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Phylogenetic structure of arbuscular mycorrhizal community shifts in response to increasing soil fertility

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Abstract

Understanding the underlying mechanisms driving responses of belowground communities to increasing soil fertility will facilitate predictions of ecosystem responses to anthropogenic eutrophication of terrestrial systems. We studied the impact of fertilization of an alpine meadow on arbuscular mycorrhizal (AM) fungi, a group of root-associated microorganisms that are important in maintaining sustainable ecosystems. Species and phylogenetic composition of AM fungal communities in soils were compared across a soil fertility gradient generated by 8 years of combined nitrogen and phosphorus fertilization. Phylogenetic patterns were used to infer the ecological processes structuring the fungal communities. We identified 37 AM fungal virtual taxa, mostly in the genus *Glomus*. High fertilizer treatments caused a dramatic loss of *Glomus* species, but a significant increase in genus richness and a shift towards dominance of the lineage of *Diversispora*. AM fungal communities were phylogenetically clustered in unfertilized soil, random in the low fertilizer treatment and over-dispersed in the high fertilizer treatments, suggesting that the primary ecological process structuring communities shifted from environmental filtering (selection by host plants and fungal niches) to a stochastic process and finally to competitive exclusion across the fertilization gradient. Our findings elucidate the community shifts associated with increased soil fertility, and suggest that high fertilizer inputs may change the dominant ecological processes responsible for the assembly of AM fungal communities towards increased competition as photosynthate from host plants becomes an increasingly limited resource.

Keywords: Symbiotic fungi; Phylogenetic structure; Ecological process; Competition; Environmental filtering; Resource availability

1. Introduction

Worldwide increases in fertilizer application and nutrient deposition raise the question of how nutrient enriched soil influences biotic communities. Numerous field studies have shown that improved soil fertility increases plant productivity, but also decreases plant species diversity and changes plant community composition mainly through the enhanced process of competitive exclusion (Rajaniemi, 2002; Hautier et al., 2009; Borer et al., 2014; Liu et al., 2015). Diverse belowground communities of microorganisms interact with plants and serve as key players in biogeochemical cycling (Fitter et al., 2005; Philippot et al., 2013), but in comparison with that of plant community, relative little is known about the patterns and underlying mechanisms of belowground community responses to soil nutrient enrichments. Filling this knowledge gap can help guide management decisions and facilitate predictions of ecosystem sustainability in a changing world.

Arbuscular mycorrhizal (AM) fungi in the phylum Glomeromycota are widespread root-associated microorganisms that are known to be sensitive to fertilization. In general, plants supply associated AM fungi with carbohydrates, and in return, fungi provide soil phosphorus (P) and possibly nitrogen (N) to their host plants (Hodge et al., 2010; Selosse and Rousset, 2011). Mycorrhizal symbioses can also provide plants with other benefits such as protection against root pathogens (Lewandowski et al., 2013) and several types of abiotic stress (Aroca et al., 2007). Furthermore, increasing evidence shows that mycorrhizas influence the structure of plant communities (Klironomos et al., 2011; Yang et al., 2014), the rhizosphere microbiome (Vestergård et al., 2008; Veresoglou et al., 2012), soil structure (van der Heijden et al., 2006; Leifheit et al., 2015), and nutrient cycles (Cheng et al., 2012; Bender et al., 2015). Many experiments have shown that fertilization reduces AM fungal diversity and shifts the community toward dominance of particular species or lineages (e.g. Johnson, 1993; Egerton-Warburton et al., 2007; Alguacil et al., 2010; Liu et al., 2012, 2015; Camenzind et al., 2014). However, neutral or even positive effects of fertilization on AM fungal diversity have also been reported (e.g. Antoninka et al., 2011; van Diepen et al., 2011; Gosling et al., 2013), suggesting that the responses of AM fungal communities to fertilization are influenced by complex interactions of many factors (van Diepen et al., 2011; Vályi et al., 2015).

Although it has long been recognized that fertilization influences the structure of

AM fungal communities, the mechanisms responsible for these impacts remain largely unclear. In theory, there are several possible mechanisms by which increasing soil fertility may influence the composition of AM fungal communities including competition among AM fungi and environmental filtering because of fungal preferences or host plant selection. First, fertilization may enhance competition among coexisting AM fungi because plants generally reduce the amount of carbohydrate supplied to mycorrhiza when they are not limited by soil nutrients (Johnson, 2010; Olsson et al., 2010); and, enhanced competition may result in loss of fungal diversity and a shift of the species composition towards dominance of AM fungi that are superior competitors when carbohydrates are limited (Hepper et al., 1988; Johnson, 1993). Second, because species of AM fungi have different niches and are known to prefer to inhabit different soil conditions (Schechter and Bruns, 2008; Alguacil et al., 2010; Dumbrell et al., 2010), fertilization may directly select taxa that grow best in the enriched conditions. Third, there is good evidence that plants actively select AM fungal taxa that best provision nutrients (Parniske, 2008; Kiers et al., 2011; Bever, 2015) and many field studies have shown that certain plants select particular AM fungi (e.g. Vandenkoornhuyse et al., 2003; Veresoglou and Rillig, 2014); thus, the loss of plant species caused by fertilization can lead to significant changes in the AM fungal community (Liu et al., 2012).

Analysis of phylogenetic structure has been used to infer the relative importance of competitive exclusion, environmental filtering, or stochastic process in driving community assembly (Webb et al., 2002). If the phylogenetic niches of organisms are conserved such that more closely related taxa have similar niche-related traits, then competitive exclusion and environmental filtering will generate patterns of over-dispersion and phylogenetic clustering, respectively (Webb et al., 2002). This method was first used in the studies of plant communities (e.g. Webb, 2000; Dinnage, 2009; de Freitas et al., 2014; Parmentier et al., 2014), but is now increasingly used to analyze the community assembly of mycorrhizal fungi (e.g. Lim and Berbee, 2013; Grilli et al., 2014; Horn et al., 2014; Rincón et al., 2014; Shi et al., 2014). A factorial field study of the influence of light and nutrient availability analyzed the phylogenetic structure of AM fungal communities inside roots and determined that environmental filtering structured communities under unfertilized conditions but random process was most important under a low level of fertilization (Liu et al., 2015). The purpose of our current study is to analyze the phylogenetic structure of AM fungal communities

across a long-term fertilization gradient and test the hypothesis that increasing fertilization will cause plants to allocate less photosynthate to their fungal symbionts and increase the relative importance of competitive interactions for structuring AM fungal communities.

