition that microbiome data from highthroughput sequencing (HTS) represents a random sample of the DNA molecules in an environment and not absolute counts of the molecules dictates that the data be treated as compositional [48] and not as counts, as commonly has been done. Therefore, we use one compositional and two traditional approaches to analyze our AMF data to both analyze the data as compositional and to permit comparisons with prior studies. For the first traditional approach (dataset 1), we rarefied the number of AMF sequences per sample to 100 using the rrarefy command in vegan in R [49, 50], an approach designed to eliminate the effects of different read numbers among the samples on the deduced AMF community composition. For the second traditional approach (dataset 2), we rarefied all fungal reads to 2743 and then extracted the AMF subset of this normalized fungal data, an approach designed to eliminate the effects of different fungal read numbers but retain the abundance variation of AMFs among the samples. For the compositional method (dataset 3), we imputed zeros in AMF compositional count data sets based on a Bayesianmultiplicative replacement using the cmultRepl command in zCompositions [51], and then converted these data to the centered log-ratio (CLR) using the codaSeq.clr command in CoDaSeq (https://github.com/ggloor/CoDaSeq) [48]. We present analyses of the three datasets in figure and supplemental figures to invite comparison. Direct comparison is possible with permutation tests for ANOVA (PERM ANOVA), but not for other analyses because the statistical methods for compositional datasets are different from those for traditional count datasets, e.g., Bray-Curtis dissimilarity for counts v. Aitchison distance for compositional, and principal coordinate (PCo) analysis for counts v. principal component (PC) analysis for compositional [48]. For some of our analyses, methods are not yet available for compositional datasets, e.g., partition of nestedness and turnover components of beta diversity [52].

By plotting time and AMF richness (dataset 1), we demonstrated the temporal dynamics of AMF diversity. To assess the phylogenetic relatedness of AMF OTUs within every sample, the net relatedness index (NRI) was calculated based on the above-mentioned phylogenetic trees and community composition data using the ses.mpd command (\times -1) in picante package [53]. Relationships between time and abundance of initially dominant and initially rare OTUs (dataset 1, 2, 3) were explored by linear mixed-effects models, including random effects of OTU identity using the lme command in the lme4 package [54]. The variance explained (conditional R^2) by the mixed effect models was calculated by the r.squaredGLMM function in MuMIn Package [55].

Bray-Curtis dissimilarities were calculated for dataset 1 and 2 to construct distance matrices of the AMF community (Hellinger transformed) using the vegdist command in vegan [49], and Aitchison distances were calculated for dataset 3 [48]. PERM ANOVA were carried out to assess the effect of compartment (soil, rhizosphere or root), time period and cultivar on the AMF community variation either detected by Bray-Curtis dissimilarities or Aitchison distances using the adonis command in vegan [49]. Euclidean dissimilarities were calculated to construct distance matrices of geographic, temporal, temperature, and solar radiation distances respectively in vegan [49]. Mantel tests were carried out to explore the correlations between these distance matrices [49]. Partial Mantel tests were carried out to explore the relationships between AMF community dissimilarity and temporal distance, after excluding the influence of geographic distance. Conversely, partial Mantel tests were carried out to explore the relationships between AMF community dissimilarity and geographic distance, after excluding the influence of temporal distance. Structural equation models (SEM) using Mantel R values as input were constructed in AMOS 25.0 [56] to explore the causal relationships among time, solar radiation, temperature, plant biomass and AMF community composition. Based on a priori and theoretical knowledge, we assumed a conceptual model in which time and solar radiation affect temperature, which in turn affects plant biomass, which further influences AMF community composition. To test the homogeneity of AMF community during succession [57], beta dispersion of AMF communities was explored by the betadisper function in vegan [49]. To graphically illustrate the AMF community composition, AMF Bray-Curtis dissimilarity matrices were ordinated by PCo analysis using the pcoa command in the Ape package [58], and AMF Aitchison distance were ordinated by PC analysis using the prcomp command in stats package [50]. The turnover and nestedness components of AMF community were calculated based on the presence/absence data using the beta.pair command (index.family = 'sorensen') in the betapart package [59], and were fitted with temporal distance using the Mantel test in vegan [49]. The nestedness of AMF community was graphically illustrated by the nestedtemp command in vegan package [49].

To test how the AMF succession might be influenced by the AMF OTU cutoff, the OTU delineation processes were repeated by changing the OTU cutoff from the defaulted 97 to 80% in increments of 1%. We calculated the AMF community Bray-Curits dissimilarity of every OTU cutoff, and fitted it with temporal distance using Mantel test, as mentioned above.

To compare the temporal dynamics of AMF communities in our study with those previously reported by Bainard et al. [30], Han et al. [29] and Voříšková et al. [28], we calculated, for the three previous studies, Bray–Curtis dissimilarities of Hellinger transformed AMF community data, and Euclidean dissimilarities of temporal distance in terms of simulated weekly sampling. Mantel tests were carried out to explore the correlations between AMF community dissimilarity and the temporal distances in vegan [49].

Results and discussion

Recognition of AMF OTUs by ITS2

To recognize AMF OTUs that approximate species more closely than SSU OTUs we use the ITS2 region of the RNA repeat [10, 32, 37, 60]. Here, using Illumina Miseq of fungal ITS2 amplified by dual-barcoded Lee Taylor's fungal specific primers [38], we successfully recognized 52

AMF OTUs with 167,749 AMF reads, belonging to *Glomus* (21 OTUs), *Rhizophagus* (13 OTUs), *Claroideoglomus* (8 OTUs), *Funneliformis* (5 OTUs), *Paraglomus* (4 OTUs), and unidentified Glomeraceae (1 OTUs) (Fig. S2). The thorough sampling (312 samples) produced a species accumulating curve that reached its plateau for the species-poor AMFs (52 OTUs) in a relative small (<5000 m²), simple agricultural field (Fig. S3). In line with this result, of the 52 AMF OTUs, only five occurred in fewer than 10 samples, suggesting a lack of rare OTUs in our study (Fig. S4).

As described in the section on statistical methods, to both recognize the compositional nature of HTS microbiome data [48] and to permit comparisons of our results with previous studies that treat HTS data as counts, we analyzed the data both as counts and compositional. We employed two count methods: in data set 1 we rarefied to equal AMF reads and in dataset 2 we rarefied to equal fungal reads. For compositional analysis, in dataset 3 we transformed the data by the CLR method [48]. The largest difference is the detected effect of time, $R^2 = 0.438$ for dataset 1, $R^2 = 0.339$ for dataset 2 and $R^2 = 0.232$ for dataset 3 as explored by PERM ANOVA (all P < 0.001) (Fig. S6). Despite these differences, analyses of the three different datasets generated remarkably consistent results in all applicable analyses that similarly supported the main conclusions of our study (Fig. S5-S7).

In light of recent reports of ITS2 variation as high as 6 to 12% in AMF species-level clades [16, 61, 62], we also investigated the effect on ecological analyses of reducing, in 1% increments, the threshold of OTU recognition by ITS2. We found that the rate of AMF succession was not substantially changed until the cutoff was reduced from 97 to 85% (15% intra-OTU variation, Fig. S8), therefore, our findings are not affected by the potential intraspecific variation reported for AMF species.

The use of ITS has been questioned due to reports showing that one AMF individual can contain more than one, independently evolving rDNA repeat [10, 16, 32]. Mindful of the possibility of amplifying and sequencing more than one rDNA repeat in a single species of Glomeromycotina, we searched for possible intra-individual rDNA polymorphism by correlating read abundance for the different ITS2-OTUs over the 17 weeks of sampling. Strongly correlated ITS2 read abundance (Fig. S9) was seen for three Rhizophagus OTUs (118, 161, 132). Therefore, due to the possibility that they might represent a single AMF species, we treated these three Rhizophagus OTUs as a single species in our analysis, reducing the number of ITS2-OTUs from 52 to 50. Two other Rhizophagus OTUs showed similar read-abundance patterns (Fig. S9A) but the unequal abundance of reads (Fig. S9B) indicated that they represented distinct OTUs and we retained them in our analyses. To assess the effect of reducing the number of OTUs from 52 to 50, we repeated the following ecological analyses with all 52 ITS2-OTUs, finding no differences in ecological results, their significance or our subsequent conclusions (Figs. S10–S15).

Succession of AMF community

Our analyses showed a strong, positive, Mantel correlation (R = 0.617 - 0.753, P < 0.001) between temporal distance (graphed on the x-axis as weeks between sampling times) and AMF community Bray-Curtis (dataset 1, 2) or Aitchison (dataset 3) dissimilarity in root, rhizosphere, and soil samples (Fig. 1d-f; Fig. S5). The slope of the change in dissimilarity per week found here (0.034-0.041 units of Bray-Curtis dissimilarity per week, dataset 1), is 34-41 times greater than the first previously mentioned study (0.001 units of Bray–Curtis dissimilarity per week, Fig. 1a) [28], 8.5–10.25 times greater than the second (0.004 units of Bray-Curtis dissimilarity per week, Fig. 1b) [29], and 5.6-6.8 times greater than the third (0.006 units of Bray-Curtis dissimilarity per week, Fig. 1c) [30]. This change in AMF community composition can also be visualized by ordination (PCo for dataset 1, 2; PC for dataset 3) analysis (Fig. 2a; Fig. S6), by a proportional bar plot of AMF relative abundance (dataset 1, Fig. 2b), or by a bar plot of percentage of AMFs in total fungal reads (dataset 2, Fig. 2c), in addition to the graph of community dissimilarity and temporal distance (Fig. 1d-f; Fig. S5; Fig. S8; Fig. S10). This strong AMF succession was also seen using PERM ANOVA ($R^2 = 0.232 - 0.438$; P < 0.001, Fig. 2a; Fig. S6). This succession is not confounded by beta dispersion in root, rhizosphere, and soil (Fig. S16). We recognize that the concept of succession, which was developed for plant communities, is controversial when applied to microbial communities. Here, we adopt a recent definition of succession as, "... somewhat orderly and predictable manner by which communities change over time following the colonization of a new environment..." [22], by treating a newly emerged plant root, as well its associated rhizosphere and soil, as new environments for AMFs to colonize and initiate succession.

Geographic distance is a factor known to have a major effect on AMF community composition [12, 27, 63]. In contrast to temporal distance, our analysis of the effect of geographic distance using Mantel and partial Mantel tests showed a small effect (slope of the change in dissimilarity over distance = 0 to 0.001 per meter, R never greater than 0.15) on the variation of AMF community dissimilarity in root, rhizosphere, and soil (Fig. 1g–i). Thus, we can infer that agricultural cultivation of a single plant species (*S. bicolor*) homogenizes AMF communities over at a range of from 10 to 60 m, but we cannot rule out environmental

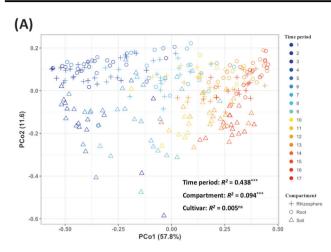


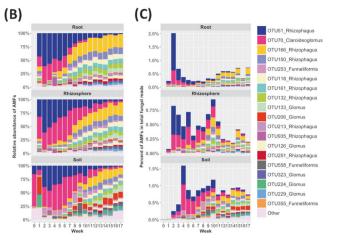
Fig. 2 Change in composition of arbuscular mycorrhizal fungal communities in three compartments (root, rhizosphere, and soil) over 17 weekly time period (TP) samplings. **a** Principal coordinate (PCo) analysis by PERM ANOVA showing significant association of community composition with time period (TP) and compartment but not cultivar (***P < 0.001; ns: not significant). Note that TP accounts for nearly half the variance, which is far more than is accounted for by compartment (root, rhizosphere or soil) or plant genotype (sorghum

heterogeneity that might occur at finer scales and that could affect AMF community composition.

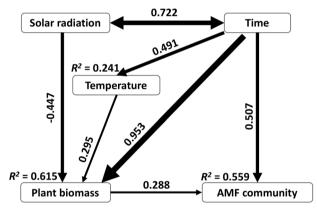
AMF community ecology follows approaches developed for plants with a major difference being the immediate source of energy, insolation for plants and symbiotic partners for AMF [1], sorghum in our case. Of course, temporal variation in insolation that directly affects the plant symbiont should have an indirect effect on AMF. Our SEM results showed that AMF community was directly affected by time and plant biomass, and also indirectly by temperature and solar radiation (Fig. 3). Surprisingly, solar radiation negatively affected plant biomass. It might be that at 36°N latitude in Central California, energy from insolation is not a limiting resource for sorghum growth, but UV radiation and drought stress associated with high insolation might detrimentally affect accumulation of sorghum biomass.

Nestedness and turnover during AMF community succession

There are two, divergent patterns describing the change in community composition: turnover (where some species are replaced by others over time) and nestedness (where the earlier community is a subset of the latter community, or vice versa) [52]. Our demonstration of AMF community succession (Figs. 1d–f, 2) was accompanied by an increase in richness (Fig. 4a–c) over the 17 weeks from emergence of seedlings to maturation of grain in sorghum, so we expected nestedness to predominate but questioned if replacement (turnover) also was involved.



cultivar RTx430 or BTx642). **b** Bar graph of AMF operational taxonomic unit (OTU) relative abundance at each TP and **c** Bar graph of AMF OTUs percentage in total fungal reads at each TP for the three compartments, root, rhizosphere and soil. Note the strong change in AMF community composition over time. Analysis in **a** treats sequence data as counts rarefied among AMF fungi and is nearly identical to analyses treating data as counts rarefied among all fungi or treating data as compositional (Fig. S6)



Chi square = 6.41, Df = 3, P = 0.093, AIC = 30.41

Fig. 3 Structural equation model (SEM) demonstrates that the succession of arbuscular mycorrhizal fungal (AMF) communities was directly affected by time and aboveground biomass of sorghum, in addition to indirect (via plant biomass) effects of solar radiation and temperature. The numbers above the arrows indicate the magnitude of path coefficients (λ), and this magnitude is also depicted by the width of the lines. R^2 values represent the proportion of variance explained for each variable

Mantel tests showed that temporal distance was significantly correlated with both the components of turnover (R = 0.193, P < 0.001) and nestedness (R = 0.214, P < 0.001) of AMF community composition variation (Fig. 5; Figs. S17–S19). The co-occurrence of these two divergent patterns of change in community composition suggested that there also would be more than a single, ecological process underlying succession in the AMF community.

