Nitrogen deposition and precipitation induced phylogenetic clustering of arbuscular mycorrhizal fungal communities

Yong-Liang Chena, b, *, Zhu-Wen Xuc, Tian-Le Xub, d, Stavros D. Veresogloue, f, Gao-Wen Yangg, Bao-Dong Chenb, d, **

State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
University of Chinese Academy of Sciences, Beijing 100049, China
Freie Universität Berlin-Institut für Biologie, Dahlem Center of Plant Sciences, Plant Ecology, Berlin, Germany
Berlin-Brandenburg Institute of Advanced Biodiversity Research, Berlin, Germany
College of Agro-grassland Science, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Article history:
Received 1 March 2017
Received in revised form
26 July 2017
Accepted 23 August 2017
Available online 4 September 2017

Keywords:
Glomeromycota
Global climate change
Nitrogen
Precipitation
Phylogenetic clustering

ABSTRACT

Despite the inarguable importance of arbuscular mycorrhizal fungi (AMF) in terrestrial ecosystems, we know little about how AMF communities shift in response to climate changes. In this study, we investigated the impacts of seven years of precipitation increment and nitrogen (N) addition on the taxonomic and phylogenetic diversity of AMF communities in a temperate steppe of northern China. Phylogenetic patterns were also used to elucidate the ecological processes structuring AMF communities. By 454-pyrosequencing, we detected a total of 71 AMF operational taxonomic units (OTUs), consisting mainly of *Glomeraceae*. In general, N addition reduced but precipitation increment increased AMF abundance including root colonization and fungal biomass. Nitrogen addition also decreased AMF alpha-diversity, including OTU richness, Chao 1 and Faith’s phylogenetic diversity. Moreover, permutational multivariate analysis of variance showed that AMF community composition shifted in response to both N addition and precipitation increment. AMF communities were phylogenetically clustered across all experimental treatments, suggesting that environmental filtering was the primary driver of AMF community assembly. Taken together, these findings supported that both N and precipitation shaped the AMF communities, but not altered the ecological processes responsible for the assembly of AMF communities in the temperate steppe.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the majority of land plants, and provide multiple benefits to their plant hosts, including increased nutrient uptake, improved tolerance to abiotic stress and protection from pathogens (Smith and Read, 2008). In return, AMF receive photosynthates to meet their demands on carbon resources. The outcome of the symbiosis for the plant host may vary depending on the cost-benefit balance, and can range from mutualism to commensalism or parasitism (Johnson et al., 2015). AMF communities play several important roles in ecosystem functioning through regulating biogeochemical nutrient cycling (Rillig, 2004; Veresoglou et al., 2012), carbon (C) storage (Cheng et al., 2012), soil structure (Rillig and Mummey, 2006), and plant productivity (van der Heijden et al., 1998, 2008).

Obviously, our knowledge of the assembly of AMF community could provide valuable clues for understanding ecosystem functioning and dynamics associated with changing environments.

Studies on the community assembly of AMF range considerably in terms of spatial resolution, which could be at local (Dumbrell
et al., 2010; Caruso et al., 2012; Horn et al., 2014), regional (Hazard et al., 2013) and continental or global (Kivlin et al., 2011; Opik et al., 2013; Davison et al., 2015, 2016; Bouffaud et al., 2016) scales. Through these studies, it is apparent that the assemblages of AMF communities in natural ecosystems are shaped by multiple processes such as host specificity, environmental filtering, dispersal and stochasticity, while the relative importance of each driver may vary with spatial scales (Vályi et al., 2016). Environmental filters such as soil nutrient availability, soil pH, soil moisture and climate gradient appear to be important predictor for AMF community composition at large spatial scales (Kivlin et al., 2011; Moora et al., 2014; Davison et al., 2015). Besides environmental gradients, plant host is another important variable in driving AMF community assembly. Different grass species or even plant individuals of the same species could harbor different AMF communities (Vandenkornhuyse et al., 2003; Gosling et al., 2013). Finally, neutral processes such as dispersal limitation also contributed to AMF communities (Kivlin et al., 2011; Davison et al., 2015, 2016). By studying patterns across various environmental gradients, we could have a better understanding about the determinants of AMF community assembly over broad scales. However, up to date little is known about the processes that drive the AMF communities under global climate changes.

Ecological impacts of climate changes on the structure and functioning of an ecosystem can be systematically investigated under experimental manipulations, which could allow a better mechanistic resolution than observational approaches do. Many studies have examined the responses of AMF community composition to environmental manipulations such as nitrogen (N) enrichment (Porras-Alfaro et al., 2007; Chen et al., 2014; Zheng et al., 2014; Kim et al., 2015) and water additions (Gao et al., 2015; Li et al., 2015). It was found that N and water addition could modulate AMF community possibly through influences on soil N availability and soil pH (van Aarle et al., 2002; Liu et al., 2012). However, most of these studies relied primarily on taxon-based approaches, while AMF phylogeny may provide additional information about trait-based processes driving the community assembly (Webb et al., 2002), as the functional traits of AMF community are generally conserved (Powell et al., 2009). Environmental filtering might lead to communities in which species are more closely related than expected by chance (phylogenetically clustering), while competitive exclusion might lead to species are less closely related (phylogenetically over-dispersion) (Webb et al., 2002). Some recent studies addressed how experimental drivers mimicking climate change influence the phylogenetic structure of the AMF community (Liu et al., 2015a, b; Mueller and Bohannan, 2015) with inconclusive results. For instance, CO2 enrichment resulted in phylogenetic clustering of AMF communities while N addition induced no obvious shift in AMF community assembly (Mueller and Bohannan, 2015). By contrast, AMF communities were phylogenetically clustered in unfertilized soil, random under low fertilization treatment and over-dispersed upon high fertilization treatment, suggesting that nutrient availability may change the dominant ecological processes associated with the assembly of AMF communities (Liu et al., 2015a). Obviously, there is still an urgent need for more systematic studies to reveal the potential impacts of climate changes on AMF communities and underlying mechanisms.