We have previously shown that eight years of combined N and P fertilization of an alpine meadow reduces the abundance and diversity of AM fungal communities inside roots (Liu et al., 2012). However, the ecological processes driving these community shifts were not examined. Our current study uses community phylogenetic analysis to study the AM fungal communities in the soil of the same fertilization experimental system as Liu et al. (2012) to better understand the mechanisms by which increasing soil fertility structures AM fungal communities. We chose to identify communities from soil DNA rather than roots because soil contains both active (extraradical hyphae) and dormant (spore) structures of AM fungi and thus may reflect a larger proportion of the total species pool (Alguacil et al., 2014). We predicted that, as in the root-derived results reported in Liu et al. (2012), increasing soil fertility would significantly reduce the diversity of soil-borne AM fungi; and additionally, this reduction should be largely attributed to the enhanced competition process. In particular, the current study aimed to answer the following questions: (1) how do species composition and phylogenetic structure of AM fungal communities respond to a fertilization gradient? (2) Which is the primary ecological process structuring AM fungal communities across a fertilization gradient?

2. Materials and methods

2.1. Description of study site and fertilization treatment

This study was conducted at the Walaka experimental site (34°00'N, 102°00'E; 3500 m above sea level) of the Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University in the eastern Qinghai-Tibetan Plateau of China. This region is a typical alpine meadow ecosystem, where the mean annual temperature is 1.2 °C, the mean annual precipitation is 620 mm, the dominant plants consist mainly of Cyperaceae and Poaceae, the growing season is from May to September, and the diversity of both plants and AM fungi is relatively high (Liu et al., 2012). Since 2001, the experimental site has been fenced and only grazed by yak and

sheep every winter from October to April.

The long-term fertilization experiment, with 25 plots (5 rows by 5 columns) consisted of five fertilization levels and five replicates using a randomized block design. Plots were established on a flat area on March 2002. Each plot was $10 \times 6\text{-m}^2$ and separated from the others by a 1-m buffer strip. Five fertilization treatments were generated with 0, 30, 60, 90 and $120\text{ g m}^{-2}\text{ yr}^{-1}$ of $(\text{NH}_4)_2\text{HPO}_4$ fertilizer applied annually from 2002 at the beginning of the growing season, and these treatments are hereafter referred to as F0, F30, F60, F90 and F120, respectively. The corresponding N and P inputs of each treatment are as follows: F0, control; F30, 6.4 g N and $7\text{ g P m}^{-2}\text{ yr}^{-1}$; F60, 12.7 g N and $14.1\text{ g P m}^{-2}\text{ yr}^{-1}$; F90, 19.1 g N and $21.1\text{ g P m}^{-2}\text{ yr}^{-1}$; F120, 25.4 g N and $28.2\text{ g P m}^{-2}\text{ yr}^{-1}$. After eight years of fertilization treatment (in 2010), soil available N and P concentrations gradually increased across the fertilization gradient, while plant species richness decreased dramatically and plant community shifted towards dominance by *Elymus nutans* (Table S1). More details of the changes in soil and plant properties across the fertilization gradient are described in Liu et al. (2012).

2.2. Soil sampling and DNA extraction

Soil samples were collected on 20 May, 10 July and 5 September 2010, concurrently with the samples collected for Liu et al. (2012). At each sampling date, nine replicate soil cores (3.8 cm diameter, 25 cm depth) were collected randomly from each plot and samples were manually homogenized. From each composite sample, about 50 g root-free soil was subsampled and stored in a pre-sterilized aluminum container and used for DNA extraction (75 soil samples in total). The remaining soil and roots were analyzed in Liu et al. (2012). All samples were transported to the laboratory within 36 h using an ice box.

Soil DNA was extracted from a 0.25-g subsample of each of the 75 soil samples using the PowerSoilTM DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Extracted DNA was examined on 0.8% (w/v) agarose gel with ethidium bromide staining to confirm the extracted efficiency. All extracted DNA was stored at -80°C until further use.

2.3. Molecular analysis of AM fungal community

Before analysis, the soil DNA collected from each plot was combined across the three sampling dates, resulting in 25 pooled DNA samples. The AM fungal community in soil was analyzed by a PCR-cloning-sequencing approach using the procedure and conditions described by Liu et al. (2012). Briefly, partial 18S rRNA gene sequences of AM fungi were amplified from soil DNA via a nested PCR, using GeoA2-Geo11 (Schwarzott and Schüßler, 2001) and NS31-AML2 (Simon et al., 1992; Lee et al., 2008) as the first and second primer combinations, respectively. The second PCR products were purified and used to construct clone libraries (25 in total), and then the DNA inserts were re-amplified with NS31-AML2 (48 putative positive clones per clone library) and screened using restriction fragment length polymorphism (RFLP) with restriction enzymes *Hinf*I and *Hin*II. The RFLP patterns were only compared within the five replicate samples within the same fertilization treatment. One representative clone of each RFLP type in each treatment was sequenced (179 sequences in total), and the remaining clones were classified by RFLP typing. All DNA sequences were edited using the ContigExpress software (InforMax Inc., MD, USA) and compared with published nucleotide sequences using the online BLAST search tool (<http://blast.ncbi.nlm.nih.gov>; accessed 10 March 2011). Non-AM fungi and possible chimeric sequences were eliminated from the dataset, and the remaining 136 AM fungal sequences were analyzed further. In total, 835 AM fungal clones were identified from 1200 clones (c. 70%). All AM fungal sequences obtained in this study have been deposited in the GenBank database under accession numbers JN009482-JN009617.