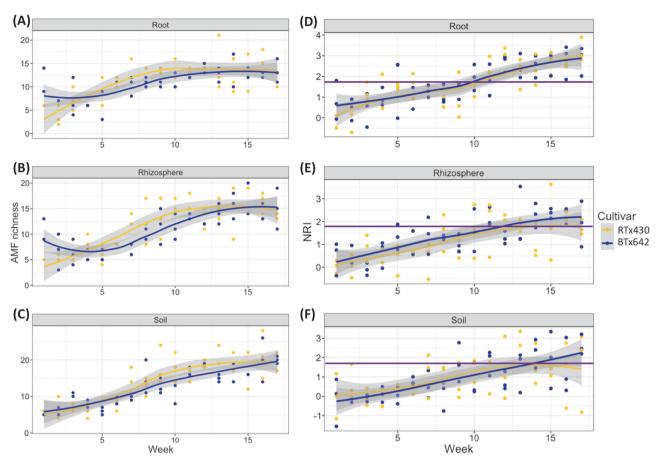


Fig. 4 Temporal dynamics of (a–c) richness and (d–f) phylogenetic relatedness of AMF communities on two sorghum cultivars. Richness shows a consistent increase over time for all three compartments (root, rhizosphere, and soil). Phylogenetic relatedness (net relatedness index, NRI) also increases over time, eventually showing significant

underdispersion as it rises above the threshold of significance (horizontal, purple line). Note that the threshold is reached earlier inside roots than outside them in the rhizosphere and soil and that both cultivars (RTx430 and BTx642) behave similarly in terms of richness and NRI, consistent with the analyses in Fig. 2a

Immigration and extinction in AMF community succession

Immigration and extinction are the two fundamental processes responsible for the patterns of succession [18]. Although immigration and extinction are far more easily observed for plants than microscopic fungi, our comparison of the first week (Time Period 1, TP01) and the last week (Time Period 17, TP17) provide evidence for both processes. Two initially dominant TP01 OTUs with indicator values (indval) strong enough to make them significant indicators of the initial time period (OTU51_Rhizophagus, *P* < 0.001; OTU70 Claroideoglomus, indval = 0.894,indval = 0.809, P < 0.001) were subsequently lost and 13 initially rare OTUs (five Rhizophagus, indval = 0.667-0.811, *P* < 0.001; eight *Glomus*, *indval* = 0.311–0.816, *P* < 0.05) became significant indicators by the final sampling at TP17 (Table S4; Fig. 2b). This result was seen with abundance of AMF alone or abundance of AMF relative to all fungal, although in the later analysis, the initial dominance was delayed from TP01 to the 2nd week in root and rhizosphere and the 4th week in soil (Fig. 2c).

Again acknowledging the difficulty of asserting the absence of a microscopic fungus, the loss of the initially dominant OTUs is consistent with the action of forces causing extinction and the rise of the initially rare OTUs is consistent with the action of forces causing immigration. These two processes can be deterministic or stochastic and, in light of the expected, dramatic effect on AMF community composition of the emergence and growth of the sorghum monoculture, determinism would seem the more likely explanation. Similarly, other factors argue against chance as the dominant force, including the paucity of rare OTUs in our communities (Fig. S3-S4), which minimizes the number of OTUs most susceptible to stochastic extinction [64], and the similarity in AMF community composition throughout the sorghum field (Fig. 1g-i), which limits the local pool of potential, stochastic immigrants.

The emergence of 13 significant indicator OTUs (Table S4) by the final time period, TP17, raises the

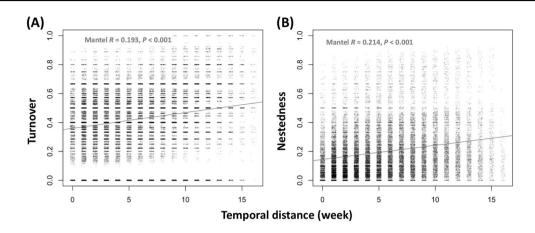


Fig. 5 Role of two patterns, (a) turnover and (b) nestedness in the change in AMF community composition over time. The compositional variance of AMF community measured by Sorenson pair-wise dissimilarity was partitioned into a turnover component (Simpson pair-wise dissimilarity) and a nestedness component (Sorenson pair-wise dissimilarity minus Simpson pair-wise dissimilarity) following Base-lga [52]. Subsequently, Mantel tests were carried out to explore the

correlation of temporal distance and either the turnover or nestedness components of AMF compositional variance. Both AMF turnover and nestedness showed significant and biologically meaningful associations with temporal distance. Visualization of the superimposed points was enhanced by rendering them semi-transparent and adding a small amount of noise to the temporal distances

question of coexistence of multiple species during succession. The creation of distinct niches by a developing host plant would favor coexistence of dissimilar species that could avoid competition by exploiting divergent niches (i.e., stabilizing niche differentiation, a process consistent with phylogenetic overdispersion) [65]. Conversely, the expansion of the same niche, as expected of a growing sorghum crop, would facilitate the immigration and coexistence of species adapted to the same environment. Successful immigrant taxa would be expected to show equal fitness in this expanding niche and, assuming that fitness traits are phylogenetically conserved, exhibit a phylogenetic underdispersion due to evolutionary relatedness [65]. We find a phylogenetic underdispersion of indicator AMF in the genera Rhizophagus and Glomus at TP17 (Table S4) in roots, rhizosphere, and soil (Fig. 4d-f) based calculation of the net relatedness index (NRI) from an ITS2 phylogeny (Fig. S2). The lack of significant phylogenetic underdispersion early in the season (Fig. 4d-f), indicative of stochastic community assembly, is consistent with our having planted sorghum in a fallowed field that was previously planted to oats and having no previous exposure to sorghum. Development of underdispersion, indicative of phylogenetic similarity of AMF community members, later in the season supports coexistence by equalizing fitness, likely due to the expanding niche, rather than avoiding competition by exploiting niche differences. A similar shift from initially random to significant phylogenetic relatedness has been reported for AMF communities of crop plants characterized by SSU OTUs in four soil samples taken over 9 weeks, but not for root samples, where the pattern was nonlinear over time [30]. The interpretation of phylogenetic underdisperson with equalizing fitness similarity relies on the phylogenetic conservation of traits [66], but evidence of specific, adaptive traits in AMF remains rare [67, 68].

Initial density-dependent AMF population demography

A role for population density in the decline of initially dominant OTUs and the rise of initially rare OTUs is suggested from our data, which document a decrease in relative abundance of the two OTUs dominant at TP01 (Rhizophagus 51, Claroideoglomus 70), and an increase in relative abundance of 13 OTUs rare at the same initial time period (Fig. 2b, c). In line with these observation, time is significantly negatively correlated with initial dominant OTUs and positively correlated with initial rare OTUs, as detected by linear mixed-effect modeling of all three datasets, whether rarefied for AMF reads, for all fungal reads, or not rarified and transformed by the CLR method (Fig. 6; Fig. S7). In the case of the two initially dominant OTUs whose relative abundance declined, they may have experienced a fitness disadvantage associated with high population density and their decline would be the result of competitive exclusion of species due to a disadvantage in fitness as compared to the rest of the community. Conversely, the population increases seen in the 13 initially rare OTUs may have been due to a fitness advantage at low population density, the magnitude of which would decrease as their populations grew [64]. Our results echo the only other studies to report replacement over time of dominant AMF OTUs [69, 70], in which the authors used SSU OTUs and five years of annual sampling to show that AMF OTUs dominant in newly

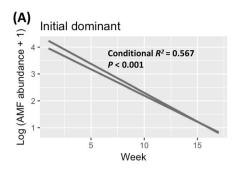
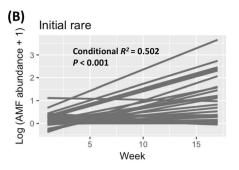


Fig. 6 Steep (**a**) decline of initially dominant OTUs and (**b**) rise of at least 13 initially rare OTUs. Relationships between time and AMF OTU abundances were explored by linear mixed-effects models, including random effects of AMF identity. The conditional R^2 calculated here can be interpreted as the variance explained by the

germinated seedlings were almost entirely replaced by previously rare types; however, with few samples and broad OTU recognition, they were unable to correlate the replacement with population density [69, 70].

The negative density-dependent population growth observed here is explained in plant communities by two mechanisms, resource partitioning and escape from natural enemies [65]. Resource partitioning posits that different species either use different resources or partition the use of shared, limited resources [71]. As a result, species with large populations should experience limited population growth due to strong intraspecific competition, whereas species with small populations should experience high population growth due to the lack of intraspecific competition. However, support for the partitioning mechanism is not seen in the case of the six, closely related Rhizophagus OTUs (Table S4) that were shown, above, to be similar enough in fitness to avoid competitive exclusion and, therefore, too similar to occupy different niche spaces. Neither does partition theory appear to explain the inability of Rhizophagus OTU 51 to maintain population size in the final time period, likely due to competitive exclusion, because this process would not be expected to occur with effective partitioning [71]. Under the mechanism of natural enemy escape, species with large populations experience limited population growth rate due to the attraction and accumulation of more specific predators and pathogens, whereas species with small population experience high population growth rates due to the escape from host-specific natural enemies [65]. Alas, we do not have any data on predators and pathogens of AMF from our study, although these organisms must exist [72, 73].

The negative, density-dependent population growth seen for at least 13, initially rare, OTUs, indicates that populations of these AMF are able to increase in size while cooccurring with stable populations of other species. This invasibility, together with the facts that these fungi live at the



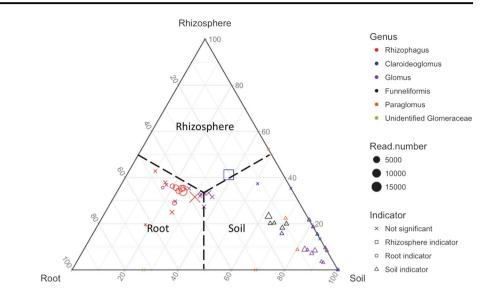
mixed-effects models. Analyses in (\mathbf{a}, \mathbf{b}) treat sequence data as counts rarefied among AMF fungi and are nearly identical to analyses treating data as counts rarefied among all fungi or treating data as compositional (Fig. S7)

same trophic level and inhabit the same roots (Figs. 2 and 6; Table S4), suggests that these representatives of two AMF genera, *Rhizophagus* and *Glomus*, not only co-occur, but also co-exist [74]. These 13 AMF OTUs represent the first microbes where negative density-population growth in support of co-existence has been demonstrated over a long period [74], but further research will be needed to determine which phenotypic trade-offs may be associated with co-existence, such as, aspects of colonization and life-history strategy, differential interaction with host plants, peers and antagonists, and variation in adaptation to features of the abiotic environmental.

Different AMF in root, rhizosphere, and soil

AMF are obligately dependent upon carbon from the roots of plants, so we expected that the AMF communities of the rhizosphere and soil would follow those seen in the root. This pattern was evidenced by similar trends for AMF in roots, soil, and rhizosphere in terms of temporal distance and succession, geographic distance, richness, and phylogenetic relatedness, as mentioned above. For example, the lag in response to nutrients provided by sorghum from roots to soil could be seen in the percentage of total fungal reads attributable to AMF, which peaked at TP02 in root, but peaked at TP04 in soil (Fig. 2c). Our data also suggest that different AMF species display different proportions of their thalli across the compartments of root, rhizosphere, and soil. In roots, six Rhizophagus OTUs were more commonly detected than in other compartments and, when detected, were more abundant (*indval* = 0.054 - 0.399, *P* < 0.05; Table S5; Fig. 7). In rhizosphere, one Claroideoglomus OTU (indval = 0.419, P < 0.001) was more common and abundant than in other compartments (Table S5; Fig. 7). In soil, five Funneliformis OTUs (indval = 0.103-0.520, P < 0.01), three Claroideoglomus (indval = 0.051-0.167, P < 0.01), two Paraglomus (indval = 0.047-0.144, P < 0.05)

Fig. 7 Ternary plot demonstrating the distribution of arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs) recovered from root, rhizosphere and soil. Note a bias toward roots for *Rhizophagus* OTUs, toward rhizosphere for a Clariodeoglomus OTU, and toward soil for *Glomus*, *Claroideglomus*, *Funneliformis* and *Paraglomus* OTUs



and 11 *Glomus* (*indval* = 0.062–0.566, P < 0.01) were more common and abundant (Table S5; Fig. 7) than in other compartments. These results are consistent with observations that *Rhizophagus* species form abundant spores in the roots of vascular plants, whereas *Funneliformis* species form spores in the soil [75]. This variation in AMF morphology in nature also raises the possibility that AMF morphology could change over time, thereby adding variation associated with function [1] to studies of community composition.

Conclusion

Our ability to demonstrate a strong signal of succession in AMF community composition over the sorghum growing season almost certainly rests on our choice of an experimental system with fewer variables than other studies (Table S1) as well as characterization of OTUs by ITS2, which recognizes species-level taxa [2, 10, 11, 31, 32]. Treating DNA sequence data as counts or as compositional showed no loss of statistical significance of results. Our approach also found that succession in AMF communities of sorghum showed the pattern of turnover in addition to strong patterns of nestedness, as has been reported in other studies of AMF (Table S6). Unlike previous studies of AMF that reported stochastic assembly of AMF communities [76-78], we provide analyses that both immigration and extinction are deterministic in this relatively homogenous environment, based on the disappearance of initial dominant OTUs rather than rare OTUs and the homogeneity of AMF communities throughout the sorghum field, which fails to provide a pool of potential immigrants that might enter communities by chance. The increase in phylogenetic similarity (underdispersion) of the many OTUs that immigrated is consistent with equalized fitness rather than niche differentiation, as might be expected with one soil type and one host plant, although phylogenetic underdispersion of AMF has been reported for more complex systems (Table S7).