The temperate grassland in northern China represents a typical arid and semi-arid region, where N and water are two key factors constraining plant productivity and associated ecosystem functioning (Yang et al., 2011). Moreover, this region is predicted to suffer from a projected higher N deposition and frequency of extreme climatic changes such as increased precipitation (Ni and Zhang, 2000). In order to examine the potential influences of climate changes on AMF, here we examined the effect of long-term artificial precipitation increment and N addition on AMF diversity, community composition and phylogenetic structure in this temperate grassland. We tested two hypotheses: 1) Nitrogen addition and precipitation increment could alter composition and phylogenetic structure of AMF communities; and 2) Nitrogen addition and precipitation increment can induce shifts in the ecological processes shaping the AMF communities.

2. Materials and methods

2.1. Study site and sampling

The experimental site is located in a semiarid steppe in Duolun County, Inner Mongolia, China (42°02′ N, 116°17′ E). Mean annual precipitation (MAP) is 385.5 mm and mean annual temperature (MAT) is 2.1 °C. The soil is classified as Haplic Calcisols according to the Food and Agricultural Organization (FAO) of the United Nations classification. The vegetation is typical steppe, and is located in an open high plain with an altitude reaching approximately 1324 m. The experiment was initiated in 2005 with a split-plot experimental design, which involves two levels of precipitation (ambient and increased) applied at the plot scale and four N levels applied to subplots. This experimental design was replicated in seven blocks, and each subplot was 8 m × 8 m with a 1 m wide buffer zone. Thus, there were eight treatments as follows: N0 (control), N5 (5 g N m⁻² yr⁻¹), N10 (10 g N m⁻² yr⁻¹), N15 (15 g N m⁻² yr⁻¹), PN0 (precipitation increment only), PNS (precipitation increment and 5 g N m⁻² yr⁻¹), PN10 (precipitation increment and 10 g N m⁻² yr⁻¹), and PN15 (precipitation increment and 15 g N m⁻² yr⁻¹). Nitrogen in form of urea was applied twice a year with half in early May and the other half in late June. The precipitation increment plots received 15 mm of simulated precipitation weekly via sprinkler irrigation from June to August. A total of 180 mm (15 mm per week for 12 weeks), equal to 50% of MAP in the study area, was uniformly added to plots during the growth season from 2005 to 2012.

We used a subset of plots (four randomly selected replicates) from each treatment to assess AMF diversity. On August 22, 2012, five soil cores (15 cm deep, 3.5 cm diameter) were randomly collected from each plot and combined into one composite sample, resulting in a total of 32 samples. Composite soil samples were passed through a 2.0 mm sieve to remove roots and debris. Subsamples were stored at 4 °C for soil chemical analysis, and –80 °C for fatty acid and DNA extraction. At the same time, vegetation coverage was visually estimated for all vascular plant species in each subplot. We calculated Shannon-Wiener index for plant alpha diversity. In each of the sampling plots, plant species richness (SR) was recorded at 6 locations using a 1 m × 1 m quadrat. Above-ground biomass (AGB) were then cut at the ground level, dried at 75 °C for 48 h after being transported to the laboratory and weighed.

2.2. Soil chemophysical properties

Soil samples were dried for 48 h at 105 °C to assess soil moisture. Soil pH was determined with a soil to water ratio of 1: 2.5. Soil nitrate and ammonium were extracted with 2 M KCl (soil to water ratio of 1:5), and then measured with a continuous flow analyzer (SAN++, Skalar, Breda, Holland). Soil available phosphorus (AP) was measured using the ammonium molybdate method (Bao, 2005) after extraction with 0.5 M NaHCO₃. Soil available potassium (AK) was extracted by NH₄OAc and analyzed by flame atomic
The quality shorter than 200 bp in length were excluded from further analysis. Sequences with ambiguous nucleotides, a quality score below 30, and assessed for the presence or absence of AMF structures (arbuscules, vesicles and thick hyphae) using a stereomicroscope. Intensity of the total mycorrhizal colonization (M%) and arbuscule abundance in the root system (a%) were evaluated according to Trouvelot et al. (1986).

The phospholipid fatty acid (PLFA) 16:1ω5c is used to measure the live AMF biomass (van Diepen et al., 2007). Phospholipids were extracted from 8 g of soil using a previously described method (Bossio and Scow, 1998; Chen et al., 2016a,b). The concentration of each PLFA was quantified against a 19:0 (Methyl Nonadecanoate, C_{20}H_{40}O_{2}) internal standard.