To facilitate the comparison between our AM fungal sequences with other studies, we delimited our sequences into different molecular virtual taxa (VTs, similar with molecular operational taxonomic unit or phylotype) using the online MaarjAM database (<http://maarjam.botany.ut.ee>; accessed 15 November 2013). This database has collected most published 18S rRNA gene sequences of AM fungi and phylogenetically defined sequence groups as AM fungal VTs with sequence identity $\geq 97\%$ (corresponding to species-level taxa roughly; Öpik et al., 2010). The VT taxonomy of AM fungi is increasingly used in identification of environmental sequences (e.g. Öpik et al., 2014; Xiang et al., 2014; Liu et al., 2015). After BLAST against the MaarjAM database, each obtained sequence was grouped into a corresponding VT according to the sequence identity ($\geq 97\%$), query coverage ($\geq 97\%$)

and BLAST bit score. To further confirm the BLAST-based VT delimitation, we aligned our AM fungal sequences and the representative sequences of VTs in MaarjAM database using ClusterW and constructed a maximum likelihood (ML) tree using MEGA 5.0 with Tamura 3-parameter model and 1000 bootstrap replications (Tamura et al., 2011). Some sequences with ambiguous VT delimitation (i.e. one sequence could be grouped into more than one VT after BLAST search) were corrected according to the sequence identity, bootstrap value and the topology of the phylogenetic tree (Liu et al., 2015).

To elucidate the phylogenetic relationships between our VTs and published AM fungal sequences, we constructed a ML phylogenetic tree using DNA sequences including the representative sequences of our VTs, the representative sequences of major AM fungal genera and the most closely related sequences from GenBank database. Each VT obtained in this study was grouped into a corresponding AM fungal genus according to the phylogenetic tree, and if a VT could not be placed in a known genus, we regarded this as a new genus-like clade. The VT/genus compositions of AM fungi were calculated on the basis of the clone numbers of each VT/genus in each clone library.

2.4. Statistical analysis

As our sampling was concurrent with the sampling work reported in Liu et al. (2012), the data of soil characteristics and plant variables used in this study were derived from that study. Before analysis, a principal component analysis ('prcomp' function of R package 'stats', R Core Team., 2015) was carried out using the $\ln(x+1)$ transformed data of soil characteristics (including soil available N, available P, N:P ratio, total N, organic C, C:N ratio, moisture and pH) in order to generate fewer compound variables (principal components, PC) that characterize the soil properties. PCA results show that 74% of total variance could be summarized in the first two components (Fig. S1): the PC1 (44%) describes a main gradient of soil available N (loading value = 0.47) and available P (0.44); the PC2 (30%) reflects a main gradient of soil moisture (0.51) and organic carbon (0.50). In the following analyses, we used the scores of PC1 and PC2 to assess the relationships between AM fungal variables and soil properties.

Based on the community matrices of VT/genus compositions of AM fungi, the

VT/genus richness as well as the relative abundance of each VT/genus in each sample were calculated, and the sampling effort curves of VT richness were examined using 'rarecurve' function of the R package 'vegan' (Oksanen et al., 2015). To test if the spatial autocorrelations were present in our measured variables, we calculated the Moran's I autocorrelation coefficients using 'Moran.I' function in R library 'ape' (Paradis et al., 2004) with a matrix of plot distance weights and our AM fungal variables. We did not observe significant spatial autocorrelations being present in all tested AM fungal variables; thus, the effects of spatial autocorrelation were not taken into account in below analyses. The effects of fertilization on AM fungal variables were analyzed using a linear mixed effects model, with fertilization as a fixed effect and block as a random effect. These analyses were conducted using the 'lme' (model fitting) and 'anova.lme' (ANOVA summary of the linear mixed effects model) functions in the R package 'nlme' (Pinheiro et al., 2015). Based on the 'lme' model, we also summarized the post-hoc pairwise comparisons (method = Tukey) to illustrate the differences of each variable between fertilization treatments using 'glht' function of R package 'multcomp' (Hothorn et al., 2008). Relationships between AM fungal variables with two extracted PCA components of soil properties, plant species richness and the relative abundance of *Elymus nutans* were tested using correlation and regression analyses (SPSS Inc., IL, USA).

The dissimilarity of communities of AM fungal VT across samples was analyzed by non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity measurement using 'metaMDS' function, and the treatments (represented by F0 to F120) were fitted as centroids onto the two dimensional ordination plot using 'ordiellipse' function. Relationships between AM fungal community composition with two extracted PCA components of soil properties, plant species richness and the relative abundance of *Elymus nutans* were tested using 'envfit' function. All of these statistical analyses were done using the R package 'vegan' (Oksanen et al., 2015).

The community phylogenetic structure was analyzed using the R package 'picante' (Kembel et al., 2010). We used the mean pairwise phylogenetic distance (MPD) to quantify the relatedness of co-occurring AM fungal VTs within a community (Webb et al., 2002; Horn et al., 2014). Based on the MPD, we calculated the inter-community MPD (betaMPD) using 'comdist' function, and again we performed NMDS and 'envfit' using the data of betaMPD. The difference in community phylogenetic composition between a given pair of samples can be clearly

depicted on the two dimensional NMDS plot (Liu et al., 2015). To evaluate the degree of non-random phylogenetic structure of our communities, we calculated the nearest relative index (NRI) using ‘ses.mpd’ function (NRI is equivalent to -1 times the output of ‘ses.mpd’; Kembel et al., 2010), in which the MPD for each VT was weighted by its abundance, and the observed MPD was compared with the null distribution of MPD generated by the 1000 randomizations of ‘phylogeny.pool’ null model. The NRI is a standardized measure of the mean pairwise phylogenetic distance of taxa in a sample (Webb et al., 2002), and through which we could infer the importance of niche-based and neutral processes in driving community assembly (Kembel, 2009). In general, a mean NRI across all samples that is significantly greater than zero is correlated with phylogenetic clustering, equal to zero with random, and less than zero with over-dispersion (Kembel, 2009). The significant difference between NRI and null expectation of zero was tested using two-tailed *T* test at the 95% confidence level (SPSS Inc., IL, USA).

3. Results

3.1. AM fungal taxa detected in this study

In total, 37 AM fungal VTs were identified in all soil samples, of which eleven VTs were related to sequences of morphologically described species (Table 1). These VTs belong to nine known genera and two new genus-like clades within five families (Fig. 1): Glomeraceae (including 27 VTs in 6 genera), Diversisporaceae (5 VTs in 2 genera), Claroideoglomereae (3 VTs in 1 genus), Archaeosporaceae (1 VT in 1 genus) and Ambisporaceae (1 VT in 1 genus). Inspection of the VT accumulation curves showed that the curves from F60, F90 and F120 were closer to asymptotic than the curves from F0 and F30 (Fig. S2), suggesting that a higher proportion of the AM fungal diversity was captured in high fertilizer treatments. Overall, VT62 (related to an unknown *Diversispora* sequence; 16% of all AM fungal clones) and *Glomus* (38%) were the most frequently detected VT and genus, respectively.