The energies supporting succession or, more broadly, temporal change in community composition, are different for the two partners of the arbuscular mycorrhizal symbiosis; the autotrophic plant community is supported by solar radiation and the heterotrophic AMF fungal community is supported by carbon fixed by the plant. For plants, the insolation inputs can be relatively consistent over the scale of plant community succession, but the energy provided to the AMF by the growing crop is clearly expanding with time. Therefore, when the abundance of specific AMF species declines during the season, the reduction can be a combination of both absolute reduction and, owing to the expanding resource provided by the plant, reduction relative to increasing abundance of other AMF species. Keeping this caveat about population density in mind, the disappearance of two initially dominant taxa suggests activity promoted by high population density, whereas the population growth of 13 immigrant OTUs suggests the opposite, activity promoted by low population density. For most of our ecological analyses, soil and rhizosphere showed the same results as our primary focus, sorghum roots. However, a difference in OTU abundance between roots on one hand and soil plus rhizosphere on the other correlates with the behavior of AMF genera, some of which live and sporulate predominately in the root and others that are known to sporulate prolifically outside the root, as has been reported in other studies of AMF (Table S8). Our study provides a foundation for more ambitious studies of AMF community ecology, where our simple experimental system would be enlarged to include diversity in hosts, soil, hydration and fertilization, with the eventual goal of effectively studying natural systems.

Due to our inability to cultivate AMF apart from plants, many ghosts have haunted our understanding of these fungi. Just as genomics is showing that the AMF life cycle is typical of other fungi in terms of sex [79] and nuclear variation within an individual [14, 15], mycobiome ecology is showing that AMF community assembly is not a matter of chance, but a process determined by biotic and abiotic factors [80]. The several studies noted above that also found patterns of nestedness (Table S6) and genetic similarity inferred from phylogenetic underdispersion (Table S7) suggest that there may be general rules for assembly of AMF communities that await discovery. The succession of AMF fungi seen here suggests that some AMF species could be more beneficial to sorghum production than others and that these species might be added to agricultural fields along with seeds or applied later in the season. Our approach would also be useful in monitoring the persistence and effects of such additions on the AMF communities of crop plants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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1 Supplementary methods

2 **Experiment site**

3 This experiment was conducted at the Kearney Agricultural Research and Extension (KARE) Center in Parlier, CA, USA (36.6008° N, 119.5109° W). KARE is located in the Central Valley, a 4 5 semiarid zone with a mean annual temperature of 17.8°C and mean annual precipitation of 325 6 mm, almost all of which falls between November and April. During the course of our experiment (27th May to 28th September, 2016), no precipitation occurred; the daily minimum temperature 7 8 ranged from 7.8 to 22.8°C, and the daily maximum temperature ranged from 22.8 to 40.5°C 9 (http://ipm.ucanr.edu/WEATHER/index.html). Soils at KARE were plowed before seeding and 10 are characterized as Hanford sandy loam with a silky substratum and pH 7.37.

11 Experiment design and sampling

Two sorghum [*Sorghum bicolor* (L.) Moench] cultivars with similar flowering times, RTx430 and BTx642, were planted in three, separate, $16 \times 8 \text{ m}^2$ plots (each with ten rows) with 3 m borders between plots (Fig. S1). The seeds were sown into pre-watered fields and left unirrigated for two weeks, which is standard agricultural management practice for sorghum in the Central Valley. From the 3rd week until the final harvest, the plants were watered using drip irrigation with 80% of calculated evapotranspiration on a weekly basis.

The trial was planted on 27th May, 2016 and plants emergence was recorded on 1st June. Weekly samples of root, rhizosphere and soil were taken in 2016 on June 8, 15, 22, 29; July 6, 13, 20, 27; August 3, 10, 17, 24, 31; and September 7, 14, 21, 28. Between 10:00 and 14:00 of every sampling date, ten or more individual sorghum plants were removed from randomly chosen locations within one of the central eight rows in each plot. Both rhizosphere and root samples were taken from the pool of these ten individuals. Roots were removed from the ten plants, mixed 24 together, transferred to 50 ml tubes with detergent-phosphate buffer (6.33 NaH₂PO₄•H₂O and 8.525 g Na₂HPO₄•anhydrous in 1 L water, autoclaved; cooled, 200µl Silwet-77 added; pre-cooled in ice-26 water mixture), and vortexed at full speed for 2 min. The roots were removed from the tube, the 27 liquid-filled tube was saved, and the roots were transferred to a 200-ml plastic cup with phosphate 28 buffer without detergent (6.33 NaH₂PO₄•H₂O and 8.5 g Na₂HPO₄•anhydrous in 1 L water, 29 autoclaved; pre-cooled in ice-water mixture), vortexed at full speed for 1 min twice, dried by clean 30 paper towels, put into aluminum packet and frozen in liquid nitrogen. The saved, liquid-filled tube 31 containing the rhizosphere was centrifuged at full speed for 3 min, the buffer discarded and the 32 rhizosphere pellet frozen in liquid nitrogen. Simultaneously, soil at 6" depth was collected adjacent 33 to the ten sampled plants using 6" soil collection tubes. Ten samples were mixed, transferred to a 34 50-ml centrifuge tube, and frozen in liquid nitrogen. Thus, a total of 312 samples were taken, which 35 comprise 17 weekly samples of the two cultivars, and three compartments (root, rhizosphere and 36 soil), all with three replicates, plus six soil samples collected prior to planting. The frozen root, 37 rhizosphere and soil samples were transferred to dry ice and transported by 18:00 on the day of 38 collection to laboratories at the University of California, Berkeley where they were stored at -80°C 39 until grinding.

40 Molecular analysis

Root samples were ground, separately, with liquid nitrogen in a cryogenic grinder (6875D Freezer/Mill, SpexSamplePrep, Stanmore, UK), and root DNA was extracted from 0.2 g ground sample using the MoBio PowerSoil DNA kit (MoBio, Carlsbad, CA, USA) with all centrifugation conducted at 4°C. Rhizosphere and soil DNA was extracted from 0.2 g samples using the MoBio PowerSoil DNA kit (MoBio, Carlsbad, CA, USA) following the manufacture's protocol. DNA concentration was measured with a Qubit dsDNA HS kit (Life Technologies Inc., Gaithersburg, 47 MD, USA) and DNAs were adjusted to 5 ng/µl with ddH₂O. In preparation for Illumina Miseq 48 sequencing of amplicons of the fungal internal transcribed spacer 2 (ITS2) region, PCR was 49 performed on all samples using forward and reverse primers designed to contain a 29 (forward) or 50 25 (reverse) base linker, a 12 base barcode, a 29 (forward) or 34 (reverse) base pad, a 0-8 base 51 heterogeneity spacer (Fadrosh et al 2014), and either the fungal ITS2 specific 5.8SFun primer or 52 ITS4Fun primer (Taylor et al 2016) (Table S2). We used Lee Taylor's ITS2 primers(Taylor et al 53 2016) because the 5.8SFun and ITS4Fun matched well with all Glomeromycotina lineages when 54 we matched the primers with the SSU-ITS-LSU alignment (Krüger et al 2012) (Database S1-S2). The 5.8Fun primer starts at the 2078th base of the SSU-ITS-LSU alignment (Database S1); and the 55 56 ITS4Fun primer starts at the 3508th base of the reverse complementary of SSU-ITS-LSU 57 alignment (Database S2). PCR amplification employed the one-step PCR method in the Gene 58 Amplification PCR System (BioRad Laboratories Inc.) with initial denaturation at 96°C for 2 min, followed by 35 cycles of 94°C for 30 s, 58°C for 40 s and 72°C for 2 min, and a final extension at 59 60 72°C for 10 min. Each amplification was carried out in a 25 µl reaction mixture containing 10 µl 61 5PRIME HotMaster Mix (Eppendorf-5Prime, Gaithersburg, MD, USA), 2.5 µl forward primer, 62 2.5 µl reverse primer, 2 µl template DNA, and 8 µl nuclease-free water. Amplicon libraries were 63 produced from a pool of three different PCRs. The yields of PCR products were measured using a 64 Qubit dsDNA HS kit (Life Technologies Inc., Gaithersburg, MD, USA) and 200 ng of DNA from 65 each of the 312 samples were randomly assigned to four different pools. The pooled products were 66 purified using AMPure magnetic beads (Beckman Coulter Inc., Brea, CA, USA) following the 67 manufacturer's instructions. Libraries were quality checked for concentration and amplicon size 68 using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) at the Vincent 69 J. Coates Genomics Sequencing Laboratory (GSL, University of California, Berkeley, CA, USA).

Pyrosequencing was performed on the Illumina Miseq PE300 sequencing platform (Illumina, Inc.,
CA, USA) at the GSL. All the raw sequences are deposited in Sequence Read Archive (raw data)
with the accession codes: Bioproject PRJNA412410 Biosamples SAMN07711256 SAMN07711567.

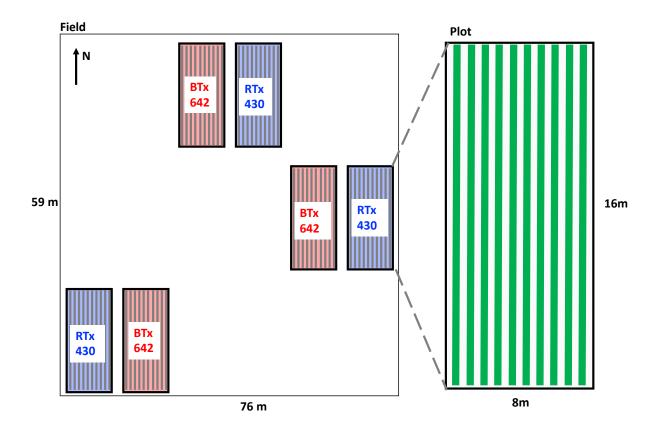
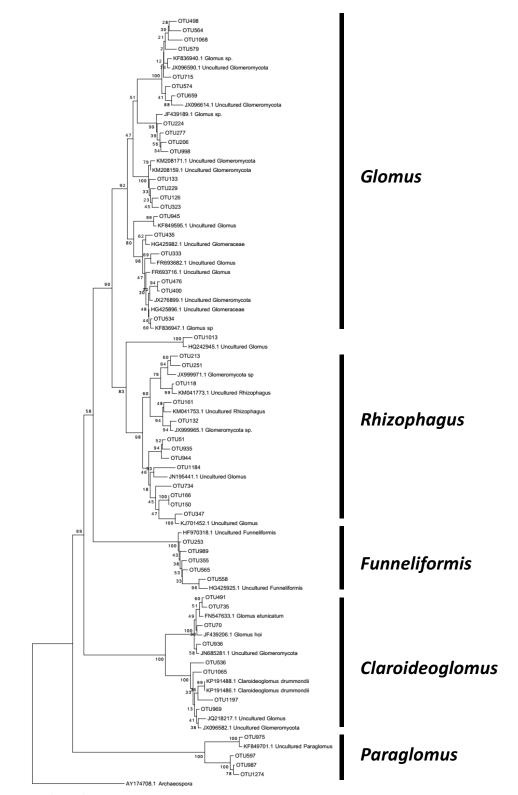
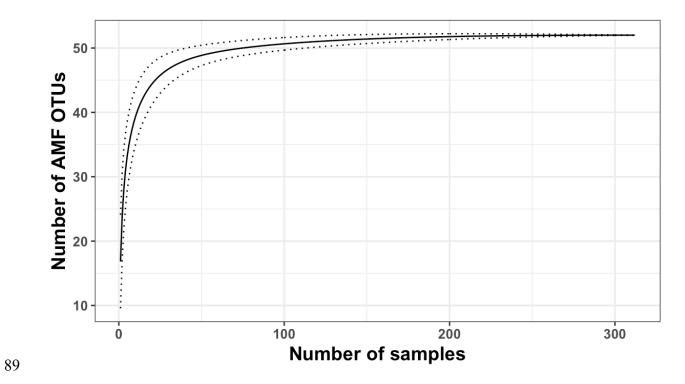


Fig. S1 Field layout of the six plots (16 x 8 m²) of two sorghum cultivars (RTx430 and BTx642)
in a field (76 x 59 m²). Each plot consisted of ten rows of sorghum, each containing approximately
200 plants spaced 10cm apart. At each sampling time, plants were removed from randomly chosen
locations within one of the central eight rows in each plot.



0.050

Fig. S2 Phylogenetic analysis of AMF ITS2 sequences of operational taxonomic units (OTUs) obtained in this study combined with named sequences from UNITE and NCBI. Generic names are applied to clades and the OTUs they contain based on named sequences that share the clade. Representative sequences of AMF OTUs were deposited in GenBank with the accession codes: MG008508 - MG008559. The phylogram is rooted with *Archaeospora* based on (BŁaszkowski et al 2006).



90 Fig. S3 The AMF species accumulation curve reaching a plateau of 48.86 ± 1.57 of 52 taxa after

91 50 samples.

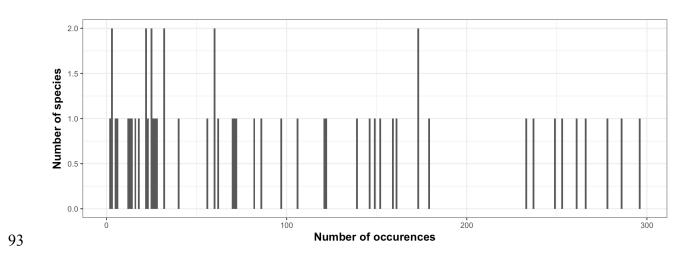




Fig. S4 The frequency of AMF OTUs found in as few as 2 to as many as 296 of all 312
communities sampled. Of the 52 AMF OTUs, only five were found in fewer than 10 samples and
six were found in at least 250 samples.