2.4. DNA extraction, PCR and 454 pyrosequencing

DNA was extracted from 0.5 g frozen soil by a direct bead-beating extraction method using the Fast DNA® SPIN Kit for Soil (Q BIogene Inc., Carlsbad, CA, USA). Genomic DNA for 454 pyrosequencing was amplified using a two-step PCR procedure. The primer pair AML1 and AML2 (Lee et al., 2008) were used in the first PCR to amplify the small submit ribosomal DNA (SSU rDNA) region. The first amplification product was diluted ten-fold with distilled water and used as templates for the second PCR using the primers NS31 (Simon et al., 1992) and AM1 (Helgason et al., 1998). The latter primer pair was augmented with the 454 pyrosequencing adapters and 7-bp-long barcodes for multiplexing. The PCR was performed in a 25 μl reaction mixture containing 1 μl diluted DNA template (1/10), 2.5 μl 10 × PCR buffer, 2 μl 2.5 mM dNTPs, 0.25 μl Ex Taq polymerase (5 U ml⁻¹, Takara Biotechnology, Dalian, China), and 1 μl 10 μM of each primer using a thermal cycler (Mastercycler Ep Gradient, Eppendorf, Hamburg, Germany). The PCR protocol consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of denaturation for 30 s at 94 °C, annealing at 58 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The second PCR amplification was carried out under the same condition. Amplification products were examined on 1.5% (w/v) agarose gels with 1.0 × TAE buffer (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA, and pH 8.0), visualized by staining with Goldview (Applied Biosystems, USA), and were purified using the Wizard® SV Gel and PCR Clean-Up Kit (Promega, San Luis Obispo, CA, USA). The purified PCR products were mixed at equimolar concentrations and sequenced on a Roche 454 FLX system.

2.5. Bioinformatics

We used the Quantitative Insights Into Microbial Ecology (QIIME) pipeline to process the 18S molecular data. In brief, sequences with ambiguous nucleotides, a quality score below 30, shorter than 200 bp in length were excluded from further analysis. The quality filtering and chimera detection was performed using UCHIME (Edgar, 2010). The remaining sequences were clustered into different operational taxonomic units (OTUs) at a 97% identity threshold using UCLUST (Edgar, 2010). Taxonomic assignment was performed by blasting the representative sequences against the Silva 124 and MaarJAM database (Opik et al., 2010). The number of sequences per sample was normalized to the smallest sample size (887 sequences) to correct for the sampling effort. Then we calculated the AMF alpha diversity (observed OTU richness, Shannon-Wiener index, Chao 1 and Faith’s phylogenetic diversity). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database under the accession number SRR5816453.

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) was performed to examine the significance of N, precipitation treatments and their interactions on AMF alpha diversity. Significant differences between treatments were confirmed using Tukey’s HSD test at P < 0.05. The relationships between AMF alpha diversity and environmental factors were assessed by Pearson correlation. These statistical analyses were performed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA).

To determine the dissimilarity between plant communities, Bray-Curtis distance metrics were calculated. To assess how N and precipitation influence AMF communities, a permutational multivariate analysis of variance (PERMANOVA) was carried out using the adonis function in the package “vegan” in R (R Core Team, 2015). The taxon community table for AMF was converted to a distance table via calculation of Bray-Curtis distances. Phylogenetic dissimilarity between samples was assessed using weighted UniFrac distance. The relationships between AMF communities and soil/plant variables and plant communities were assessed using Mantel test with the “ecodist” and “vegan” packages (Goslee and Urban, 2007). Variation partition analysis was carried out to separate the effects of plant species richness, soil pH and N (available nitrogen, ammonium and nitrite) on AMF communities using the “packfor” and “vegan” packages in R.

Structural equation models (SEM) were further constructed with the Amos 21.0 software (SPSS Inc., IBM Co., Armonk, NY, USA) to examine direct and indirect paths through which N, precipitation, soil pH, available N and plant species richness influence AMF richness and community composition. AMF community composition was characterized by the first component of PCoA. Model adequacy was determined through χ² tests, goodness-of-fit index (GFI), Akaike information criteria (AIC), and root mean square error of approximation (RMSEA). Low χ² values (P > 0.05), high GFI (>0.90), low AIC, and low RMSEA (<0.05) indicated sound fit of the model (Hooper et al., 2008). Standardized total effects of N, precipitation, soil pH, available N and plant species richness on AMF richness and community composition were calculated for SEM.

To characterize the phylogenetic structure of AMF communities within each sample, we calculated the mean nearest taxon distance (MNTD) and the mean pairwise phylogenetic distance (MPD) (Webb et al., 2002) using the ‘picante’ package (Kembel et al., 2010). MNTD stands for the mean phylogenetic distance to the nearest relative for all species occurring together in a sample, while MPD stands for the mean phylogenetic distance separating all pairwise combinations of species occurring within a sample (Kembel and Hubbell, 2006). We calculated the standardized effect size (SES) of observed MNTD and MPD distances using the ses.mntd and ses.mpd functions respectively (Kembel et al., 2010). NTI and NRI stands for negative output of ‘ses.mntd’ and ‘ses.mpd’, respectively. The values across all samples that are significantly higher than zero indicated phylogenetic clustering (species more closely
related than expected by chance), equal to zero indicate random, and lower than zero indicate phylogenetic over-dispersion (Kembel, 2009). Significant deviation of observed patterns from the null expectation of zero was determined using a two-tailed t-test at $P < 0.05$.