3.2. Fertilization effects on AM fungi

The relative abundance of most VTs varied among treatments (Table 1), with

some VTs decreased (e.g. VT222 and VT371) while other increased (e.g. VT62 and VT 64) across the fertilization gradient. Similarly, the abundance of most AM fungal genera, especially the *Glomus* (decreased by fertilization; $F_4 = 24.74$, $P < 0.001$) and *Diversispora* (increased by fertilization; $F_4 = 18.08$, $P < 0.001$), were very sensitive to fertilization treatment. The abundance of *Glomus* was negatively correlated with that of *Diversispora* ($r = -0.653$, $P < 0.001$) and, the former significantly decreased but the latter increased with increasing soil N and P fertility and the dominance of *Elymus nutans* (Fig. 2a,c); moreover, the *Glomus* and *Diversispora* abundance were correlated positively and negatively with plant species richness, respectively (Fig. 2d).

The VT richness of both all lineages ($F_4 = 5.71$, $P = 0.005$) and *Glomus* ($F_4 = 19.24$, $P < 0.001$) declined but genus richness increased ($F_4 = 3.05$, $P = 0.048$) with increasing fertilizer inputs (Fig. 3a,b), and all were correlated significantly with soil PC1 scores and the abundance of *Elymus nutans* (all negatively with VT richness and positively with genus richness; Table 2). NMDS ordinations revealed that both the VT and phylogenetic compositions of AM fungal communities varied among treatments (Fig. 4), with the exception of similar phylogenetic compositions under treatments of unfertilized control and F30 (Fig. 4b). Both community ordinations were highly related with plant species richness, *Elymus nutans* dominance and soil N and P fertility (soil PC1 scores; Fig. 4), but not with the soil moisture and organic C gradient (soil PC2 scores; VT composition: $R^2 = 0.207$, $P = 0.084$; phylogenetic composition: $R^2 = 0.05$, $P = 0.574$).

The NRI of the AM fungal community also declined dramatically across the fertilization gradient ($F_4 = 12.23$, $P < 0.001$; Fig. 3c) and, it was correlated negatively with soil PC1 scores and *Elymus nutans* dominance, and positively with plant species richness (Table 2); moreover, NRI was negatively correlated with AM fungal genus richness ($r = -0.689$, $P < 0.001$) but marginally positively with VT richness ($r = 0.392$, $P = 0.052$). The highest NRI occurred in unfertilized control (NRI > 0), middle in F30 treatment (NRI = 0), and the lowest in other three high fertilizer treatments (NRI < 0; Fig. 3c). These results indicate that the phylogenetic structure of AM fungal communities were clustered in unfertilized control, random in low level of fertilization (F30), and over-dispersed in high levels of fertilization (F60 to F120).

4. Discussion

4.1 Fertilization effects on AM fungal richness and community composition

Negative impacts of fertilization on AM fungal diversity have been widely reported in many ecosystems (e.g. Egerton-Warburton et al., 2007; Alguacil et al., 2010; Camenzind et al., 2014; Chen et al., 2014). Our results corroborate this and provide new evidence showing that *Glomus* species but not the non-*Glomus* species are largely lost by increasing soil N and P fertility in an alpine meadow (Fig. 3 and Table 2). In contrast to our first expectation, surprisingly, genus richness of AM fungi was increased significantly across our fertilization gradient (Fig. 3). Richness at the genus level has rarely been measured, although some studies have shown that AM fungal responses to changing environments might be different at the genus level (Klironomos et al., 1998; Bainard et al., 2014). In our study, the relative abundance of *Glomus* dramatically declined (from 81% in F0 to 14% in F120) while *Diversispora* increased (4.6% to 43%) with increasing soil N and P fertility (Fig. 2); moreover, the highest level of fertilization caused the VT number of *Glomus* to decline by near three-fold (Fig. 3a). Thus, a possible explanation for our observed increase in genus richness is that the loss of dominance by *Glomus* caused by high fertilizer treatments may have provided more opportunities for successful colonization by VTs belonging to other genera.

As expected, both species and phylogenetic compositions of our AM fungal communities varied among treatments (Fig. 4). These results are consistent with previous studies in other ecosystems showing significant impacts of N and/or P fertilization on the species composition of AM fungal communities (e.g. Egerton-Warburton et al., 2007; Alguacil et al., 2010; van Diepen et al., 2011; Camenzind et al., 2014), and also corroborate a nearby study (Liu et al., 2015). Interestingly, we found that a low level of fertilization caused significant turnover in species composition but not in phylogenetic composition. This phenomenon was also found in a study of soil bacterial community responses to N fertilization (Fierer et al., 2012), and indicates that the phylogenetic turnover of a given community might be unlikely under slight environmental change. It has been suggested that shifts of AM fungal community structure in a local scale might be related with the changes in environmental conditions and/or competition dynamics of AM fungi (Dumbrell et al., 2010, 2011; Caruso et al., 2012; Maherali and Klironomos, 2012; Horn et al., 2014). This idea is supported by our results showing that both species and phylogenetic compositions

were significantly correlated with those plant and soil variables that were very sensitive to fertilizer treatments and the increasing importance of fungal competition across the fertilization gradient (see discussion below). Future studies are necessary to address the ecological consequences of the increasing dominance of VT62 (unknown VT within *Diversispora*) and VT193 (*Claroideoglossum etunicatum* related VT) with fertilization, because high soil fertility may be expected to select AM fungi that form less mutualistic or even parasitic mycorrhiza (Johnson, 1993; Johnson et al., 2015).