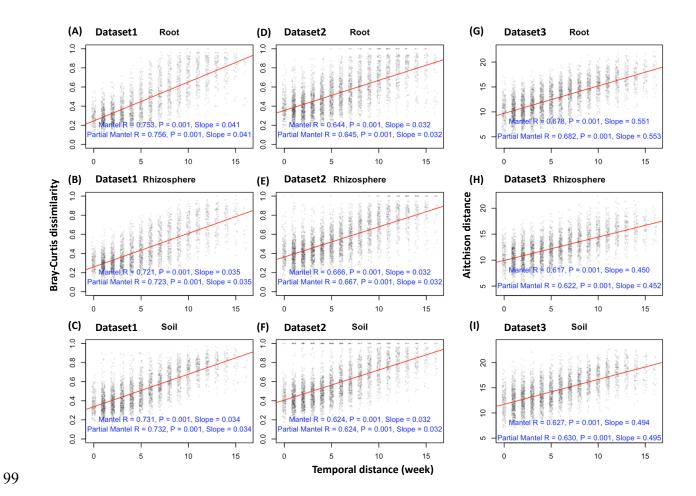


Fig. S5 Comparison of the correlation between AMF community change and temporal distance when treating the DNA sequence data as counts (A-F) or compositional (G-I) (Gloor et al 2017). Note that the result of strong succession is seen regardless of the analytical treatment. AMF datasets (A-C; See Figure 1) rarefied to equal AMF reads (dataset 1), (D-F) rarefied to equal fungal reads (dataset 2), and (G-I) transformed by the centered log-ratio method (dataset 3).

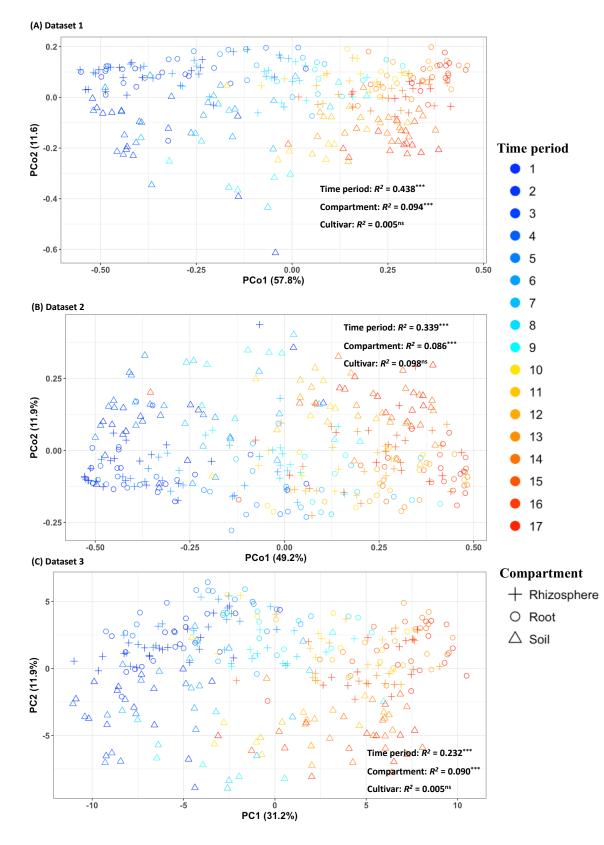


Fig. S6 Comparison of change in 17 weekly samples of composition of arbuscular mycorrhizal fungal communities of soil, rhizosphere and root when treating the DNA sequence data as counts (A, B) or as compositional (C). AMF datasets (A) rarefied to equal AMF reads (dataset 1) (see Figure 2A), (B) rarefied to equal fungal reads (dataset 2), and (C) transformed by the centered logratio method (dataset 3). As seen in Figure 2A, the strongest correlation is between community composition and time period, a result returned by all three methods of analysis. PCo: principal

112 coordinate; PC: principal component.

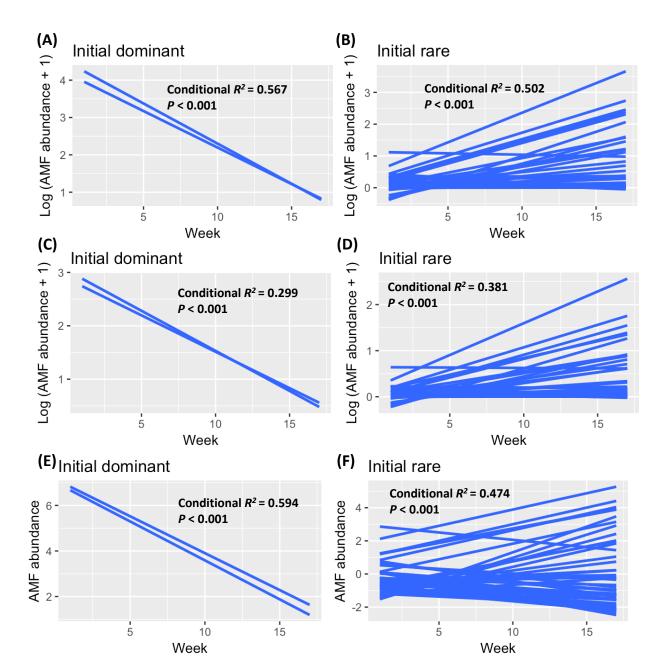
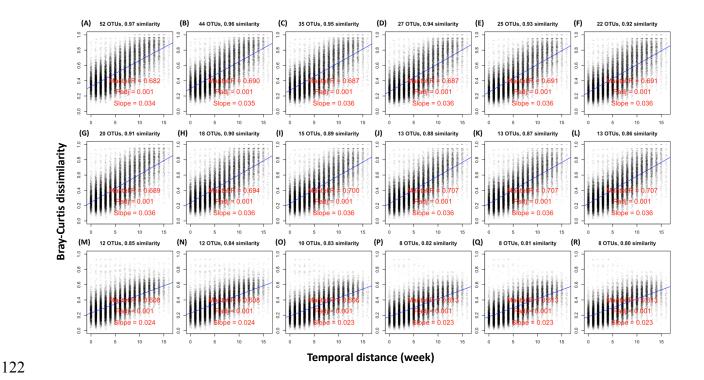
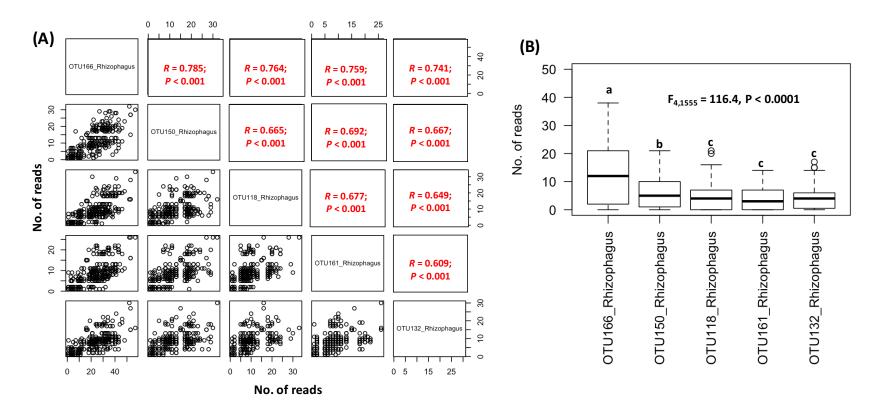


Fig. S7 Comparison of temporal change in initial dominant and initial rare arbuscular mycorrhizal fungal OTUs when treating the DNA sequence data as counts (A-D) or as compositional (E-F). AMF datasets (A-B, See Fig. 6) rarefied to equal AMF reads (dataset 1), (C-D) rarefied to equal fungal reads (dataset 2), and (E-F) transformed by the centered log-ratio method (dataset 3). Note

- 119 that the results are almost identical whether the data are treated as counts or considered to be
- 120 compositional.



123 Fig. S8 Mantel correlation between temporal distance and Bray-Curtis dissimilarity of arbuscular 124 mycorrhizal fungal (AMF) communities determined by reducing the sequence similarity used to 125 delineate OTUs. Sequence similarities: (A) 97%, (B) 96%, (C) 95%, (D) 94%, (E) 93%, (F) 92%, (G) 126 91%, (H) 90%, (I) 89%, (J) 88%, (K) 87%, (L) 86%, (M) 85%, (N) 84%, (O) 83%, (P) 82%, (Q) 81% and 127 (R) 80%. To improve visualization, we added a small amount of noise to the temporal distance 128 and rendered the points transparent. The P value was adjusted by the Bonferroni method, to 129 avoid the type-I error of multiple testing. Note the sharp drop of association between 130 community dissimilarity and temporal distance that occurred between the 86% cutoff (L) (slope 131 = 0.036) and the 85% cutoff (M) (slope = 0.024). The slopes were stable prior to this point, 97% 132 (A) to 86% (L), (slope = 0.034 -0.036) and after it, 85% (M) to 80% (R) (slope = 0.023 - 0.024).



134

Fig. S9 Using the pattern and abundance of internal transcribed spacer 2 (ITS2) reads to identify possible cases of more than one ITS2 sequence in a single arbuscular mycorrhizal fungal (AMF) operational taxonomic unit (OTU). (A) Strong correlation (all P < 0.001) among abundance of five *Rhizophagus* OTUs. (B) Differences in the abundance among five *Rhizophagus* OTUs. Bars without shared letters indicate significant differences as determined by Tukey HSD. Based on their strongly correlated (A) and equalized abundance

- 139 (B), three OTUs (118, 161 and 132) were treated as a single species. Other two OTUs (166 and 150) were treated as different species
- 140 because their abundances were unequal, despite their similar behavior.

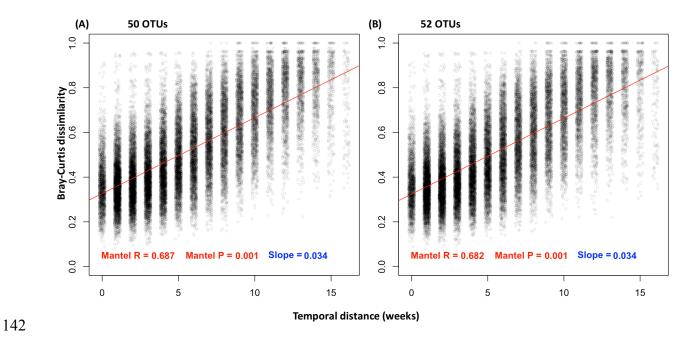
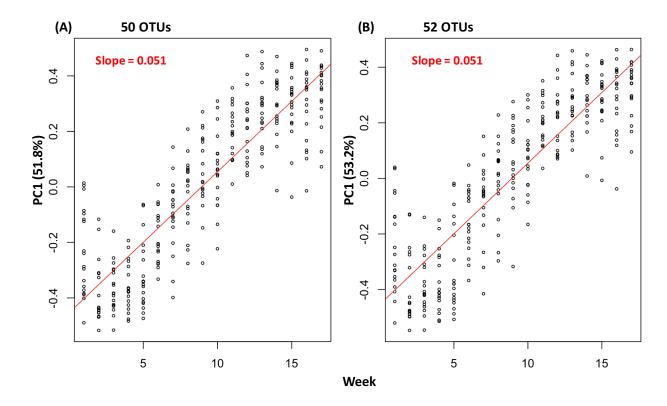
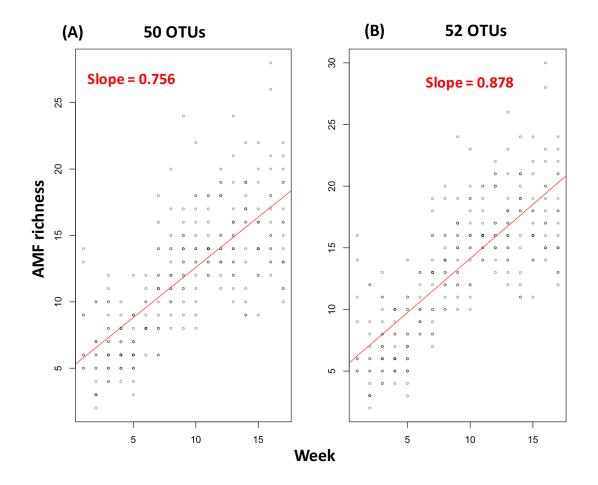


Fig. S10 Comparison of succession analyses with 50 or 52 arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs). No substantial difference in the succession pattern of AMF communities with three OTUs, i.e., 118, 161, 132 possibly belonging to one species, were (A) combined (50 OTUs dataset) or (B) not (52 OTUs dataset), as demonstrated by Mantel test between temporal distance and AMF Bray-Curtis dissimilarity. To improve visualization, we added a small amount of noise to the temporal distance and rendered the points transparent.



149

Fig. S11 Comparison of principal coordinate (PC) analyses with 50 or 52 arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs). No substantial difference in the temporal dynamic of AMF communities with three OTUs, i.e., 118, 161, 132 possibly belonging to one species, were (A) combined (50 OTUs dataset) or (B) not (52 OTUs dataset), as demonstrated by correlation between time and the first axis of PC analysis of AMF community



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Fig. S12 Comparison of richness with 50 or 52 arbuscular mycorrhizal fungal (AMF) operational
taxonomic units (OTUs). No substantial difference in the temporal dynamic of AMF richness
with three OTUs, i.e., 118, 161, 132 possibly belonging to one species, were (A) combined (50
OTUs dataset) or (B) not (52 OTUs dataset), as demonstrated by correlation between time and
the AMF richness.

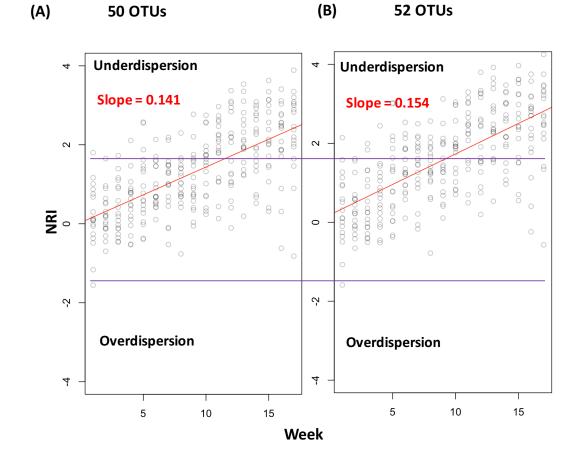


Fig. S13 Comparison of phylogenetic relatedness analyses with 50 or 52 arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs). No substantial difference in the temporal dynamic of net relatedness index (NRI) of AMF with three OTUs, i.e., 118, 161, 132 possibly belonging to one species, were (A) combined (50 OTUs dataset) or (B) not (52 OTUs dataset), as demonstrated by correlation between time and the AMF NRI. Note both datasets showed increases of NRI over time resulting in eventual, significant (above the upper purple horizontal line) underdispersion.