### 3. Results

#### 3.1. Plant and soil properties

Soil pH significantly declined as a result of N addition and precipitation increment ($P < 0.001$; Table 1). Nitrogen additions

| Treatment | Soil moisture (%) | pH | NH$_4^+$-N (mg kg$^{-1}$) | NO$_3^-$-N (mg kg$^{-1}$) | Available nitrogen (mg kg$^{-1}$) | Available phosphorus (mg kg$^{-1}$) | Available potassium (mg kg$^{-1}$) | Above-ground plant biomass (g m$^{-2}$) | Plant species richness |
|-----------|------------------|----|--------------------------|--------------------------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|---------------------|
| N0        | 4.16             | 7.35 | 15.5 | 2.91 | 18.4 | 5.78 | 27.1 | 198 | 16.3 |
| N5        | 4.89             | 7.03 | 20.0 | 5.27 | 25.3 | 5.22 | 28.6 | 313 | 14.5 |
| N10       | 4.43             | 6.47 | 18.6 | 3.86 | 22.5 | 6.25 | 27.0 | 315 | 12.8 |
| N15       | 4.13             | 6.31 | 22.2 | 8.98 | 31.2 | 5.43 | 34.1 | 364 | 9.3  |
| PN0       | 4.51             | 7.02 | 17.8 | 3.25 | 21.1 | 6.69 | 30.5 | 210 | 18.5 |
| PN5       | 4.79             | 6.53 | 17.5 | 5.62 | 23.1 | 4.29 | 38.8 | 242 | 16.5 |
| PN10      | 4.70             | 5.87 | 20.1 | 3.48 | 23.6 | 5.83 | 28.9 | 318 | 15.0 |
| PN15      | 6.27             | 5.64 | 21.5 | 4.47 | 26.0 | 5.62 | 31.2 | 285 | 11.3 |

Significance of N, P, interaction of nitrogen addition with precipitation increment: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

N0, N5, N10 and N15, nitrogen addition with 0, 5, 10 and 15 g N m$^{-2}$ yr$^{-1}$ respectively; PN0, PN5, PN10 and PN15, precipitation increment and nitrogen addition with 0, 5, 10 and 15 g N m$^{-2}$ yr$^{-1}$ respectively. Significant effects ($P < 0.05$) are highlighted in bold as determined by two-way ANOVA. N, the effect of nitrogen addition; P, the effect of precipitation increment; N × P, interaction of nitrogen addition with precipitation increment.
increased soil NH$_4^+$-N and available nitrogen concentrations, but reduced plant species richness ($P < 0.05$). By contrast, precipitation increment did not have obvious effect on NH$_4^+$-N, available nitrogen and plant species richness. Neither N addition nor precipitation increment showed significant effects on soil moisture, NO$_3^-$-N, available phosphorus (AP), available potassium (AK) and above-ground biomass (Table 1). Non interactive effects of N addition and precipitation increment on soil proprieties, aboveground biomass and plant species richness were detected (Table 1).

3.2. AMF abundance and diversity

The 454 pyrosequencing yielded a total of 124, 519 AMF sequences after extensive quality filtering (Table S1). The rarefaction analysis showed that the curves reached stable plateaus, indicating the number of sequences analyzed was sufficient to detect the AMF diversity (Fig. S1). According to the results of two-way ANOVA, N addition significantly decreased AMF richness, Chao 1, Faith’s phylogenetic diversity (PD), and AMF biomass indicated by 16:1+5 ($P < 0.05$; Fig. 1). By contrast, precipitation increment significantly increased root colonization and AMF biomass ($P < 0.05$; Fig. 1). Both N addition and precipitation increment have no significant effect on Shannon-Wiener diversity index (Fig. 1). There was also no significant interactive effect of N and precipitation on root colonization, AMF biomass, OTU richness, Shannon-Wiener diversity index, Chao 1, and Faith’s PD (Fig. 1).

3.3. AMF community composition

The 71 AMF OTUs belonged to four families: Glomeraceae (66), Claroideoglomeraceae (2), Paraglomeraceae (1) and Gigasporaceae (2) (Table S2; Fig. 2a). There were significant differences in taxonomic and phylogenetic composition of AMF communities (Fig. 2b and c). PERMANOVA outputs showed that AMF taxonomic communities were significantly altered by N ($F = 5.2211, P < 0.001$) and precipitation ($F = 2.2431, P < 0.05$; Table 3). AMF phylogenetic communities were only significantly affected by N fertilization ($F = 3.3553, P < 0.05$), but not by precipitation ($F = 1.7632, P = 0.124$; Table 3). Mantel test showed that AMF communities were significantly correlated with SR, available nitrogen, NH$_4^+$-N and NO$_3^-$-N ($P < 0.05$; Table 2). Variation partitioning showed that SR, pH, available nitrogen, NH$_4^+$-N and NO$_3^-$-N explained 17.7% and 13.6% of the variation in taxonomic and phylogenetic composition of AMF communities, respectively (Fig. 3).