4.2 Fertilization effects on phylogenetic structure of AM fungi and the inferred ecological processes

We found that AM fungal communities were phylogenetically clustered in unfertilized soil, random in the low fertilizer treatment (F30) and over-dispersed in the other three high fertilizer treatments (Fig. 3c), suggesting that the co-occurring AM fungal taxa were more closely related in unfertilized soil and more distantly related in highly fertilized soil (Webb et al., 2002). Few studies have examined the phylogenetic patterns of soil-derived AM fungal communities, but existing evidence from colonized roots shows that communities are frequently clustered in natural undisturbed ecosystems (e.g. Horn et al., 2014; Saks et al., 2014; Shi et al., 2014; Liu et al., 2015). In fact, AM fungal communities in both roots (data not shown) and soil were phylogenetically clustered in our unfertilized control, suggesting that the phylogenetic structure in root- and soil-derived communities might be similar in natural ecosystems (but see Saks et al., 2014).

Because the functional traits of AM fungi are phylogenetically conserved (Powell et al., 2009; Maherali and Klironomos, 2012), we can confidently infer that the primary ecological processes determining AM fungal communities shifted from environmental filtering (in F0) to a stochastic process (F30) and finally to competitive exclusion (F60, F90 and F120) across our fertilization gradient. Our previous observations in this experimental system revealed that both plant allocation to mycorrhiza and plant species richness decreased significantly with increasing soil fertility (Liu et al., 2012). Thus, the shifts of phylogenetic patterns observed in our study are likely to be related to increasing dominance of competition for host plant carbohydrates. The shift of phylogenetic structure in our case was tightly linked with a large loss of *Glomus* species and emergence of other genera. However, whether

Glomus species in our study site are weak competitors for carbohydrates or whether they just have very strong host specificity remain to be determined through further investigation. Although competition can sometimes produce a phylogenetically clustered pattern even when functional traits are phylogenetically conserved (Mayfield and Levine, 2010), in our case, a clustered pattern in unfertilized soil can be largely attributed to environmental filtering, because plants under extremely low P availability ($<3 \text{ mg kg}^{-1}$ soil in our study site) can be expected to actively select AM fungi with high P-uptake ability (Kiers et al., 2011; Bever, 2015), which has been shown to be phylogenetically conserved (Powell et al., 2009).

4.3 Comparison of AM fungal taxa detected from soil- and root-derived DNA

Molecular identification of AM fungal communities are usually based on root-derived DNA (e.g. Liu et al., 2011; Öpik et al., 2013; Camenzind et al., 2014; Grilli et al., 2014; Mao et al., 2014), but this method may underestimate AM fungal diversity because of the omission of dormant spores. In the present study, we used soil DNA to identify the AM fungal communities and expected that it may be superior to than root-derived DNA. Our finding of 37 AM fungal VTs is the same as the number detected from mixed roots that were sampled synchronously in the same experimental plots (Liu et al., 2012), although fewer clones were examined in this study (1200 vs. 3600 clones). We can expect that more VTs would be detected from soil than from roots if the same number of clones had been screened. However, comparison of the detected VTs here and in Liu et al. (2012) showed that some dominant VTs in roots (e.g. *Glomus intraradices* related VT) were rarely or never detected in this study, and vice versa (e.g. *Septoglomus constrictum* related VT, Table 1). These differences support previous findings of varying AM fungal taxa identified from root- and soil-derived DNA (e.g. Hempel et al., 2007; Yang et al., 2013; Bainard et al., 2014; Saks et al., 2014) and also suggest that assessment of AM fungal biodiversity might be more accurate using both root- and soil-extracted DNA (Chen et al., 2014).

4.4 Conclusions

Our study reveals the compositional changes of AM fungal communities and infers the ecological processes driving these changes across a long-term fertilization

gradient in an alpine meadow ecosystem. In our experiment, increasing soil N and P fertility significantly reduced the VT richness (especially the *Glomus* VT richness) but increased genus richness of AM fungi, and the shifts of AM fungal community structure implies an increasing importance of competition among fungi for limited host resources. These findings support our research hypothesis and highlight the contribution of competition to AM fungal community changes under increasing soil fertility. To our best knowledge, this is the first study to analyze phylogenetic structure to explore the ecological processes driving AM fungal responses to an environmental gradient. Given the increasing availability of phylogenetic data, merging phylogenetic information with studies of belowground community ecology should be encouraged in future research to help us more fully understand changes in diversity and to predict the dynamics of biotic communities under changing environments (Cavender-Bares et al., 2009).

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References

- Alguacil, M.M., Lozano, Z., Campoy, M.J., Roldán, A., 2010. Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system. *Soil Biology and Biochemistry* 42, 1114-1122.
- Alguacil, M.M., Torrecillas, E., García-Orenes, F., Roldán, A., 2014. Changes in the composition and diversity of AMF communities mediated by management practices in a Mediterranean soil are related with increases in soil biological activity. *Soil Biology and Biochemistry* 76, 34-44.
- Antoninka, A., Reich, P.B., Johnson, N.C., 2011. Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem. *New Phytologist* 192, 200-214.
- Aroca, R., Porcel, R., Ruiz-Lozano, J.M., 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* 173, 808-816.
- Bainard, L.D., Bainard, J.D., Hamel, C., Gan, Y., 2014. Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. *FEMS Microbiology Ecology* 88, 333-344.
- Bender, S.F., Conen, F., van der Heijden, M.G.A., 2015. Mycorrhizal effects on nutrient cycling, nutrient leaching and N₂O production in experimental grassland. *Soil Biology and Biochemistry* 80, 283-292.
- Bever, J.D., 2015. Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. *New Phytologist* 205, 1503-1514.
- Borer, E.T., Seabloom, E.W., Gruner, D.S., et al., 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508, 517-520.
- Camenzind, T., Hempel, S., Homeier, J., Horn, S., Velescu, A., Wilcke, W., Rillig, M.C., 2014. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Global Change Biology* 20, 3646-3659.
- Caruso, T., Hempel, S., Powell, J.R., Barto, E.K., Rillig, M.C., 2012. Compositional divergence and convergence in arbuscular mycorrhizal fungal communities.

Ecology 93, 1115-1124.

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A., Kembel, S.W., 2009. The merging of community ecology and phylogenetic biology. *Ecology Letters* 12, 693-715.

Chen, Y.-L., Zhang, X., Ye, J.-S., Han, H.-Y., Wan, S.-Q., Chen, B.-D., 2014. Six-year fertilization modifies the biodiversity of arbuscular mycorrhizal fungi in a temperate steppe in Inner Mongolia. *Soil Biology and Biochemistry* 69, 371-381.