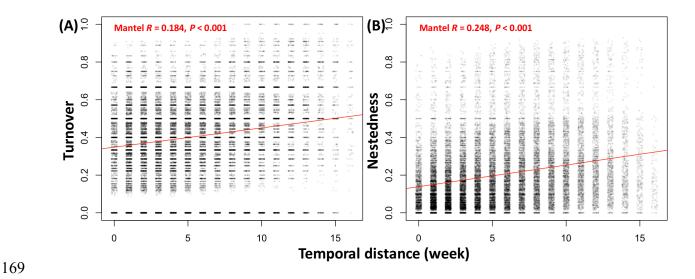


Fig. S14 Comparison of temporal distance and turnover and nestedness with 50 (Fig. 5) or 52 arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs). Both patterns of (A) turnover and (B) nestedness of AMF community over time are detected by Mantel test of the correlation between temporal distance and turnover and nestedness. To improve visualization, we added a small amount of noise to the temporal distance and rendered the points transparent. Note three OTUs, i.e., 118, 161, 132 possibly belonging to one species, were not combined in this analysis, and the results are not substantially different from those that were combined (Fig. 5).

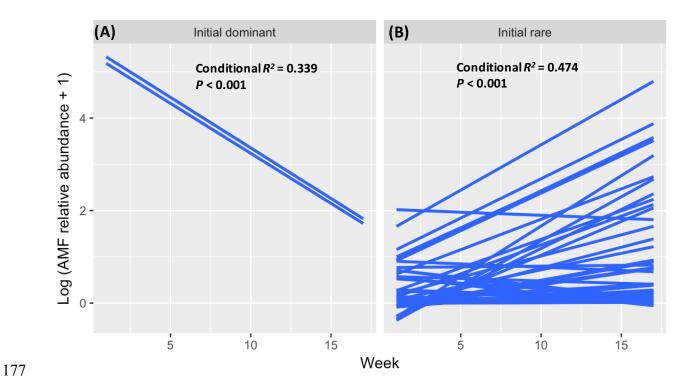


Fig. S15 Comparison of time and abundance analyses with 50 (Fig. 6) or 52 arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs). Relationships between time and abundance of initial dominant AMF OTUs, and initial rare AMF OTUs, as explored by linear mixed-effects models, including random effects of AMF identity. The conditional R^2 that can be interpreted as the variance explained by the mixed effect model was calculated. Note three OTUs, i.e. 118, 161, 132 possibly belonging to one species, were not combined in this analysis, and the results are not substantially different from those that were combined (Fig. 6)

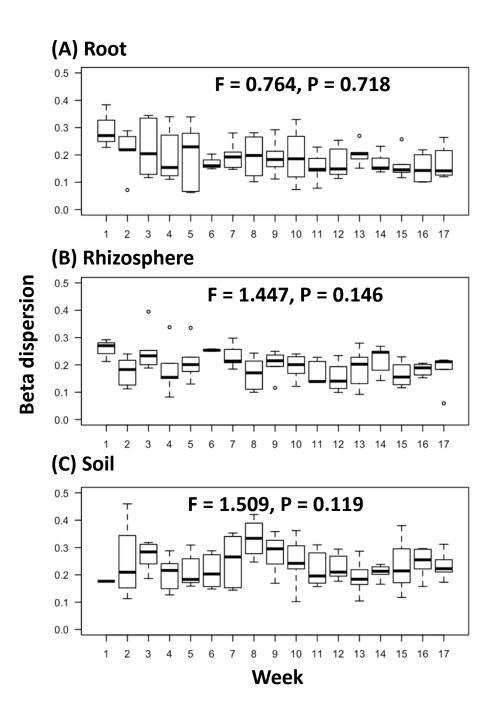




Fig. S16 Permuted beta-dispersion to test the community homogeneity of the arbuscular
mycorrhizal fungal (AMF) communities within every sampling time period (TP) in (A) root, (B)
rhizosphere, and (C) soil. Note the homogeneity of AMF community variances across all TPs in
root, rhizosphere and soil, due to the lack of significant differences among TPs.

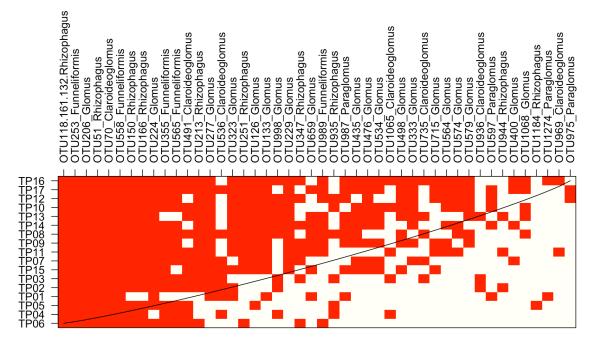
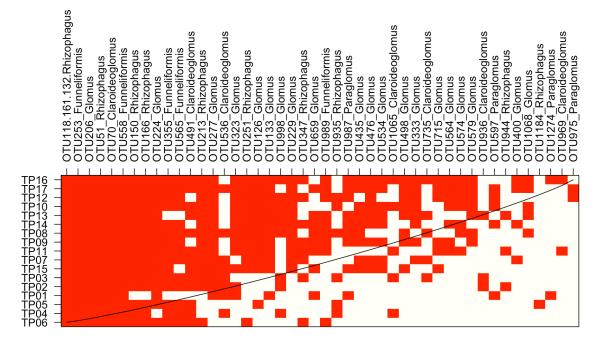


Fig. S17 Root samples showing a nested pattern of arbuscular mycorrhizal fungal (AMF)
operational taxonomic units (OTUs) occurrence (in red) with time period (TP). Matrix of time ×
AMF is sorted to maximize nestedness by nestedtemp function in vegan package in R. The curved
line shows isoclines of prefect nestedness.



196 **Fig. S18** Rhizosphere samples showing a nested pattern of arbuscular mycorrhizal fungal (AMF)

- 197 operational taxonomic units (OTUs) occurrence (in red) with time period (TP). Matrix of time \times
- 198 AMF is sorted to maximize nestedness by nestedtemp function in vegan package in R. The curved
- 199 line shows isoclines of prefect nestedness.

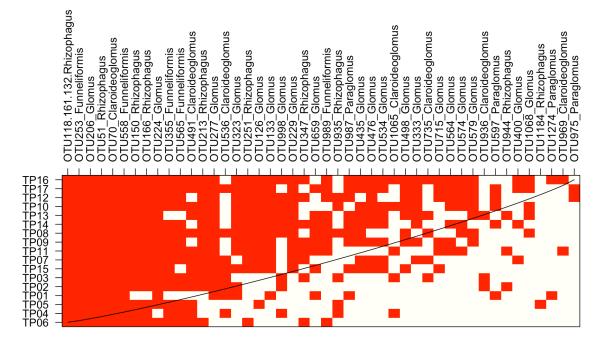


Fig. S19 Soil samples showing a nested pattern of arbuscular mycorrhizal fungal (AMF)
operational taxonomic units (OTUs) occurrence (in red) with time period (TP). Matrix of time ×
AMF is sorted to maximize nestedness by nestedtemp function in vegan package in R. The curved
line shows isoclines of prefect nestedness.

Table S1 A list of studies investigating the temporal dynamics of arbuscular mycorrhizal fungal (AMF) communities. Note succession of AMF communities often cannot be fully acknowledged due to (i) the lack of intensity sampling, (ii) poor AMF recognition resolution, and (iii) confounding influences by history, geography, climate and environments

Study	Ecosystem	AMF recognition	recognition Sampling time Sample		Temporal change
				type	
(Bainard	Farmed pea, lentil	454 sequencing of	Four, tri-week	Root,	AMF richness, community composition, and net
et al	and wheat in	AMF SSU	samples	soil	relatedness index changed by time.
2014)**	temperate semiarid				
	prairie				
(Yu et al	Pea grown in	454 sequencing of	Vegetative growth,	Root	Abundance of a Glomus AMF decreased, a
2012)	climate chamber	fungal ITS	flowering,		Paraglomus AMF increased, and two Glomus
			senescence		AMF not changed with time
(Zeng et	Maize farmed in	tRFLP	Seedling, large ball,	Root,	AMF diversity and community composition were
al 2014)	subtropical China		matured	soil	not influenced by sampling time
(Liu et al	Maize planted in	tRFLP	6-leaf, 13-leaf,	Root	AMF community richness and composition
2016)	temperate China		kernel dough		changed by plant develop stage
(Bainard	Maize monocropped	tRFLP	Four, monthly	Root	AMF community composition changed by time
et al 2012)	or intercropped by		samples (May-		
	tree		August)		
(Turrini et	Crop-maize	Sanger sequencing	April and June	Root	AMF community composition differed between
al 2016)	succession	of AMF SSU			these two seasons
(Higo et al	Soybean field with	Sanger sequencing	Five years (the	Root	AMF community composition differed by year
2014,	different winter	of AMF LSU	flowering time of		
Higo et al	rotation type in		every year)		
2015)	Japan				
(Davison	Temperate mixed	454 sequencing of	Four, monthly	Soil	AMF was not changed by time in ref.; AMF
et al	forest	AMF SSU	samples		community composition changed by time weakly,
2012)*					as demonstrated by reanalysis of ref

(Voříškov á et al 2014)**	Temperate oak woodland	454 sequencing of fungal ITS	Four, seasonal samples	Soil	AMF community composition changed by time, as demonstrated by reanalysis of ref
(Helgason et al 2014)*	Maples (eight spp.) in England garden	tRFLP	April, June, October	Root	Both AMF richness and composition affected by the three seasons
(López- García et al 2014)	Rosemary seedlings, in a mesocosm system	tRFLP	Every three months in two years	Root	AMF community composition was affected by season
(Varela- Cervero et al 2016)	Temperate forest, 5 tree species	tRFLP	Autumn, spring	Root Soil	AMF community composition was reported to be influenced by season in root, but not in soil.
(Dumbrell et al 2011)	Temperate grassland	454 sequencing of AMF SSU	11 samplings in 8 months	Root	AMF community composition differed between summer and winter; AMF beta diversity declined from Nov. to July.
(Montero Sommerfe ld et al 2013)*	Grasslands in Chile	tRFLP	August, January	Root	AMF richness and composition significantly different between winter and summer
(Hazard et al 2014)	Pasture and arable field in Ireland	tRFLP	Six samplings in two years (Mar, June, Oct, Jan, Mar, Oct)	Root	AMF richness and composition changed by season
(Barnes et al 2016b)	Short rotation coppice willow plantation in UK	tRFLP	Four times a year (Oct, June, Aug, Oct)	Root	The spatial distance-decay pattern of AMF community changed by time
(Barnes et al 2016a)	<i>Miscanthus</i> <i>giganteus</i> plantation in UK	tRFLP	Four times a year (Oct, June, Aug, Oct)	Root	AMF richness and community composition changed over time
(Bouffaud et al 2017)	Four long-term observatories in Europe	454 sequencing of AMF ITS2	Spring, autumn	Soil	AMF community composition is weakly affected by season

(Husband	Tropical forest	Sanger sequencing	Two years	Root	AMF community composition changed between
et al	(seedlings of two	of AMF SSU			years
2002a)	spp. in two sites)				
(Taylor et	Boreal forest	Sanger sequencing	Two years	Soil	AMF community composition not changed by
al 2014)*		of ITS			time, as reanalyzed by
(Husband	Tropical forest	Sanger sequencing	Four samplings (3	Root	AMF community composition changed by time
et al	(seedling)	of AMF SSU	month, 1, 2 and 5 yr)		
2002b)					
(Helgason	Woodland in UK	RFLP	July vs Dec.	Root	AMF community composition show difference
et al 1999)					between seasons
(Kabir et	Corn grown in	Hyphae	Apr, Jun, Aug and	Root	The levels of intra- and extraradical fungal
al 1997)	Canada		Oct of two years	Soil	colonization always increased from spring to
					silking and decreased thereafter.
(Kivlin	Monoculture stands	454 sequencing of	Dry and wet season,	Soil	AMF richness and PD affected by time
and	of four tree species	fungal LSU	two years		
Hawkes	in Costa Rica				
2016)					
(Daniell et	Arable fields around	Sanger sequencing	Nine sample times	Root	AMF composition changed by time
al 2001)	North Yorkshire,	of SSU	in 14 months		
	UK				
(Bencherif	Algerian steppic	Spore	Four seasons	Soil	Season affect AMF spore abundance and root
et al 2016)	area			Root	colonization rate, but not diversity
(Herrman	Tropical rubber tree	454 sequencing of	Chronosequence (3,	Root	AMF community composition differed between 3
n et al	plantation	AMF SSU	6, 16 yr)		and 16 yr trees in ordination constrained by soil
2016)					variables, but not in unconstrained ordination
(Krüger et	Temperate	454 sequencing of	Spring autumn,	Root	AMF richness and community composition not
al 2017)	woodland recovered	AMF LSU	Chronosequence		affected by the chronosequences, but community
	from brown-coal		(12, 20, 30, 50 yr)		variation increased along the chronosequences
	mining				