There were significant differences in the relative abundance of specific taxa in different treatments (Fig. 4). The relative abundance of OTU 6, OTU 9, OTU 17, and OTU 32 decreased with N gradients (Fig. 4c, d, f, i), while the relative abundance of OTU 10, OTU 19, OTU 28 increased along the N gradient (Fig. 4e, g, h). Precipitation significantly increased the relative abundance of OTU 4, and OTU 6 (Fig. 4a, c), but significantly decreased the relative abundance of OTU 5, OTU 9 and OTU 10 (Fig. 4b, d, e). The interactive effect of N and precipitation was only observed on the relative abundance of OTU 10 and OTU 40 (Fig. 4e, j).

SEM was used to evaluate the direct and indirect effects of environmental variables on AMF richness and communities (Fig. 5). The fitted models explained 37%, 74% and 44% of the variance in AMF richness, taxon and phylogenetic communities, respectively (Fig. 5a, b, c). Soil pH and plant SR showed significant correlations with AMF richness, whereas soil pH, SR and available nitrogen showed significant correlations with AMF communities. Standardized total effects derived from the SEM revealed that fungal richness was mainly driven by soil pH, followed by SR (Fig. 5d). By contrast, AMF taxon communities were mainly driven by nitrogen gradient, and then by soil pH, while phylogenetic communities mainly by soil pH (Fig. 5e and f).

3.4. Phylogenetic structure

The null model analyses showed that NRI and NTI were all significantly higher than zero, indicating that AMF species were more closely related than expected by chance across all treatments (Fig. 5a and b). The highest NTI and NRI occurred in treatment with precipitation increment only (PN0), and the lowest NRI and NTI value occurred in control (N0) and highest nitrogen with precipitation increment (PN15), respectively. In general, NTI increased significantly with NO$_3$N content (Table S4).
4. Discussion

4.1. The effect of nitrogen and precipitation on AMF abundance

The experimental results indicated that N addition significantly reduced AMF biomass as assessed by PLFA 16:1o5c, and also decreased root colonization under ambient precipitation. This is consistent with previous studies showing that N fertilization negatively impacts AMF abundance (hyphal length density, root colonization) (van Diepen et al., 2007, 2010; Liu et al., 2012; Camenzind et al., 2014; Kim et al., 2015) across different ecosystems. According to the functional equilibrium model, the N enrichment in P-rich soils like our study site (Chen et al., 2014) could reduce the plant C allocated to AMF biomass (Johnson et al., 2003, 2015), considering that N uptake represents a major benefit provided by AMF symbiosis. However, the neutral effect of N on root colonization has also been reported in a temperate steppe (Chen et al., 2014; Li et al., 2015). These results suggested that the effects of N fertilization on AMF colonization, intraradical and extraradical fungal structures are more variable, and are possibly caused by site-level differences in C allocation to AMF, which are dependent on the soil nutrient status (e.g. N:P ratio) and the associated mycorrhizal phenotypes (Johnson et al., 2003, 2015).

In contrast to the negative effects of N, precipitation increment increased the AMF abundance as assessed by both root colonization and the PLFA marker 16:1o5c. This is also consistent with previous studies showing that increased precipitation has positive effects on AMF extraradical hyphal density and spore density (Gao et al., 2015). A very popular concept in mycorrhizal ecology is the Law of Minimum, which was used for predicting mycorrhizal function in different ecosystems (Johnson et al., 2015). The soil in semiarid grassland of Inner Mongolia was limited by water resources, and thus precipitation increment could alleviate drought stress on AMF and also on host plant (Johnson et al., 2015). Moreover, changes in AMF communities induced by precipitation increment might also affect AMF abundance, because the Glomeromycota exhibit distinct colonization strategies among different families (Hart and Reader, 2002; Powell et al., 2009). For instance, Glomeraceae has been shown to have the ability to rapidly and extensively colonize host roots while Gigasporaceae and Acaulosporaceae tended to be poor colonizers of both soil and roots.

4.2. The effect of nitrogen and precipitation on AMF richness and community compositions

We additionally detected a negative influence of N additions on AMF richness and diversity. This was in agreement with many previous experiments which found negative effect of N addition on AMF richness and diversity in grassland (Liu et al., 2012; Chen et al., 2014), forest (Camenzind et al., 2014) and agricultural ecosystems (Lin et al., 2012). As expected, N addition significantly influenced AMF communities, supporting our first hypothesis. Many previous studies also demonstrated that AMF community composition are affected by N addition in different ecosystems (Porras-Alfaro et al., 2007; van Diepen et al., 2011; Zheng et al., 2014; Tian et al., 2013; Kim et al., 2015). There are three possible mechanisms responsible for the effect of N on the AMF community compositions. Firstly, N additions can increase N availability and reduce soil pH, both of which could impact AMF communities (Egerton-Warburton et al., 2007; Dumbrell et al., 2010; Liu et al., 2012). It is likely that a low soil pH selects for AMF species producing less dense mycelia networks (van Aarle et al., 2002; Dumbrell et al., 2010). High soil N availability may also have direct influences on AMF resulting in lower root colonization and vesicle abundance (van Diepen et al., 2007). Secondly, N addition may intensify the competition among