Cheng, L., Booker, F.L., Tu, C., Burkey, K.O., Zhou, L., Shew, H.D., Rufty, T.W., Hu, S., 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO₂. *Science* 337, 1084-1087.

de Freitas, C., de Sales Dambros, C., Eiserhardt, W., Costa, F., Svenning, J.-C., Balslev, H., 2014. Phylogenetic structure of a palm community in the central Amazon: changes along a hydro-edaphic gradient. *Plant Ecology* 215, 1173-1185.

Dinnage, R., 2009. Disturbance alters the phylogenetic composition and structure of plant communities in an old field system. *PLoS ONE* 4, e7071.

Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H., Helgason, T., 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist* 190, 794-804.

Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., Fitter, A.H., 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME Journal* 4, 337-345.

Egerton-Warburton, L.M., Johnson, N.C., Allen, E.B., 2007. Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. *Ecological Monographs* 77, 527-544.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME Journal* 6, 1007-1017.

Fitter, A.H., Gilligan, C.A., Hollingworth, K., et al., 2005. Biodiversity and ecosystem function in soil. *Functional Ecology* 19, 369-377.

Gosling, P., Mead, A., Proctor, M., Hammond, J.P., Bending, G.D., 2013. Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. *New Phytologist* 198, 546-556.

Grilli, G., Urcelay, C., Galetto, L., Davison, J., Vasar, M., Saks, Ü., Jairus, T., Öpik, M., 2014. The composition of arbuscular mycorrhizal fungal communities in the

roots of a ruderal forb is not related to the forest fragmentation process.
 Environmental Microbiology, doi:10.1111/1462-2920.12623.

Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant
 biodiversity loss after eutrophication. *Science* 324, 636-638.

Hempel, S., Renker, C., Buscot, F., 2007. Differences in the species composition of
 arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland
 ecosystem. *Environmental Microbiology* 9, 1930-1938.

Hepper, C.M., Azcon-Aguilar, C., Rosendahl, S., Sen, R., 1988. Competition between
 three species of *Glomus* used as spatially separated introduced and indigenous
 mycorrhizal inocula for leek (*Allium porrum* L.). *New Phytologist* 110, 207-215.

Hodge, A., Helgason, T., Fitter, A.H., 2010. Nutritional ecology of arbuscular
 mycorrhizal fungi. *Fungal Ecology* 3, 267-273.

Horn, S., Caruso, T., Verbruggen, E., Rillig, M.C., Hempel, S., 2014. Arbuscular
 mycorrhizal fungal communities are phylogenetically clustered at small scales.
ISME Journal 8, 2231-2242.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general
 parametric models. *Biometrical Journal* 50, 346-363.

Johnson, N.C., 1993. Can fertilization of soil select less mutualistic mycorrhizae?
Ecological Applications 3, 749-757.

Johnson, N.C., 2010. Resource stoichiometry elucidates the structure and function of
 arbuscular mycorrhizas across scales. *New Phytologist* 185, 631-647.

Johnson, N.C., Wilson, G.W.T., Wilson, J.A., Miller, R.M., Bowker, M.A., 2015.
 Mycorrhizal phenotypes and the Law of the Minimum. *New Phytologist* 205,
 1473-1484.

Kembel, S.W., 2009. Disentangling niche and neutral influences on community
 assembly: assessing the performance of community phylogenetic structure tests.
Ecology Letters 12, 949-960.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly,
 D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating
 phylogenies and ecology. *Bioinformatics* 26, 1463-1464.

Kiers, E.T., Duhamel, M., Beesetty, Y., et al., 2011. Reciprocal rewards stabilize
 cooperation in the mycorrhizal symbiosis. *Science* 333, 880-882.

Klironomos, J., Zobel, M., Tibbett, M., et al., 2011. Forces that structure plant
 communities: quantifying the importance of the mycorrhizal symbiosis. *New*

Phytologist 189, 366-370.

Klironomos, J.N., Ursic, M., Rillig, M., Allen, M.F., 1998. Interspecific differences in the response of arbuscular mycorrhizal fungi to *Artemisia tridentata* grown under elevated atmospheric CO₂. New Phytologist 138, 599-605.

Lee, J., Lee, S., Young, J.P., 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 65, 339-349.

Leifheit, E.F., Verbruggen, E., Rillig, M.C., 2015. Arbuscular mycorrhizal fungi reduce decomposition of woody plant litter while increasing soil aggregation. Soil Biology and Biochemistry 81, 323-328.

Lewandowski, T.J., Dunfield, K.E., Antunes, P.M., 2013. Isolate identity determines plant tolerance to pathogen attack in assembled mycorrhizal communities. PLoS ONE 8, e61329.

Lim, S., Berbee, M.L., 2013. Phylogenetic structure of ectomycorrhizal fungal communities of western hemlock changes with forest age and stand type. Mycorrhiza 23, 473-486.

Liu, Y., He, J., Shi, G., An, L., Öpik, M., Feng, H., 2011. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. FEMS Microbiology Ecology 78, 355-365.

Liu, Y., Mao, L., Li, J., Shi, G., Jiang, S., Ma, X., An, L., Du, G., Feng, H., 2015. Resource availability differentially drives community assemblages of plants and their root-associated arbuscular mycorrhizal fungi. Plant and Soil 386, 341-355.

Liu, Y., Shi, G., Mao, L., Cheng, G., Jiang, S., Ma, X., An, L., Du, G., Johnson, N.C., Feng, H., 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytologist 194, 523-535.

Maherali, H., Klironomos, J.N., 2012. Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. PLoS ONE 7, e36695.

Mao, L., Liu, Y., Shi, G., Jiang, S., Cheng, G., Li, X., An, L., Feng, H., 2014. Wheat cultivars form distinctive communities of root-associated arbuscular mycorrhiza in a conventional agroecosystem. Plant and Soil 374, 949-961.

Mayfield, M.M., Levine, J.M., 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecology Letters 13, 1085-1093.

