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Six-year fertilization modifies the biodiversity of arbuscular mycorrhizal fungi in a temperate steppe in Inner Mongolia

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) play key roles in supporting ecosystem sustainability, stability and function, but little is known about how fertilization practices affect AMF abundance and community composition in the grassland ecosystems. In the present study, a field trial was established to examine the effects of 6 years of nitrogen (N) and phosphorus (P) fertilization on the community structure of AMF both in soils and plant roots in a typical temperate steppe in Inner Mongolia, northern China. The AMF small-subunit (SSU) rRNA genes were subjected to PCR, cloning, sequencing, and phylogenetic analyses. A total of 1554 sequenced SSU rRNA clones, including 919 clones from the soil and 635 clones from the roots, were analyzed. The 31 AMF sequence types belonging to Glomeromycota were identified: 17 to *Glomus* group A and 14 to *Glomus* group B. The experimental results indicated that N fertilization significantly altered the AMF communities in both soils and mixed roots but had no obvious influence on AMF abundance. However, P fertilization showed no significant influence on the AMF community structure, but induced a significant decrease in mycorrhizal colonization rate, arbuscule colonization and hyphal length density. Furthermore, N and P application showed significant interactions in affecting AMF species compositions in soils but not in roots. Generally the AMF diversity in the soil was higher than that in the roots. The study suggested that N fertilization predominantly altered AMF species composition, while P fertilization influenced AMF abundance in this steppe.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) belonging to the phylum Glomeromycota can form symbiotic associations with the roots of over 80% of all terrestrial plant families (Schüßler et al., 2001). They play a vital role in plant growth by providing mineral nutrients such as phosphorus (P), nitrogen (N), and also trace elements to their host plants (Smith and Read, 2008). Moreover, AMF can also protect their host plants from pathogen infections (Newsham et al., 1995), drought stress (Li et al., 2013) and heavy metal contaminations (Leyval et al., 1997).

It has been well demonstrated that AMF diversity can influence the biodiversity and productivity of plant communities (Van der Heijden et al., 1998). The growing evidence of the multi-function and ecological importance of AMF has triggered research interests to identify their abundance and distributions in natural ecosystems. As AMF were obligate symbionts associated with host plants, the community composition of plant and AMF could be highly correlated (Oehl et al., 2003; Landis et al., 2004). However, some studies showed that highly diverse plant communities may not supported a higher AMF diversity compared with depauperate plant communities (Johnson et al., 2003a; Börstler et al., 2006), suggesting that factors other than plant diversity are also important in determining AMF communities. Distinctions between AMF communities may be partially caused by ecological specificity among fungal–plant pairs (Helgason et al., 1998; Vandenkoornhuyse et al., 2003) or effects of season and host plant development stage (Husband et al., 2002a), and also possibly a result of environmental disturbance. For example, farming practices may play a key role in determining the AMF communities at both the local and regional scales (van der Gast et al., 2011).

A common agricultural practice influencing the AMF abundance and community structures is the application of fertilizers. The response of AMF communities to fertilizer addition may depend on the types, dozes and duration of fertilization. Previous studies using

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morphological (Egerton–Warburton and Allen, 2000, 2007; Bhadalung et al., 2005) and molecular identifications of AMF (Jumpponen et al., 2005; Porras-Alfaro et al., 2007; Lin et al., 2012; Liu et al., 2012) have shown that increased N fertilizer induced changes in AMF species richness and community structure. P fertilizer applications can also lead to changes in AMF community structures (Alguacil et al., 2010; Lin et al., 2012; Liu et al., 2012). However, most of these studies have examined the response of AMF communities to either single elements (N or P) or a combination of N and P, but the interactive effects of the two elements have rarely been examined.

Furthermore, most studies have focused on AMF diversity either in roots (Helgason et al., 1999; Daniell et al., 2001; Vandenkoornhuyse et al., 2002a) or in soils (Alguacil et al., 2012; Lin et al., 2012), and few studies examined both simultaneously. However, the overlap between the AMF species composition in soil and the functionally active AMF communities within mycorrhizal roots has been shown to be relatively low (Clapp et al., 1995; Hempel et al., 2007). It was reported that the colonizing strategies of AMF differ considerably and this variation is taxonomically based at the family level (Hart and Reader, 2002). Therefore, a systematic investigation of AMF species composition in roots and soils is required.