![Fig. 3. Variation partition of AMF taxonomic composition (a) and phylogenetic composition (b). AN, available nitrogen.](image)
AMF species for plant C if the host plant reduces the amount of C allocated to mycorrhiza under non-N-limiting conditions (Johnson et al., 2015). Competition between AMF species may result in a loss of fungal diversity, and a dominance of the AMF taxa which can compete more effectively under C-limited and N-enriched environments (Johnson, 2010; Johnson et al., 2015; Liu et al., 2015a). For example, N fertilization intensified AMF community convergence due to the increased abundance of rare AMF species (e.g. Diversispora spurca) and the loss of Glomus species (Liu et al., 2012, 2015a). In our study, we also observed that the relative abundance of Glomus species increased (OTU 10, OTU 19 and OTU 28) or decreased (OTU 4, OTU 5, OTU 6, OTU 9, OTU 17 and OTU 32) in response to N additions. Thirdly, N addition may indirectly influence AMF communities via changes in plant community composition (Liu et al., 2012). However, we did not find significant ecological linkages between plant community structure and below-ground AMF communities based on the Mantel test, but significant associations between plant species richness and AMF richness were noted in
this study (Fig. 5a; Table S5). Therefore, we cannot exclude the possibility that plant species may select certain preferential AMF taxon (Vandenkoornhuyse et al., 2003; Helgason et al., 2007; Veresoglou and Rillig, 2014), which would subsequently alter AMF communities.

In support of our first hypothesis, we also observed shifts in AMF community composition induced by precipitation increment. On one hand, precipitation would influence AMF communities by affecting soil pH. Water may wash away H\(^+\) ions from the surface layer to deep soil horizons, and affect surface soil pH. Shifts in soil pH could lead to niche differentiation and structure AMF communities (van Aarle et al., 2002; Dumbrell et al., 2010). On the other hand, precipitation may also affect AMF communities via alterations in plant species richness. Precipitation increment can increase the availability of soil nutrients and water, promote plant growth and alter plant community compositions (Xu et al., 2015), and subsequently shape AMF communities (Vandenkoornhuyse et al., 2003; Helgason et al., 2007; Veresoglou and Rillig, 2014). Anyway, the present study strongly supported that precipitation is a major influencing factor on AMF communities possibly through altering soil pH and plant communities.

4.3. The ecological processes structuring AMF communities

It is interesting to note that AMF communities were phylogenetically clustered across all experimental treatments, suggesting that the AMF taxa were more closely related and thus habitat filtering was the major process structuring AMF community. Our study was consistent with the existing evidence that AMF communities are frequently clustered in natural ecosystems (Horn et al., 2004; Dumbrell et al., 2010). On the other hand, precipitation may also affect AMF communities via alternations in plant species richness. Precipitation increment can increase the availability of soil nutrients and water, promote plant growth and alter plant community compositions (Xu et al., 2015), and subsequently shape AMF communities (Vandenkoornhuyse et al., 2003; Helgason et al., 2007; Veresoglou and Rillig, 2014). Anyway, the present study strongly supported that precipitation is a major influencing factor on AMF communities possibly through altering soil pH and plant communities.

5. Conclusions

The study describes how N addition and precipitation increment impact AMF communities, and the main processes structuring AMF assemblages. In support to our first hypothesis, we found that N addition and precipitation increment significantly altered AMF communities. This indicates that the functioning of arbuscular mycorrhizas in semiarid grasslands might change by future climate changes. Considering atmospheric nitrogen deposition is predicted to increase in this temperate grassland in the coming decades (Liu et al., 2011), the N gradients in this study could allow better understanding of the potential influence of environmental changes on belowground AMF communities. It was also noticeable that AMF communities were phylogenetically clustered in all experimental treatments, indicating that habitat filtering such as lower soil pH, increased N availability or changed plant community composition served as the primary driver of AMF community assembly, which denied our second hypothesis. These findings supported that phylogenetic clustering in unfertilized soil, random under low fertilization level, to over-dispersion upon high fertilization treatments in an alpine meadow ecosystem (Liu et al., 2015a). This is possibly because the N addition levels in this study were generally lower compared with Liu et al. (2015a), which found high N level caused significant loss of Glomus species and emergence of other genera, and ultimately a shift in AMF phylogenetic pattern. Consistent with this assumption, low N addition (7 g N m\(^{-2}\) yr\(^{-1}\)) did not resulted in shifts in phylogenetic structure of AMF in a grassland ecosystem (Mueller and Bohannan, 2015). Moreover, the AMF communities were dominated by Glomeraceae in all experimental treatments in this study, which possibly explained non-shift in AMF phylogenetic structure and implied similar functional traits of these AMF species (Powell et al., 2009).
environmental filtering have selected AMF species with similar functional traits, which allowed them to survive the changed environments.

Acknowledgments

This research was supported by National Key Research and Development Program of China (2016YFC0500701; 2016YFC0500702) and National Natural Science Foundation of China (41371264; 41501265; 31200349).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.08.024.