(Yu et al	Semiarid grassland	Spore	Chronosequence (12 Soil		No significant differences in community
2017)	and woodland in		yr v.s. 30yr planted		composition and diversity of AM fungi were
	China		Caragana		recorded at the dunes with different revegetation
			microphylla)		duration.
(Liu et al	Temperate tree	DGGE	Three seasons	Root	AMF richness was not influenced by the
2009)	plantation in China		Chronosequence (5,		chronosequences, but AMF community
			13, 20, 42 yrs)		composition was affected by season
(Sheng et	Temperate black	454 sequencing of	Chronosequence	Root	AMF community composition changed by plant
al 2017)	locust plantations in	SSU	(11, 23, 35, 46 yr)	Soil	age. AMF spore density increased with plant age.
	China				AMF richness not linearly related to plant age
(Hart et al	Tropical long-lived	454 sequencing of	Chronosequence (5-	Root	AMF richness increased with age in root, but not in
2014)	perennial breadfruit	AMF SSU	6, 20-21, 42-40 yr)	Soil	soil; AMF community composition differ between
	trees				young and old trees
(Guadarra	Mexican seasonal	Spore	Chronosequence (<5	Soil	AMF diversity was affected by season and age
ma et al	dry forests		yr, 11-23 yr, >30 yr)		
2014)			Wet vs dry season		
(García de	Temperate alvar	454 sequencing of	Chronosequence	Soil	AMF community composition differed between
León et al	grasslands	AMF SSU	(young (20 yr),	Root	young and mature grasslands, but not between
2016b)			intermediate (50 yr),		intermediate and mature grasslands.
			mature)		
(Honnay	Grassland of	454 sequencing of	Chronosequence	Root	AMF richness and community composition were
et al 2017)	Belgium	SSU	(Forested, 8-11yr,	Soil	affected by successional stages
			12-20yr, ancient)		
(Roy et al	Recultivation after	Illumina	Chronosequence	Soil	AMF community composition differed among the
2017)	open-cast mining in	sequencing of	(2013-2015, 2011-		three phases
	Germany	AMF LSU	2012, 1964-2006)		
(Johnson	Abandoned fields in	Spore	Chronosequence (12	Soil	AMF richness was not influenced by the
et al 1991)	Minnesota		samples of 1-60		chronosequence, but AMF Shannon's diversity
			years)		index increased

(Kowalch	Dunes in	DGGE	Chronosequence	Root	AMF diversity is lower in the later, degraded
uk et al	Netherlands		(Vigorous vs	Soil	stages
2002)			degenerating stand)		
(Oba et al	Recovery from	Spore	Chronosequence	Soil	AMF diversity and composition were not
2004)	volcanic deposits in		(Sites with sparse or		significantly differed between two sites
	Philippines		dense vegetation)		
(Pezzani	Two-phase mosaics	Spore	Chronosequence	Soil	Spore density was higher in late than in early
et al 2006)	in Mexican		(Pioneer vs late-		successional stages
	Chihuahuan Desert		successional		
			grasses)		
(Wu et al	Primary	Spore	Chronosequence	Root	AMF spore abundance, richness of morphtypes
2007)	successional		(Different altitudes)	Soil	increased with decreasing altitue
	volcanic desert in				
	Japan				
(Oehl et al	Retreat of Glacier in	Trap culture	Chronosequence	Soil	AMF diversity increased with succession
2011)	Alps		(1875–1900, 1940–		
			1950, 1970–1980		
			and 1990–2000)		
(Sikes et	Sand dune in	Sanger sequencing	Chronosequence	Soil	AMF isolated from early succession were more
al 2012)	Michigan, USA		(10- 35 yr,		phylogenetically diverse relative to intermediate
			235- 295 yr, 450-		and late succession while late successional fungi
			845 yr)		consistently produced more soil hyphae and
					arbuscules.
(Gorzelak	Temperate	454 sequencing of	Chronosequence	Root	No differences in richness along the host
et al 2017)	rainforests of British	LSU	Young, mature and	Soil	chronosequence. AMF community composition
	Columbia		old)		was affected by age weakly. All host age classes
					harboured AMF communities that were
					overdispersed
(Bennett	Re-analysis of and		Chronosequence		Succession affect connectance and H2, sampling
et al 2013)			(Young, old)		time affect link/specie of plant-AMF symbiotic
			June, July, October		network

(Krüger et al 2015)	Dunes in Australia	454 sequencing of LSU	Chronosequence (1000 yr, 120 000 yr, > 2 000 000 yr soil)	Root Soil	AMF richness peaked in the middle age. AMF community composition differed among the three stages
(Martínez- García et al 2015)	New Zealand	tRFLP, Sanger sequencing and 454 sequencing of SSU	Chronosequence (15 yr, 5000 yr, 12 000 yr, 60 000 – 120 000 yr)	Root	AMF community composition changed by successional stage
(Koziol and Bever 2016)	12 plant spp. forming a successional gradient				Mycorrhizal responsiveness change with plant successional status
(Senés- Guerrero and Schüßler 2016)	Potato Bolivia, Ecuador and Peru	454 sequencing of LSU	emergence, flowering and senescence 105 samples = 3 sites * 3 stages * 3 replicates * 4 altitudes	Root	a surprisingly conserved AMF core-species community structure in Andean potatoes, regardless of different plant stages and environmental factors

207 *Re-analyzed by Bahram et al (2015); **Also re-analyzed by this study in Fig. 2. SSU: small subunit; LSU, large subunit; ITS: internal

208 transcribed spacer; DGGE: Denaturing Gradient Gel Electrophoresis; tRFLP, terminal restriction fragment length polymorphism.

Table S2 The forward and reverse primers that we constructed to be used for Illumina Miseq PE300 in this study. Note we constructed 24 forward and 24 reverse primers that, via a dual-indexing approach, enable us to sequence up to 576 samples in a Miseq PE 300 lane.

Prime				SP		
		BARC		AC		
r	LINUZED		DID			
Name	LINKER	ODE	PAD	ER	PRIMER	COMPLETE
5.8SF	AATGATACGG	CCTA	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACCCTAAAC
un_S_	CGACCACCGA	AACT	CGACGCTCTTCC		CAAYGGATC	TACGGTCTTTCCCTACACGACGCTCTTCCGATCTAAC
24 01	GATCTACAC	ACGG	GATCT		WCT	TTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	GTGG	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACGTGGTAT
un S	CGACCACCGA	TATG	CGACGCTCTTCC		CAAYGGATC	GGGAGTCTTTCCCTACACGACGCTCTTCCGATCTTA
24 02	GATCTACAC	GGAG	GATCT	Т	WCT	ACTTTYRRCAAYGGATCWCT
	Giffefficite	00110		-		
5.8SF	AATGATACGG	TGTT	ТСТТТСССТАСА		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACTGTTGCG
un S	CGACCACCGA	GCGT	CGACGCTCTTCC		CAAYGGATC	TTTCTTCTTTCCCTACACGACGCTCTTCCGATCTGTA
		TTCT		СТ		
24_03	GATCTACAC	IICI	GATCT	GT	WCT	ACTTTYRRCAAYGGATCWCT
- 007						
5.8SF	AATGATACGG	ACAG	TCTTTCCCTACA	~~~	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACACAGCCA
un_S_	CGACCACCGA	CCAC	CGACGCTCTTCC	CG	CAAYGGATC	CCCATTCTTTCCCTACACGACGCTCTTCCGATCTCGA
24_04	GATCTACAC	CCAT	GATCT	Α	WCT	AACTTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	GTTA	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACGTTACGT
un S	CGACCACCGA	CGTG	CGACGCTCTTCC	AT	CAAYGGATC	GGTTGTCTTTCCCTACACGACGCTCTTCCGATCTATG
24 05	GATCTACAC	GTTG	GATCT	GA	WCT	AAACTTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	TACC	TCTTTCCCTACA	TG	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACTACCGGC
un S	CGACCACCGA	GGCT	CGACGCTCTTCC	CG	CAAYGGATC	TTGCATCTTTCCCTACACGACGCTCTTCCGATCTTGC
24 06	GATCTACAC	TGCA	GATCT	A	WCT	GAAACTTTYRRCAAYGGATCWCT
21_00	Giffefficite	10011	Giffel		wer	
5.8SF	AATGATACGG	TGCA	ТСТТТСССТАСА	GA	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACTGCAGAT
	CGACCACCGA	GATC	CGACGCTCTTCC	GAGT	CAAYGGATC	CCAACTCTTTCCCTACACGACGCTCTTCCGATCTGAG
un_S_						
24_07	GATCTACAC	CAAC	GATCT	GG	WCT	TGGAACTTTYRRCAAYGGATCWCT
				CC		
5.8SF	AATGATACGG	TTAA	TCTTTCCCTACA	TG	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACTTAACTG
un_S_	CGACCACCGA	CTGG	CGACGCTCTTCC	GA	CAAYGGATC	GAAGCTCTTTCCCTACACGACGCTCTTCCGATCTCCT
24_08	GATCTACAC	AAGC	GATCT	G	WCT	GGAGAACTTTYRRCAAYGGATCWCT

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5.8SF	AATGATACGG	TACC	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACTACCGCC
un S	CGACCACCGA	GCCT	CGACGCTCTTCC		CAAYGGATC	TCGGATCTTTCCCTACACGACGCTCTTCCGATCTAAC
24 09	GATCTACCGA	CGGA	GATCT		WCT	TTTYRRCAAYGGATCWCT
24_07	GAICIACAC	CUUA	GAICI		wei	
5.8SF	AATGATACGG	ACTT	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACACTTTAA
un S	CGACCACCGA	TAAG	CGACGCTCTTCC		CAAYGGATC	GGGTGTCTTTCCCTACACGACGCTCTTCCGATCTTAA
24 10	GATCTACAC	GGTG	GATCT	Т	WCT	CTTTYRRCAAYGGATCWCT
24_10	unternene	0010	Unici	1	wer	
5.8SF	AATGATACGG	CCAT	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACCCATCAC
un S	CGACCACCGA	CACA	CGACGCTCTTCC		CAAYGGATC	ATAGGTCTTTCCCTACACGACGCTCTTCCGATCTGTA
24 11	GATCTACAC	TAGG	GATCT	GT	WCT	ACTTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	GAGC	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACGAGCAAC
un S	CGACCACCGA	AACA	CGACGCTCTTCC	CG	CAAYGGATC	ATCCTTCTTTCCCTACACGACGCTCTTCCGATCTCGA
24 12	GATCTACAC	ТССТ	GATCT	Α	WCT	AACTTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	ATGT	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACATGTCCG
un S	CGACCACCGA	CCGA	CGACGCTCTTCC	AT	CAAYGGATC	ACCAATCTTTCCCTACACGACGCTCTTCCGATCTATG
$24 \overline{13}$	GATCTACAC	CCAA	GATCT	GA	WCT	AAACTTTYRRCAAYGGATCWCT
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5.8SF	AATGATACGG	TGTC	TCTTTCCCTACA	TG	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACTGTCTCG
un_S_	CGACCACCGA	TCGC	CGACGCTCTTCC	CG	CAAYGGATC	CAAGCTCTTTCCCTACACGACGCTCTTCCGATCTTGC
$24 \overline{14}$	GATCTACAC	AAGC	GATCT	Α	WCT	GAAACTTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	CGCG	TCTTTCCCTACA	GA	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACCGCGGTT
un_S_	CGACCACCGA	GTTA	CGACGCTCTTCC	GT	CAAYGGATC	ACTAATCTTTCCCTACACGACGCTCTTCCGATCTGAG
24_15	GATCTACAC	CTAA	GATCT	GG	WCT	TGGAACTTTYRRCAAYGGATCWCT
				CC		
5.8SF	AATGATACGG	GAGA	TCTTTCCCTACA	TG	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACGAGACTA
un_S_	CGACCACCGA	CTAT	CGACGCTCTTCC	GA	CAAYGGATC	TATGCTCTTTCCCTACACGACGCTCTTCCGATCTCCT
24_16	GATCTACAC	ATGC	GATCT	G	WCT	GGAGAACTTTYRRCAAYGGATCWCT
		AGGT				
5.8SF	AATGATACGG	ACGC	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACAGGTACG
un_S_	CGACCACCGA	ACGC	CGACGCTCTTCC		CAAYGGATC	CAATTTCTTTCCCTACACGACGCTCTTCCGATCTAAC
24_17	GATCTACAC	AATI	GATCT		WCT	TTTYRRCAAYGGATCWCT
		GAGG				
5.8SF	AATGATACGG	AGTA	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACGAGGAGT
un_S_	CGACCACCGA	AAGC	CGACGCTCTTCC		CAAYGGATC	AAAGCTCTTTCCCTACACGACGCTCTTCCGATCTTAA
24_18	GATCTACAC		GATCT	Т	WCT	CTTTYRRCAAYGGATCWCT