In a typical steppe in Inner Mongolia, Su and Guo (2007) characterized AMF communities in non-grazed, restored and overgrazed grasslands, and Tian et al. (2009) also studied the AMF communities associated with wild forage plants. In a desert steppe, Bai et al. (2013) investigated AMF communities associated with vegetation and soil parameters under different grazing managements. However, all these studies are based on spore counts and identification of AMF by spore morphology. It has been reported that spore populations in the soil do not reflect the AMF communities in roots (Clapp et al., 1995), and spore morphological characteristics are highly imprecise as descriptions usually depend on the identifier's experience and therefore needs to be treated with caution. Recently, the application of molecular techniques to the identification of AMF in the field has revealed an unexpectedly high diversity of AMF, with many detected sequences that cannot be related to known taxa (Vandenkoornhuyse et al., 2002a). These findings have challenged the traditional knowledge of a relatively low number of AMF species. Therefore, it is equally important to characterize the AMF species using a molecular approach.

In order to examine the potential effects of nutrient addition on AMF communities, a field fertilization experiment was established in Inner Mongolia. Mycorrhizal colonization and AMF diversity in root and soil based on molecular identification were determined so as 1) to investigate the influences of different fertilization on AMF community structure both in soils and roots; 2) to check whether there are interactive effects of N and P fertilizers in determining the AMF abundance and species compositions.

2. Materials and methods

2.1. Site description and experimental design

This experiment was conducted in a typical temperate steppe in Duolun County (E 116°17′20″, N 42°2′29″, 1324 m above sea level) in Inner Mongolia, China. Mean annual precipitation (MAP) is ~380 mm, with >80% of the precipitation occurring from June to September. Mean annual temperature (MAT) in the research area is 2.1 °C, with mean monthly temperature ranging from −17.5 °C in January to 18.9 °C in July. The soil on site is classified as Haplic Calcisol according to the FAO classification, or chestnut according to the Chinese classification. The native vegetation is a typical steppe community. The dominant plant species in this area are *Artemisia frigida*, *Stipa krylovii*, *Cleistogenes squarrosa* which are perennial herbs.

The experiment was established in 2005 with a split plot design, including four replicate plots, each containing four 44 × 30 m^2 subplots with 1 m wide buffer zone. The four subplots within one plot were randomly assigned to one of the four fertilizer addition treatments, including control (CK; no nutrient addition), N addition (N; 10 g N m^{-2} $year^{-1}$, in form of urea in 2005 and NH_3NO_4 in 2006–2011), P addition (P; 5 g P_2O_5 m^{-2} $year^{-1}$, in form of calcium superphosphate), and N together with P addition (NP). Fertilizers were supplied once a year in the middle of July from 2005 to 2011.

2.2. Sampling

Soil samples were collected in May 2011. From each subplot, five soil cores (containing soils and roots) were randomly taken from the 0–15 cm topsoil and then mixed to form one composite sample. All samples were placed in a standard ice box with a temperature of ~0 °C and transported to the laboratory. Soil samples were passed through a 2.0-mm sieve, stored at 4 °C before soil chemical analysis, and at −80 °C before DNA extraction. At the same time, the mixed roots were handpicked from the surface of the 2.0-mm sieve. They were washed thoroughly with distilled water. A sonicator was used to remove soil particles adhered to the root surface. Root samples were then divided into two subsamples. One was frozen at −80 °C for DNA extraction, and the other for monitoring mycorrhizal colonization rate and arbuscule abundance.

2.3. Soil biological, biochemical and physical analyses

Soil pH was determined at a soil to water ratio of 1: 2.5. Soil ammonium and nitrate 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). Available soil phosphate was determined by colorimetry according to a method described by Murphy and Riley (1962). The total organic C was measured according to Yeomans and Bremner (1988). Soil total N and total carbon (C) content were measured with an element analyzer (Vario EL III, Elementar, Hanau, Germany).

Acid phosphatase and urease activities were determined according to Alguacil et al. (2010). Soil microbial biomass C was determined using a fumigation–extraction method (Vance et al., 1987). Ten grams of soil at 60% of field water holding capacity were fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ for 24 h placed in a glass desiccator. After removal of residual $CHCl_3$, 40 ml of 0.5 M K_2SO_4 solution was added and the sample was shaken for 1 h before filtration of the mixture. The K_2SO_4-extracted C was determined with an automatic carbon analyzer for liquid samples and microbial biomass C was calculated as the difference between fumigated and non-fumigated samples.

2.4. DNA extraction and PCR

A total of 32 samples, consisting of 16 soil samples and 16 root samples, were subjected to molecular analysis. Soil DNA was extracted from 0.5-g soil using the Fast DNA® SPIN Kit for Soil (Q BIOgene Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Root DNA was extracted from 100-mg fine roots using a Plant Genomic DNA Kit following protocol recommended by the manufacturer (Tiangen Biotech, Beijing, China).

The extracted DNA was diluted with double-distilled water (1: 10). Partial sequences of the SSU rRNA genes were amplified using a nested PCR with a first glomeromycotan-specific primer pair AML1 and AML2 (Lee et al., 2008), and a second primer combination of universal eukaryotic primer NS31 (Simon et al., 1992) as forward