References

Bao, S.D., 2005. Agricultural Chemical Analysis of Soil. China Agriculture Press, Beijing.
Bosio, D.A., Seow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265–278.
Bouffaud, M.L., Creame, R., Stone, D., Plaisant, P., van Tuinen, D., Lemanceau, P., Wipf, D., Redeker, D., 2016. Indicator species and co-occurrence in communities of arbuscular mycorrhizal fungi at the European scale. Soil Biology & Biochemistry 103, 464–470.
Camenzind, T., Hempel, S., Homeier, J., Horn, S., Vele foc, A., Wilcke, W., Rillig, M., 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., et al., 2012. Compositional divergence and convergence in arbuscular mycorrhizal fungal communities. Ecology 93, 1115–1124.
Chen, Y.L., Chen, L.Y., Peng, Y.F., Ding, J.Z., Li, F., Yang, G.B., Kou, D., Liu, L., Fang, K., Zhang, B.B., Wang, J., Yang, Y.H., 2016a. Linking microbial C: N:P stoichiometry to microbial community and abiotic factors along a 3500-km grassland transect on the Tibetan Plateau. Global Ecol. Biogeogr. 25, 1416–1427.
Chen, Y.L., Ding, J.Z., Peng, Y.F., Li, F., Yang, G.B., Liu, L., Qin, S.Q., Fang, K., Yang, Y.H., 2016b. Patterns and drivers of soil microbial communities in Tibetan alpine and global terrestrial ecosystems. J. Biogeogr. 43, 2027–2039.
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 & Biochemistry 69, 371–381.
Cheng, L., Booker, P.L., Te, C., Burkey, K.O., Zhou, L., Shew, H.D., Ruffy, T.W., Hu, S., 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO2. Science 337, 1084–1087.
Davison, J., Moora, M., Jarius, T., Vasar, M., Opik, M., Zobel, M., 2016. Hierarchical assembly rules in arbuscular mycorrhizal (AM) fungal communities. Soil Biology & Biochemistry 97, 63–70.
Davison, J., Moora, M., Opik, M., Adholeya, A., Ainsaar, L., Bä, A., Burla, S., Diebsdorf, A.G., Hiesa, L., Jarius, T., Johnson, N.C., Kane, A., Koerem, K., Kochar, M., Ndiaye, C., Partel, R., Reier, U., Saks, Ü., Singh, R., Vasar, M., Zobel, M., 2015. Global assessment of arbuscular mycorrhizal fungal diversity reveals very low endemism. Science 349, 970–973.
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. The ISME Journal 4, 337–345.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Egan, C.P., Callaway, R.M., Hart, M.M., Pither, J., Klinoromos, J., 2017. Phylogenetic structure of arbuscular mycorrhizal fungal communities along an elevation gradient. Mycorrhiza 27, 273–282.
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.
Gao, C., Kim, Y.C., Zheng, Y., Yang, W., Chen, L., Ji, N.N., Wan, S.Q., Guo, L.D., 2015. Increased precipitation, rather than warming exerts strong influence on arbuscular mycorrhizal fungal community in a semiarid steppe ecosystem. Botany 94, 459–469.
Goslee, S.C., Urban, D.L., 2007. The ecospat package for dissimilarity-based analysis of ecological data. Journal of Statistical Software 22, 1–15.
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.
Hart, M.M., Reader, J.R., 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153, 335–344.
Hazard, C., Gosling, P., van der Gast, C.J., et al., 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. The ISME Journal 7, 498–508.
Helgason, T., Danell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 1998. Ploughing up the wood-wide web? Nature 394, 431.
Helgason, T., Merryweather, J.W., Young, J.P.W., Fitter, A.H., 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. Journal of Ecology 95, 623–630.
Hopper, D., Coughlan, J., Muller, M., 2008. Structural equation modelling: guidelines for determining model fit. Electronic Journal of Business Research Methods 6, 53–60.
Horn, S., Caruso, T., Verbruggen, E., Rillig, M.C., Hempel, S., 2014. Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. The ISME Journal 8, 2231–2242.
Johnson, N.C., 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185, 631–647.
Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warburton, L.M., Allen, E.B., 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84, 1895–1908.
Johnson, N.C., Wilsen, G.W., Wilsen, 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, 941–950.
Kembel, S.W., Cowan, P.D., Helmus, M.R., et al., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463–1464.
Kembel, S.W., Hubbell, S.P., 2008. The phylogenetic structure of a neotropical forest tree community. Ecology 87, 86–95.
Kim, Y.C., Gao, C., Zheng, Y., He, X.H., Yang, W., Chen, L., Wan, S.Q., Guo, L.D., 2015. Arbuscular mycorrhizal fungal community response to warming and nitrogen addition in a semiarid steppe ecosystem. Mycorrhiza 25, 267–276.

Kivistin, S.N., Hawkes, C.V., Treseder, K.K., 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 43, 2294–2303.

Koske, R., Tessier, B., 1983. A convenient, permanent slide mounting medium. Mycological Society of America Newsletter 34, 59.

Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 92, 486–505.

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

Li, X.L., Zhu, T.Y., Feng, F., Chen, Q., Lin, S., Christie, P., Zhang, J.L., 2015. Inner Mongolia steppe arbuscular mycorrhizal fungal communities respond more strongly to water availability than to nitrogen fertilization. Environmental Microbiology 17, 3051–3068.