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5.8SF	AATGATACGG	CGTA	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACCGTAAGA
5.85r un S	CGACCACCGA	AGAT	CGACGCTCTTCC		CAAYGGATC	TGCCTTCTTTCCCTACACGACGCTCTTCCGATCTGAGA
<u>un_s</u> 24 19	GATCTACCGA	GCCT	GATCT	GT	WCT	ACTTTYRRCAAYGGATCWCT
24_19	GAICIACAC		GAICI	GI	WCI	
5.8SF	AATGATACGG	ATCT	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACATCTAGT
	CGACCACCGA	AGTG	CGACGCTCTTCC	CG	CAAYGGATC	GGCAATCTTTCCCTACACGACGCTCTTCCGATCTAG
un_S_		GCAA	GATCT		WCT	
24_20	GATCTACAC		GAICI	Α	wu	AAACTTTYRRCAAYGGATCWCT
5.8SF	AATGATACGG	CCAG	TCTTTCCCTACA		AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACCCAGGGA
un S	CGACCACCGA	GGAC	CGACGCTCTTCC	AT	CAAYGGATC	CTTCTTCTTTCCCTACACGACGACGCTCTTCCGATCTATG
un_5_ 24 21	GATCTACCGA	TTCT	GATCT	GA	WCT	AAACTTTYRRCAAYGGATCWCT
	GAICIACAC		GAICI	GA	WCI	AAACIIIIKKUAAIGGAIUWUI
5.8SF	AATGATACGG	CACC	TCTTTCCCTACA	TG	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACCACCTTA
5.85r un S	CGACCACCGA	TTAC	CGACGCTCTTCC	CG	CAAYGGATC	CCTTATCTTTCCCTACACGACGCTCTTCCGATCTTGC
un_5_ 24 22	GATCTACCGA	CTTA	GATCT	A	WCT	GAAACTTTYRRCAAYGGATCWCT
	GAICIACAC		GAICI	A	WCI	GAAACIIIIKKCAAIGGAICWCI
5.8SF	AATGATACGG	ATAG	TCTTTCCCTACA	GA	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACATAGTTA
	CGACCACCGA	TTAG	CGACGCTCTTCC	GA GT	CAAYGGATC	GGGCTTCTTTCCCTACACGACGCTCTTCCGATCTGA
un_S_		GGCT	GATCT	GG	WCT	
24_23	GATCTACAC		GAICI	CC	wu	GTGGAACTTTYRRCAAYGGATCWCT
5 OCE		GCAC	TOTTTOOOTAOA			
5.8SF	AATGATACGG	TTCA	TCTTTCCCTACA	TG GA	AACTTTYRR	AATGATACGGCGACCACCGAGATCTACACGCACTTC
un_S_	CGACCACCGA	TTTC	CGACGCTCTTCC		CAAYGGATC	ATTTCTCTTTCCCTACACGACGCTCTTCCGATCTCCT
24_24	GATCTACAC		GATCT	G	WCT	GGAGAACTTTYRRCAAYGGATCWCT
ITS4F	CAACCACAAC	ССТА	GTGACTGGAGT		AGCCTCCGC	
	CAAGCAGAAG ACGGCATACG	AACT	TCAGACGTGTG		ТАТТБАТА	CAAGCAGAAGACGGCATACGAGATCCTAAACTACGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAG
un_S_ 24 01	ACGGCATACG					
24_01	AGAI	ACGG	CTCTTCCGATCT		TGCTTAART	CCTCCGCTTATTGATATGCTTAART
ITC/F	CAACCACAAC	GTGG	CTCACTCCACT		AGCCTCCGC	
ITS4F	CAAGCAGAAG ACGGCATACG	TATG	GTGACTGGAGT TCAGACGTGTG		ТАТТСАТА	CAAGCAGAAGACGGCATACGAGATGTGGTATGGGA GGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTG
un_S_				G	-	
24_02	AGAT	GGAG	CTCTTCCGATCT	G	TGCTTAART	AGCCTCCGCTTATTGATATGCTTAART
ITCAE	CAACCACAAC	TGTT	CTCACTCCACT		ACCCTCCCC	
ITS4F	CAAGCAGAAG ACGGCATACG	GCGT	GTGACTGGAGT TCAGACGTGTG		AGCCTCCGC TTATTGATA	CAAGCAGAAGACGGCATACGAGATTGTTGCGTTTCT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTC
un_S_		TTCT		тс		
24_03	AGAT	IICI	CTCTTCCGATCT	IC	TGCTTAART	AGCCTCCGCTTATTGATATGCTTAART
ITCAE	CAACCACAAC		CTCACTCCACT		ACCCTCCCC	
ITS4F	CAAGCAGAAG	ACAG	GTGACTGGAGT	CT	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATACAGCCACCCAT
un_S_	ACGGCATACG	CCAC	TCAGACGTGTG	СТ	TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCT
24_04	AGAT	CCAT	CTCTTCCGATCT	Α	TGCTTAART	AAGCCTCCGCTTATTGATATGCTTAART

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ITS4F	CAAGCAGAAG	GTTA	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATGTTACGTGGTTG
un S	ACGGCATACG	CGTG	TCAGACGTGTG	GA	ТТАТТБАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGA
24 05	AGAT	GTTG	CTCTTCCGATCT	TA	TGCTTAART	TAAGCCTCCGCTTATTGATATGCTTAART
24_03	nom	0110	ererrecomer	177		
ITS4F	CAAGCAGAAG	TACC	GTGACTGGAGT	AC	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATTACCGGCTTGCA
un S	ACGGCATACG	GGCT	TCAGACGTGTG	TC	ТТАТТБАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAC
24 06	AGAT	TGCA	CTCTTCCGATCT	A	TGCTTAART	TCAAGCCTCCGCTTATTGATATGCTTAART
		10011	01011000		100111111	
ITS4F	CAAGCAGAAG	CACC	GTGACTGGAGT	ТТ	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATCACCTTACCTTA
un S	ACGGCATACG	TTAC	TCAGACGTGTG	СТ	TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTT
24 07	AGAT	CTTA	CTCTTCCGATCT	СТ	TGCTTAART	CTCTAGCCTCCGCTTATTGATATGCTTAART
				CA		
ITS4F	CAAGCAGAAG	TTAA	GTGACTGGAGT	СТ	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATTTAACTGGAAGC
un S	ACGGCATACG	CTGG	TCAGACGTGTG	TC	TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCA
2408	AGAT	AAGC	CTCTTCCGATCT	Т	TGCTTAART	CTTCTAGCCTCCGCTTATTGATATGCTTAART
ITS4F	CAAGCAGAAG	TACC	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATTACCGCCTCGGA
un_S_	ACGGCATACG	GCCT	TCAGACGTGTG		TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAG
24 09	AGAT	CGGA	CTCTTCCGATCT		TGCTTAART	CCTCCGCTTATTGATATGCTTAART
ITS4F	CAAGCAGAAG	ACTT	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATACTTTAAGGGTG
un S	ACGGCATACG	TAAG	TCAGACGTGTG		TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGA
$24 \overline{10}$	AGAT	GGTG	CTCTTCCGATCT	G	TGCTTAART	GCCTCCGCTTATTGATATGCTTAART
ITS4F	CAAGCAGAAG	CCAT	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATCCATCACATAGG
un_S_	ACGGCATACG	CACA	TCAGACGTGTG		TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTC
24_{11}	AGAT	TAGG	CTCTTCCGATCT	TC	TGCTTAART	AGCCTCCGCTTATTGATATGCTTAART
ITS4F	CAAGCAGAAG	GAGC	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATGAGCAACATCCT
un_S_	ACGGCATACG	AACA	TCAGACGTGTG	СТ	TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCT
24_12	AGAT	TCCT	CTCTTCCGATCT	Α	TGCTTAART	AAGCCTCCGCTTATTGATATGCTTAART
ITS4F	CAAGCAGAAG	ATGT	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATATGTCCGACCAA
un_S_	ACGGCATACG	CCGA	TCAGACGTGTG	GA	TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGA
24_13	AGAT	CCAA	CTCTTCCGATCT	ТА	TGCTTAART	TAAGCCTCCGCTTATTGATATGCTTAART
ITS4F	CAAGCAGAAG	TGTC	GTGACTGGAGT	AC	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATTGTCTCGCAAGC
un_S_	ACGGCATACG	TCGC	TCAGACGTGTG	TC	TTATTGATA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAC
24_14	AGAT	AAGC	CTCTTCCGATCT	Α	TGCTTAART	TCAAGCCTCCGCTTATTGATATGCTTAART

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ITS4F	CAAGCAGAAG	CGCG	GTGACTGGAGT	ТТ	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATCGCGGTTACTAA
un S	ACGGCATACG	GTTA	TCAGACGTGTG	CT	ТТАТТСАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTT
24 15	AGAT	СТАА	CTCTTCCGATCT	CT	TGCTTAART	CTCTAGCCTCCGCTTATTGATATGCTTAART
24_10	nom	CIIII	erenceomer	CA	ТОСТИМИ	
ITS4F	CAAGCAGAAG	GAGA	GTGACTGGAGT	CT	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATGAGACTATATGC
un S	ACGGCATACG	CTAT	TCAGACGTGTG	TC	ТТАТТБАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCA
24 16	AGAT	ATGC	CTCTTCCGATCT	T	TGCTTAART	CTTCTAGCCTCCGCTTATTGATATGCTTAART
24_10	AGAI		CICITECOATET	1	TUCTIAARI	
ITS4F	CAAGCAGAAG	AGGT	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATAGGTACGCAATT
un S	ACGGCATACG	ACGC	TCAGACGTGTG		ТТАТТСАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAG
24 17	AGAT	AATT	CTCTTCCGATCT		TGCTTAART	CCTCCGCTTATTGATATGCTTAART
24_1/	nom		ererrecomer			
ITS4F	CAAGCAGAAG	GAGG	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATGAGGAGTAAAG
un S	ACGGCATACG	AGTA	TCAGACGTGTG		ТТАТТСАТА	CGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTG
24 18	AGAT	AAGC	CTCTTCCGATCT	G	TGCTTAART	AGCCTCCGCTTATTGATATGCTTAART
21_10	nom		ererreconter	Ū		
ITS4F	CAAGCAGAAG	CGTA	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATCGTAAGATGCCT
un_S_	ACGGCATACG	AGAT	TCAGACGTGTG		ТТАТТСАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTC
24 19	AGAT	GCCT	CTCTTCCGATCT	ТС	TGCTTAART	AGCCTCCGCTTATTGATATGCTTAART
<u> </u>	nom		ererreconter	10		
ITS4F	CAAGCAGAAG	ATCT	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATATCTAGTGGCAA
un_S_	ACGGCATACG	AGTG	TCAGACGTGTG	СТ	ТТАТТСАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCT
24 20	AGAT	GCAA	CTCTTCCGATCT	A	TGCTTAART	AAGCCTCCGCTTATTGATATGCTTAART
	nom		ererreconter	11		
ITS4F	CAAGCAGAAG	CCAG	GTGACTGGAGT		AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATCCAGGGACTTCT
un S	ACGGCATACG	GGAC	TCAGACGTGTG	GA	ТТАТТСАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGA
24 21	AGAT	TTCT	CTCTTCCGATCT	TA	TGCTTAART	TAAGCCTCCGCTTATTGATATGCTTAART
	nom					
ITS4F	CAAGCAGAAG	TGCA	GTGACTGGAGT	AC	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATTGCAGATCCAAC
un S	ACGGCATACG	GATC	TCAGACGTGTG	TC	ТТАТТСАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAC
24 22	AGAT	CAAC	CTCTTCCGATCT	A	TGCTTAART	TCAAGCCTCCGCTTATTGATATGCTTAART
	nom					
ITS4F	CAAGCAGAAG	ATAG	GTGACTGGAGT	ТТ	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATATAGTTAGGGCT
un_S_	ACGGCATACG	TTAG	TCAGACGTGTG	CT	ТТАТТБАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTT
24 23	AGAT	GGCT	CTCTTCCGATCT	CT	TGCTTAART	CTCTAGCCTCCGCTTATTGATATGCTTAART
				CA	- GOTTAINI	
ITS4F	CAAGCAGAAG	GCAC	GTGACTGGAGT	CT	AGCCTCCGC	CAAGCAGAAGACGGCATACGAGATGCACTTCATTTC
un S	ACGGCATACG	TTCA	TCAGACGTGTG	TC	ТТАТТБАТА	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCA
24 24	AGAT	TTTC	CTCTTCCGATCT	T	TGCTTAART	CTTCTAGCCTCCGCTTATTGATATGCTTAART
<u></u>	110/11		erenceomer	1 *	igernami	

Table S3 Molecular identification of arbuscular mycorrhizal fungi in this study

OTU	Coverage	E-value	Similarity	Best NCBI hit	
OTU51_Rhizophagus	99%	2.00E-159	93%	JN195441.1	Uncultured_Glomus
OTU70_Claroideoglomus	99%	0	99%	JF439206.1	Glomus_hoi
OTU166_Rhizophagus	98%	0	98%	JN936299.1	Rhizophagus_sp.
OTU150_Rhizophagus	98%	0	97%	JN936299.1	Rhizophagus_sp.
OTU253_Funneliformis	98%	0	99%	AJ919274.1	Glomus_mosseae
OTU118_Rhizophagus*	98%	4.00E-180	98%	KM041773.1	Uncultured_Rhizophagus
OTU161_Rhizophagus*	98%	1.00E-173	97%	KM041753.1	Uncultured_Rhizophagus
OTU132_Rhizophagus*	98%	0	98%	JX999965.1	Glomeromycota_sp.
OTU133_Glomus	98%	2.00E-172	96%	KM208159.1	Uncultured_Glomeromycota
OTU206_Glomus	99%	2.00E-178	97%	GQ388297.1	Uncultured_Glomus
OTU213_Rhizophagus	98%	0	98%	JX999971.1	Glomeromycota_sp.
OTU935_Rhizophagus	98%	1.00E-156	92%	JN195441.1	Uncultured_Glomus
OTU126_Glomus	98%	5.00E-173	97%	KM208171.1	Uncultured_Glomeromycota
OTU251_Rhizophagus	98%	0	98%	GQ205073.1	Glomus_custos
OTU558_Funneliformis	99%	0	98%	HG425925.1	Uncultured_Funneliformis
OTU323_Glomus	98%	1.00E-174	97%	KM208159.1	Uncultured_Glomeromycota
OTU224_Glomus	99%	7.00E-178	98%	GQ388314.1	Uncultured_Glomus
OTU229_Glomus	98%	2.00E-179	98%	KM208159.1	Uncultured_Glomeromycota
OTU355_Funneliformis	99%	0	98%	HF970318.1	Uncultured_Funneliformis
OTU347_Rhizophagus	98%	0	98%	KJ701452.1	Uncultured_Glomus
OTU476_Glomus	99%	4.00E-180	97%	HG425896.1	Uncultured_Glomeraceae
OTU277_Glomus	99%	7.00E-178	98%	GQ388297.1	Uncultured_Glomus
OTU565_Funneliformis	99%	0	98%	U49264.1	Glomus_moessae
OTU491_Claroideoglomus	99%	0	99%	KY927389.1	Claroideoglomus_etunicatum
OTU400_Glomus	99%	7.00E-178	97%	JX276899.1	Uncultured_Glomeromycota
OTU333_Glomus	99%	0	98%	FR693682.1	Uncultured_Glomus