Oksanen, J., Blanchet, F.G., Kindt, R., et al., 2015. vegan: community ecology

- package. R package version 2.2-1. <http://CRAN.R-project.org/package=vegan>.
- Olsson, P.A., Rahm, J., Aliasghar zad, N., 2010. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiology Ecology* 72, 125-131.
- Öpik, M., Davison, J., Moora, M., Zobel, M., 2014. DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. *Botany* 92, 135-147.
- Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, Ü., Zobel, M., 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* 188, 223-241.
- Öpik, M., Zobel, M., Cantero, J.J., et al., 2013. Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza* 23, 411-430.
- Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289-290.
- Parmentier, I., Réjou-Méchain, M., Chave, J., et al., 2014. Prevalence of phylogenetic clustering at multiple scales in an African rainforest tree community. *Journal of Ecology* 102, 1008-1016.
- Parniske, M., 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* 6, 763-775.
- Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* 11, 789-799.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team., 2015. nlme: linear and nonlinear mixed effects models. R package version 3.1-120. <http://CRAN.R-project.org/package=nlme>.
- Powell, J.R., Parrent, J.L., Hart, M.M., Klironomos, J.N., Rillig, M.C., Maherali, H., 2009. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B-Biological Sciences* 276, 4237-4245.
- R Core Team., 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Rajaniemi, T.K., 2002. Why does fertilization reduce plant species diversity? Testing

- three competition-based hypotheses. *Journal of Ecology* 90, 316-324.
- Rincón, A., Santamaría, B.P., Ocaña, L., Verdú, M., 2014. Structure and phylogenetic diversity of post-fire ectomycorrhizal communities of maritime pine. *Mycorrhiza* 24, 131-141.
- Saks, Ü., Davison, J., Öpik, M., Vasar, M., Moora, M., Zobel, M., 2014. Root-colonizing and soil-borne communities of arbuscular mycorrhizal fungi in a temperate forest understorey. *Botany* 92, 277-285.
- Schechter, S.P., Bruns, T.D., 2008. Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. *Molecular Ecology* 17, 3198-3210.
- Schwarzott, D., Schüßler, A., 2001. A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza* 10, 203-207.
- Selosse, M.A., Rousset, F., 2011. The plant-fungal marketplace. *Science* 333, 828-829.
- Shi, G., Liu, Y., Mao, L., Jiang, S., Zhang, Q., Cheng, G., An, L., Du, G., Feng, H., 2014. Relative importance of deterministic and stochastic processes in driving arbuscular mycorrhizal fungal assemblage during the spreading of a toxic plant. *PLoS ONE* 9, e95672.
- Simon, L., Lalonde, M., Bruns, T.D., 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Microbiology* 58, 291-295.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731-2739.
- Vályi, K., Rillig, M.C., Hempel, S., 2015. Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. *New Phytologist* 205, 1577-1586.
- van der Heijden, M.G., Streitwolf-Engel, R., Riedl, R., Siegrist, S., Neudecker, A., Ineichen, K., Boller, T., Wiemken, A., Sanders, I.R., 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist* 172, 739-752.
- van Diepen, L.T., Lilleskov, E.A., Pregitzer, K.S., 2011. Simulated nitrogen deposition affects community structure of arbuscular mycorrhizal fungi in

northern hardwood forests. *Molecular Ecology* 20, 799-811.

Vandenkoornhuyse, P., Ridgway, K.P., Watson, I.J., Fitter, A.H., Young, J.P.W., 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology* 12, 3085-3095.

Veresoglou, S.D., Rillig, M., 2014. Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. *Plant and Soil* 377, 395-406.

Veresoglou, S.D., Shaw, L.J., Hooker, J.E., Sen, R., 2012. Arbuscular mycorrhizal modulation of diazotrophic and denitrifying microbial communities in the (mycorrhizosphere of *Plantago lanceolata*. *Soil Biology and Biochemistry* 53, 78-81.

Vestergård, M., Henry, F., Rangel-Castro, J.I., Michelsen, A., Prosser, J.I., Christensen, S., 2008. Rhizosphere bacterial community composition responds to arbuscular mycorrhiza, but not to reductions in microbial activity induced by foliar cutting. *FEMS Microbiology Ecology* 64, 78-89.

Webb, C.O., 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. *American Naturalist* 156, 145-155.

Webb, C.O., Ackerly, D.D., McPeck, M.A., Donoghue, M.J., 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33, 475-505.

Xiang, D., Verbruggen, E., Hu, Y., Veresoglou, S.D., Rillig, M.C., Zhou, W., Xu, T., Li, H., Hao, Z., Chen, Y., Chen, B., 2014. Land use influences arbuscular mycorrhizal fungal communities in the farming–pastoral ecotone of northern China. *New Phytologist* 204, 968-978.

Yang, G., Liu, N., Lu, W., Wang, S., Kan, H., Zhang, Y., Xu, L., Chen, Y., 2014. The interaction between arbuscular mycorrhizal fungi and soil phosphorus availability influences plant community productivity and ecosystem stability. *Journal of Ecology* 102, 1072-1082.

Yang, W., Zheng, Y., Gao, C., He, X., Ding, Q., Kim, Y., Rui, Y., Wang, S., Guo, L.D., 2013. The arbuscular mycorrhizal fungal community response to warming and grazing differs between soil and roots on the Qinghai-Tibetan plateau. *PLoS ONE* 8, e76447.

Table 1 Taxonomic description of each arbuscular mycorrhizal fungal virtual taxon (VT) and their relative abundances (% , proportion of clone numbers) in different fertilization treatments and the ANOVA summary of the effects of fertilization on each VT (linear mixed effects model). F0, F30, F60, F90 and F120 represent different levels of fertilizer application. Significant effects are in bold.