Lin, X.G., Feng, Y.Z., Zhang, H.Y., Chen, R.R., Wang, J.H., Zhang, J.B., Chu, H.Y., 2012. Long term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in North China revealed by 454 pyrosequencing. Environmental Science & Technology 46, 5764–5771.

Liu, X.J., Duan, L., Mo, J.M., Du, E.Z., Shen, J.L., Lu, X.K., Zhang, Y., Zhou, X.B., He, C.E., Zhang, F.S., 2011. Nitrogen deposition and its ecological impact in China: an overview. Environmental Pollution 159, 2251–2264.

Liu, Y.J., Johnson, N.C., Mao, L., Shi, G.X., Jiang, S.J., Ma, X.J., Du, G.Z., An, L.Z., Feng, H.Y., 2015a. Phylogenetic structure of arbuscular mycorrhizal community shifts in response to increasing soil fertility. Soil Biology & Biochemistry 89, 196–205.

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

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

Moora, M., Davison, J., Opik, M., Metsis, M., Saks, U., Jairus, T., Vasar, M., Zobel, M., 2014. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiology Ecology 90, 609–621.

Mueller, R.C., Bohannan, B.J.M., 2015. Shifts in the phylogenetic structure of arbuscular mycorrhizal fungi in response to experimental nitrogen and carbon dioxide additions. Oecologia 179, 175–185.

Ni, J., Zhang, X.S., 2000. Climate variability, ecological gradient and the Northeast China Transect (NECT). Journal of Arid Environments 46, 313–325.

Opik, M., Vanatoo, A., Vanatoo, E., Moorea, M., Davison, J., Kalvej, J.M., Reuer, U., Zobel, M., 2010. The online database Maarjam reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytologist 188, 223–241.

Opik, 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.

Porras-Alfaro, A., Herrera, J., Natvig, D.D., Sinsabaugh, R.L., 2007. Effect of long-term nitrogen fertilization on mycorrhizal fungi associated with a dominant grass in a semiarid grassland. Plant and Soil 296, 65–75.

Powell, J.R., Parent, 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, Austria, Vienna.

Rillig, M.C., 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters 7, 740–754.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171, 41–53.

Saks, U., Davison, J., Opik, M., Vasar, M., Moora, M., Zobel, M., 2014. Root-colonizing and soil-borne communities of arbuscular mycorrhizal fungi in a temperate forest understory. Botany 92, 277–285.

Simon, L., Lalonde, M., Bruns, T.D., 1992. Specific amplification of 18S ribosomal genes from VA endomycorrhizal fungi colonizing roots. Applied and Environmental Microbiology 58, 291–295.

Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, third ed. Academic Press, London.

Tian, H., Dijker, R.A., Zhang, J.L., Li, X.L., 2013. Impact of long-term nitrogen fertilization and rotation with soybean on the diversity and phosphorus metabolism of indigenous arbuscular mycorrhizal fungi within the roots of maize (Zea mays L.). Agriculture Ecosystems & Environment 164, 53–61.

Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V., 1986. Measure detaux de mycorrhizar VA d’un systeme radiculaire. Recherche de methodes d’estimation ayant une important fonctionnelle. In: Gianinazzi-Pearson, V., Gianinazzi, S. (Eds.), Physiological and Genetic Aspects of Mycorrhizae. INRA, Paris, pp. 217–221.

Valyi, K., Mardhiah, U., Rillig, M., Hempel, S., 2016. Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. The ISME Journal 10, 2341–2351.

van Aarle, M., Olsson, P.A., Soderstrom, B., 2002. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. New Phytologist 153, 173–182.

van der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boiler, T., Wiemken, A., Sanders, L.R., 1998. Arbuscular fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 72–75.

van Diepen, L.T.A., 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.

van Diepen, L.T.A., Lilleskov, E.A., Pregitzer, K.S., Miller, R.M., 2010. Simulated nitrogen deposition causes a decline of intra and extraradical abundance of arbuscular mycorrhizal fungi and changes in microbial community structure in northern hardwood forests. Ecosystems 13, 683–695.

van Diepen, L.T.A., Lilleskov, E.A., Pregitzer, K.S., Miller, R.M., 2007. Decline of arbuscular mycorrhizal fungi in northern hardwood forests exposed to chronic nitrogen additions. New Phytologist 176, 175–183.

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

Veresoglou, S.D., Chen, B.D., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biology & Biochemistry 46, 53–62.

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.

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

Xu, Z.W., Ren, H.Y., Cai, J.P., Wang, R.Z., He, P., Li, M.H., Lewis, B.J., Han, X.G., Jiang, Y., 2015. Antithetical effects of nitrogen and water availability on community similarity of semiarid grasslands: evidence from a nine-year manipulation experiment. Plant and Soil 397, 357–369.

Yang, H.J., Li, Y., Wu, M.Y., Zhang, Z., Li, L.H., Wan, S.Q., 2011. Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. Global Change Biology 17, 2316–2344.

Zhang, Y., Kim, Y.C., Tian, X.F., Chen, L., Yang, W., Gao, C., Song, M.H., Xu, X.L., Guo, L.D., 2014. Differential responses of arbuscular mycorrhizal fungi to nitrogen addition in a near pristine Tibetan alpine meadow. FEMS Microbiology Ecology 89, 584–605.