OTU435_Glomus	99%	0	98%	HG425982.1	Uncultured_Glomeraceae
OTU534_Glomus	98%	0	99%	KF836947.1	Glomus_sp.
OTU944_Rhizophagus	99%	1.00E-154	91%	JN195441.1	Uncultured_Glomus
OTU536_Claroideoglomus	98%	0	97%	JQ218217.1	Uncultured_Glomus
OTU989_Funneliformis	99%	0	98%	EF989113.1	Funneliformis_mosseae
OTU498_Glomus	99%	3.00E-176	98%	JX096590.1	Uncultured_Glomeromycota
OTU579_Glomus	99%	6.00E-166	96%	JX096590.1	Uncultured_Glomeromycota
OTU987_Paraglomus	99%	7.00E-159	88%	AB520480.1	Uncultured_fungus
OTU734_Rhizophagus	97%	0	98%	AF185651.1	Glomus_intraradices
OTU715_Glomus	99%	1.00E-173	97%	JX096590.1	Uncultured_Glomeromycota
OTU574_Glomus	98%	1.00E-174	97%	JX096590.1	Uncultured_Glomeromycota
OTU659_Glomus	98%	5.00E-179	98%	JX096614.1	Uncultured_Glomeromycota
DTU1068_Glomus	98%	1.00E-173	97%	HM162343.1	Uncultured_Glomeromycota
DTU564_Glomus	99%	2.00E-160	95%	GU059545.1	Glomus_indicum
OTU735_Claroideoglomus	99%	0	96%	AF004682.1	Glomus_etunicatum
DTU597_Paraglomus	98%	6.00E-166	91%	AB520480.1	Uncultured_fungus
DTU998_Glomus	99%	8.00E-177	97%	GQ388297.1	Uncultured_Glomus
OTU1184_Rhizophagus	98%	0	99%	AF185650.1	Glomus_intraradices
OTU1065_Claroideoglomus	99%	0	96%	KP191488.1	Claroideoglomus_drummondii
OTU936_Claroideoglomus	100%	0	99%	JN685281.1	Uncultured_Glomeromycota
OTU1197_Claroideoglomus	99%	0	97%	KP191486.1	Claroideoglomus_drummondii
OTU1274_Paraglomus	98%	9.00E-151	85%	AB520480.1	Uncultured_fungus
OTU969_Claroideoglomus	98%	0	97%	JX096582.1	Uncultured_Glomeromycota
OTU1013_Glomeraceae	98%	4.00E-180	96%	JN195694.1	Uncultured_Glomus
OTU975_Paraglomus	99%	0	99%	KF849701.1	Uncultured_Paraglomus
OTU945_Glomus	99%	0	99%	KF849595.1	Uncultured_Glomus

*Note: These three OTUs are combined to avoid the potential more than one rDNA repeat in a single species. However, the ecological results and conclusion are not affected (Fig. S10-S15). Representative sequences of AMF OTUs were deposited in GenBank with the accession codes: MG008508 - MG008559.

- 213 Table S4 Arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs) bias occurred in the
- 214 first week (TP01) and the last week (TP17), as detected by indicator species analysis. Note the steep
- 215 decline of two initially dominant species and the rise of 13 initially rare *Rhizophagus* and *Glomus* species

AMF OTUs	Preferred	Indicator value	Р
OTU51_Rhizophagus	TP01	0.894	0.001
OTU70_Claroideoglomus	TP01	0.809	0.001
OTU166_Rhizophagus	TP17	0.855	0.001
OTU118.161.132_Rhizophagus	TP17	0.811	0.001
OTU150_Rhizophagus	TP17	0.797	0.001
OTU213_Rhizophagus	TP17	0.75	0.001
OTU251_Rhizophagus	TP17	0.667	0.001
OTU126_Glomus	TP17	0.816	0.001
OTU133_Glomus	TP17	0.788	0.001
OTU229_Glomus	TP17	0.762	0.001
OTU323_Glomus	TP17	0.711	0.001
OTU476_Glomus	TP17	0.474	0.007
OTU333_Glomus	TP17	0.415	0.01
OTU534_Glomus	TP17	0.318	0.05
OTU400_Glomus	TP17	0.311	0.035

- 217 **Table S5** Arbuscular mycorrhizal fungal (AMF) operational taxonomic units (OTUs) bias occurred in the
- 218 root, rhizosphere and soil, as detected by indicator species analysis. Note a number of *Rhizophagus* were
- 219 more common and abundant in root, whereas Funneliformis, Claroideoglomus, Paraglomus and Glomus
- 220 were more common and abundant in rhizosphere and soil

AMF OTUs	Indicator	Indicator value	Р
OTU118.161.132_Rhizophagus	Root	0.399	0.001
OTU166_Rhizophagus	Root	0.391	0.001
OTU213_Rhizophagus	Root	0.37	0.001
OTU150_Rhizophagus	Root	0.363	0.002
OTU251_Rhizophagus	Root	0.236	0.009
OTU734_Rhizophagus	Root	0.054	0.011
OTU70_Claroideoglomus	Rhizosphere	0.419	0.001
OTU206_Glomus	Soil	0.566	0.001
OTU224_Glomus	Soil	0.529	0.001
OTU277_Glomus	Soil	0.434	0.001
OTU498_Glomus	Soil	0.118	0.001
OTU659_Glomus	Soil	0.104	0.001
OTU998_Glomus	Soil	0.104	0.001
OTU579_Glomus	Soil	0.1	0.001
OTU574_Glomus	Soil	0.089	0.001
OTU564_Glomus	Soil	0.085	0.001
OTU1068_Glomus	Soil	0.068	0.001
OTU715_Glomus	Soil	0.062	0.005
OTU253_Funneliformis	Soil	0.52	0.001
OTU558_Funneliformis	Soil	0.277	0.001
OTU355_Funneliformis	Soil	0.261	0.001
OTU565_Funneliformis	Soil	0.222	0.001
OTU989_Funneliformis	Soil	0.103	0.006
OTU491_Claroideoglomus	Soil	0.167	0.001
OTU536_Claroideoglomus	Soil	0.088	0.007
OTU1065_Claroideoglomus	Soil	0.051	0.005
OTU987_Paraglomus	Soil	0.144	0.001
OTU597_Paraglomus	Soil	0.047	0.021

Table S6 A list of studies demonstrating the nestedness of arbuscular mycorrhizal fungal (AMF) community. Note nestedness is prevailing in 222

223	AMF community, but not seen in some other studies
-	

Study	Ecosystem	AMF recognition	Conclusion
(Kawahara et	Six locations along a pH	Trap culture, Sanger	AMF communities in lower pH soils were subsets of (nested in)
al 2016)	gradient in Japan	sequencing of LSU	those in higher pH soil
(van Geel et	Apple trees in 24 orchards in	454 sequencing of AMF	Degree of nestedness of the AMF communities was related to plant-
al 2015)	Belgium	SSU	available P and N content of the soil, pointing to a progressive loss
			of AMF taxa with increasing fertilization.
(Vályi et al	Land use intensity gradient	454 sequencing of AMF	Communities in medium and low land-use sites were subsets of
2015)	in Germany	SSU	high land-use communities
(Camenzind	Tropical montane forest in	454 sequencing of AMF	AMF community is highly nested
et al 2014)	Ecuador	LSU	
(Verbruggen	40 agricultural soils in the	tRFLP of LSU	Communities from species-poor fields were found to be subsets of
et al 2012)	Netherlands		those in richer fields
(Chen et al	Subtropical forest in China	454 sequencing of AMF	Plant-AMF symbiotic network is highly nested
2017a)		SSU	
(Chagnon et	Reanalysis of study in	454 sequencing of AMF	Plant-AMF symbiotic network is highly nested
al 2012)	hemiboreal forest	SSU	
(Montesinos-	Semiarid valley	Sanger sequencing of	Plant-AMF symbiotic network is highly nested
Navarro et al		AMF ITS	
2012)			
(Toju et al	Temperate forest	Sequencing of fungal ITS	No significant nestedness in plant-AMF symbiotic network
2014)	_		
(Van Geel et	European grasslands	454 sequencing of AMF	Plant-AMF symbiotic network is highly nested
al 2017a)	-	SSU	
(Van Geel et	European vineyards	454 sequencing of AMF	AMF community is highly nested
al 2017b)	1	SSU	

224 SSU: small subunit; LSU, large subunit; ITS: internal transcribed spacer; tRFLP, terminal restriction fragment length polymorphism.

Table S7 List of studies investigating the phylogenetic relatedness of arbuscular mycorrhizal fungal (AMF) community. Note phylogenetic

226	underdispersion.	overdisperion a	and stochastic are all seen	, with the underdisper	ion most prevalent
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Study	Ecosystem	AMF	Conclusion
		recognition	
(Bainard et	Farmed pea, lentil and wheat in	454 sequencing	AMF communities in general are phylogenetically underdispered, with
al 2014)	temperate semiarid prairie	of AMF SSU	exception of the first soil and second root samplings.
(Horn et al	Temperate grassland in Germany	454 sequencing	AMF communities are phylogenetically underdispersed
2014)		of AMF LSU	
(Chen et al	Temperate semiarid steppe in	454 sequencing	AMF communities are phylogenetically underdispered, regardless of
2017b)	China	of AMF SSU	precipitation and nitrogen
(Liu et al	Alpine meadow in China	Sanger	AMF shifted from phylogenetic underdispersion to overdisersion with
2015a)		sequencing of	increasing nitrogen fertilization
		SSU	
(García de	Abandoned quarry in Estonia	454 sequencing	Phylogenetic community composition of AMF was
León et al		of AMF SSU	more clustered than global, and European taxon pools
2016a)			
(Davison et	Re-analysis of global dataset of	454 sequencing	Coexisting fungi were more phylogenetically clustered than the random
al 2016)		of AMF SSU	communities defined by a variety of null models.
(Maherali	Meadow (50 x 50 m^2 with 2601	Spores	AMF communities are generally phylogenetically overdispersed, but a
and	samples) in Canada		subset of AMF communities are phylogenetically underdispersed
Klironomos			
2012)			
(Maherali	Greenhouse controlling initial	tRFLP	Initial phylogenetic overdispersion of AMF community result in high
and	AMF phylogenetic diversity		richness
Klironomos			
2007)			
(Roger et al	Pot cultures inoculated with	qPCR	When fungi were closely related, they were able to coexist in almost equal
2013)	Rhizophafus irregularis isolate		proportions
	combinations with different		
	phylogenetic relatedness		

(Liu et al 2015b)	Alpine meadow in China	Sanger sequencing of	AMF communities were phylogenetically clustered and random in unfertilized and fertilized plots, respectively.
,		SSU	
(Mueller	Grassland in California, USA	Sanger	AMF communities were generally phylogenetically underdispered, but not
and		sequencing of	significant in the nitrogen treatment
Bohannan		SSU	
2015)			
(Egan et al	Subalpine grassland, treeline and	454 sequencing	AMF communities being phylogenetically clustered at all elevations
2017)	alpine tundra along an elevational	of AMF SSU	sampled
	gradient in Montana, USA		
(Saks et al	Mature mixed boreonemoral	454 sequencing	AMF communities being frequently phylogenetically clustered compared
2013)	forest in Estonia	of AMF SSU	with local and global taxon pools
(Kivlin et	Meta-analysis of 111 published	14,961 public	AMF communities being phylogenetically clustered in the majority of
al 2011)	studies	DNA sequences	sites, and only two sites had communities that were phylogenetically
			dispersed
(Shi et al	Alpine meadow in China	454 sequencing	AMF community was phylogenetically clustered under warming, but not
2017)		of AMF SSU	in control, or plots experienced clip

SSU: small subunit; LSU, large subunit; RFLP: restriction fragment length polymorphism.

228 **Table S8** List of studies comparing arbuscular mycorrhizal fungal (AMF) communities between root and

Study	Ecosystem	AMF	Conclusion
		recognition	
(Hempel et	Farmed	Sanger	Root is abundant in Rhizophagus (Glomus group Ab);
al 2007)	meadow in	sequencing of	soil is abundant in Paraglomeraceae and
	Germany	ITS	Archaeosporaceae
(Bainard et	Farmed pea,	454	Root is abundant in <i>Rhizopahgus</i> and <i>Funneliformis</i> ,
al 2014)	lentil and	sequencing of	soil is abundant in Paraglomus
	wheat in	AMF SSU	
	temperate		
	semiarid		
	prairie		
(Wilde et al	Two salt	Both	Rhizophagus (Glomus) intraradices is more abundant
2009)	marshes in	morphological	in root,
	Germany	and molecular	Funneliformis (Glomus) geosporum is more abundant
		criteria	in soil
(Yang et al	Alpine	Sanger	One Diversisporales was more abundant in soil, two
2013)	meadow	sequencing of	Glomerales were more abundant in root
,	subjected to	SSU-ITS-	
	warming and	LSU	
	grazing in		
	China		
(Beauregard	Crop fields in	DGGE	Six out of seven ribotypes show difference between
et al 2013)	Canada		root and soil
(Liu et al	Alpine	Soil spore	<i>Rhizophagus</i> was the most abundant AMF in root;
2012)	Meadow	isolation;	Diverspora was the most abundant AMF in soil spore
	subjected to	Root, sanger	
	fertilization in	sequencing of	
	China	SSU	
(Hart and	Pot	Percent of	Glomaceae isolates had high root colonization but low
Reader	experiment to	colonization	soil colonization, Gigasporaceae isolates showed the
2002)	test the	and fungal	opposite trend whereas Acaulosporaceae isolates had
	colonization	biomass in	low root and soil colonization.
	potential of	root; Hyphal	
	21 AMF	length in soil	
	isolates from		
	three families		
(Verbruggen	40	tRFLP	No obvious different between root and soil AMF
et al 2012)	agricultural		communities
	soils in the		
	Netherlands		

soil. Note different root and soil AMF are often seen, but not in some studies

(Wu et al	Primary	tRFLP	AMF community structures detected by spore
2007)	successional		sampling were inconsistent with those from plant
	volcanic		roots.
	desert in		
	Japan		

- 230 SSU: small subunit; LSU, large subunit; ITS: internal transcribed spacer; tRFLP, terminal restriction
- 231 fragment length polymorphism; DGGE: Denaturing Gradient Gel Electrophoresis.

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