	Taxonomic information		Fertilization treatment					ANOVA summary	
	Related genus	Related species	F0	F30	F60	F90	F120	F-value	P-value
VT222	<i>Glomus</i>	<i>G. indicum</i>	7.3	15.5	0.6	–	–	14.74	<0.001
VT125	<i>Glomus</i>	–	1.2	–	–	3.4	–	3.34	0.036
VT130	<i>Glomus</i>	–	0.6	4	–	–	0.6	2.44	0.090
VT159	<i>Glomus</i>	–	–	0.6	3.6	–	–	7.50	0.001
VT129	<i>Glomus</i>	–	0.6	–	–	–	–	1.00	0.436
VT373	<i>Glomus</i>	–	10.4	–	–	–	–	40.40	<0.001
VT371	<i>Glomus</i>	–	27.4	8.6	–	–	–	19.45	<0.001
VT393	<i>Glomus</i>	–	–	4	–	0.6	–	7.68	0.001
VT172	<i>Glomus</i>	–	–	2.3	2.4	1.7	1.3	0.92	0.477
VT137	<i>Glomus</i>	–	6.1	5.7	–	3.4	–	4.46	0.013
VT143	<i>Glomus</i>	–	–	2.3	3.6	–	–	4.31	0.015
VT135	<i>Glomus</i>	–	9.8	–	8.5	–	–	19.30	<0.001
VT166	<i>Glomus</i>	–	15.9	1.1	8.5	–	5.1	13.95	<0.001
VT199	<i>Glomus</i>	<i>G. macrocarpum</i>	2.4	1.1	1.8	6.9	–	2.33	0.100
VT174	<i>Glomus</i>	–	–	0.6	–	–	–	1.00	0.436
VT304	<i>Glomus</i>	–	–	–	–	–	1.9	2.47	0.086
VT151	<i>Glomus</i>	–	–	–	–	–	5.1	2.31	0.103
VT214	new genus 1	–	–	–	6.1	–	–	9.54	<0.001
VT212	new genus 1	–	–	–	–	–	1.9	1.91	0.158
VT177	new genus 1	–	–	–	–	–	1.9	1.00	0.436
VT83	new genus 2	–	–	2.3	–	0.6	0.6	1.10	0.391
VT325	<i>Rhizophagus</i>	–	–	16.1	17	16.6	3.2	5.12	0.008
VT113	<i>Rhizophagus</i>	<i>R. intraradices</i>	0.6	–	0.6	–	–	0.76	0.566
VT295	<i>Rhizophagus</i>	–	0.6	–	–	–	–	1.00	0.436
VT64	<i>Septoglomus</i>	<i>S. constrictum</i>	–	10.9	2.4	28	3.2	7.73	0.001
VT65	<i>Funnelformis</i>	<i>F. caledonium</i>	0.6	0.6	–	–	2.5	2.71	0.068
VT67	<i>Funnelformis</i>	<i>F. mosseae</i>	–	–	–	0.6	4.5	3.61	0.028
VT356	<i>Redeckera</i>	–	–	–	0.6	2.3	–	1.76	0.187
VT380	<i>Diversispora</i>	–	–	0.6	–	–	–	1.00	0.436
VT60	<i>Diversispora</i>	<i>D. celata</i>	–	–	5.5	–	–	3.32	0.037
VT54	<i>Diversispora</i>	<i>D. aurantia</i>	4.3	–	–	–	–	5.81	0.004
VT62	<i>Diversispora</i>	–	–	5.2	17	13.7	39.5	17.64	<0.001
VT56	<i>Claroideoglomus</i>	–	1.2	6.9	21.8	10.9	–	10.24	<0.001
VT57	<i>Claroideoglomus</i>	–	–	11.5	–	10.9	–	5.58	0.005
VT193	<i>Claroideoglomus</i>	<i>C. etunicatum</i>	8.5	–	–	–	28.7	11.97	<0.001
VT245	<i>Archaeospora</i>	<i>Ar. trappei</i>	–	–	–	0.6	–	1.00	0.436
VT283	<i>Ambispora</i>	<i>Am. fennica</i>	2.4	–	–	–	–	4.46	0.013

6 **Table 2** Correlations between virtual taxon (VT) richness, genus richness and the nearest relative index (NRI) of arbuscular mycorrhizal fungal
7 community with two PCA extracted components of soil properties (soil PC1 and PC2), plant species richness and the relative abundance of
8 *Elymus nutans*. Soil PC1 reflects positively the soil N and P fertility gradient; soil PC2 reflects positively the soil moisture and organic C
9 gradient. Values are Pearson's correlation coefficients. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Significant correlations are in bold.

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	Soil PC1	Soil PC2	Plant species richness	Abundance of <i>Elymus nutans</i>
VT richness of all lineages	-0.53**	-0.05	0.38	-0.57**
VT richness of <i>Glomus</i>	-0.79***	-0.24	0.77***	-0.82***
Genus richness	0.44*	0.23	-0.64***	0.52**
NRI	-0.69***	-0.30	0.76***	-0.65***

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Figure captions

Fig. 1 Maximum likelihood phylogenetic tree of representative sequences of each arbuscular mycorrhizal fungal virtual taxon (VT371 etc.) obtained in this study and referenced sequences from the GenBank database. Arbuscular mycorrhizal fungal VTs were delimited according to the sequence similarity ($\geq 97\%$), query coverage ($\geq 97\%$) and BLAST bit score after BLAST against the online MaarjAM database. The nomenclatures of arbuscular mycorrhizal fungal genera and families are according to Schüßler and Walker, 2010 (<http://schuessler.userweb.mwn.de/amphylo/>).

Fig. 2 Linear regressions of the relative abundance of *Glomus* and *Diversispora* versus the two PCA extracted components of soil properties (PC1 and PC2), relative abundance of *Elymus nutans* and plant species richness.

Fig. 3 The virtual taxon (VT) richness, genus richness and the nearest relative index (NRI) of arbuscular mycorrhizal fungal community varied across a fertilization gradient. Bars represent means $\pm$ SEs ($n = 5$). F0, F30, F60, F90 and F120 represent different levels of fertilizer application. For (a) and (b), the significant differences between columns are indicated with dissimilar letters using Tukey's HSD test ($P \leq 0.05$) after fitting linear mixed effects models. For (c), asterisk indicates that the NRI is significantly different from zero after *T*-test (ns, non-significant; * $P \leq 0.05$; ** $P \leq 0.01$).

Fig. 4 Non-metric multidimensional scaling (NMDS) patterns of arbuscular mycorrhizal fungal community dissimilarities among fertilization treatments using the data of virtual taxon composition (a) and phylogenetic composition (betaMPD; b). Ellipses with different colors indicate 95% confidence ellipses for each treatment. Correlations between each community ordination with the two PCA extracted components of soil properties (PC1 and PC2), relative abundance of *Elymus nutans* and plant species richness were jointed and the significant vectors ($P < 0.05$) were shown. F0, F30, F60, F90 and F120 represent different levels of fertilizer application.

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Fig. 3

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