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# Fine-scale variations of fungal community in a heterogeneous grassland in Inner Mongolia: Effects of the plant community and edaphic parameters

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ABSTRACT

Soil and root-associated fungi are important in ecosystem functioning, and it is essential to understand driving factors of these fungi in natural ecosystems. In the present study, soil and root fungal communities in a fine-scale grassland were determined using high through-put sequencing, and our aims are to evaluate the relative importance of plant composition, soil elements and space factors on these two fungal communities. Our results showed that (1) fungal communities in soil and plant roots were distinct from each other, and OTU richness in soil was significantly higher than that in plant roots; (2) biomass of perennial rhizome grass, soil carbon content, and soil C/P ratio were key drivers for both soil and root fungal community; (3) dissimilarity of the soil fungal community significantly correlated with spatial distance, while no significant correlations were found between dissimilarity of root-associated fungal community and spatial distance. These results suggest the different spatial patterns of root and soil fungal communities which may contribute to a better understanding of the mechanisms maintaining root and soil fungal communities at small scales in this grassland ecosystem.

## 1. Introduction

As an important component of terrestrial ecosystems, fungal communities are involved in many ecosystem functions, such as carbon (C) and nutrient cycling (Gadd, 2007; Cheeke et al., 2017) and composition and productivity of plant community (van der Heijden et al., 1998, 2008; Roger et al., 2013). The underground fungal community can be divided into the soil fungal community and the root-associated fungal community, and the community composition of the two compartments may be driven by different factors with different underlying mechanisms. Root-associated fungi may depend to a large degree on the host plant community (Zak et al., 1994), while the soil fungal community may be determined mainly by soil elemental compositions (Eschen et al., 2013; Kim et al., 2015; Liu et al., 2015).

It has been well documented that fungal communities in natural ecosystems are coupled with plant communities and shaped by many environmental factors (Zachow et al., 2009; Valyi et al., 2016; Horn et al., 2017). The strong coupling of plant and fungal communities has been reported in forests (Peay et al., 2013; Mueller et al., 2014) and grasslands (Prober et al., 2015). The plant-fungal coupling in natural ecosystems may be from direct effects of the plant community through host-fungi specificity or preference (Lapointe and Molard, 1997; Bever, 2003) or indirect effects through litter resource inputs (Bardgett et al., 2008) or plant-driven changes in soil chemical and physical characteristics (Boyle et al., 2008). The host specificity or preference was usually considered as a key driver of symbiotic or pathogenic fungal community (e.g. Horn et al., 2017), and the indirect effects of plants through litter input may determine mainly saprotrophic fungal community.

Edaphic parameters are also considered as important determinants of fungal communities, and it has been reported that fungal communities may vary with soil depth (Fierer et al., 2003; Prober et al., 2015), soil pH (Rousk et al., 2011), and soil elemental compositions (Porras-Alfaro et al., 2007; Lin et al., 2012; Liu et al., 2012). Liu et al. (2015) demonstrated that soil carbon content drives the geographical distribution of fungal communities in northeastern China. In a semiarid steppe ecosystem in northern China, Kim et al. (2015) also showed that nitrogen addition significantly altered the fungal community composition.

While plant community composition and abiotic environmental factors have been reported as determinants of the fungal community in many studies, these conclusions were mainly based on global or

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regional studies. Since in these large-scale studies, plant and fungal species pools and climatic factors were also important contributors to variation in fungal community composition (Hawkes et al., 2011; Peay et al., 2013), it is difficult to assess the exact contributions of plant community composition and soil parameters on the fungal community. Arbuscular mycorrhizal fungi (AMF) may vary in quite small scales (Wolfe et al., 2007), and it has been reported that AMF community in a fine-scale study depended much on spatial distance and environmental factors rather than structure of plant community (Horn et al., 2017). Such fine-scale studies on the relationships among the local plant community, fungal community and their abiotic environments are helpful in better understanding the role of the plant community and soil parameters on the fungal community, since the effects of the species pool and climatic factors can be ignored within a local community.

Besides environmental filtering, dispersal limitation has also been raised as a major concept in explaining species distribution patterns, in which species distributions are explained by differences in dispersal ability and the probability of each species arriving in a local community (Peay and Bruns, 2014). Since dispersal propagules (e.g., spores and hyphal fragments) are usually quite small, fungal species have been considered as ubiquitous species with less dispersal limitations (Finlay, 2002). However, the results of some recent large-scale studies showed that dispersal limitation is still an underlying determinant of fungal communities (Peay et al., 2010; Norros et al., 2012; Peay and Bruns, 2014). As there may be different assemble rules for fungal communities at different scales (Valyi et al., 2016), the relative importance of dispersal in the fine spatial scale may be different from that of large-scale studies.

In this study, fine-scale variations of soil and root fungal communities were determined in a temperate grassland in Inner Mongolia and our hypotheses are (a) the diversity and composition of the fungal community in plant roots may be quite different from those of the soil fungal community; and (b) root-associated and soil fungal community may have different driving factors and the plant community, soil elements, and space may have different effects on the two fungal communities.

## 2. Materials and methods

### 2.1. Study site

The study site is located near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 43°38′N, 116°42′E), fenced since 1999 in a typical steppe dominated by *Leymus chinensis* and *Stipa grandis* (Lu and Han, 2010). The mean annual precipitation at the study site is 350 mm, with 80% occurring from June to September, and the mean annual temperature is 2.0 °C (Wang et al., 2006). The soil of the site is characterized as Calcis-orthic Aridisol according to the US Soil Taxonomy system (Lu and Han, 2010).

### 2.2. Plot and sample collection

We used a 10 × 10 m plot that was further divided into 10,000 0.1 × 0.1 m quadrats to investigate fine-scale spatial patterns in the plant and fungal communities. All plant species in each 0.1 × 0.1 m quadrat were identified and recorded. Totally 24 plant species were recorded in the study plot, including 11 perennial forbs (PF), six perennial bunchgrasses (PB), four annuals or biennials (AB), two shrubs or semi-shrubs (SS), and one perennial rhizome grass (PR) (Table S1). The dominant plant species were *Agropyron cristatum* (63.66% in frequency), *Stipa grandis* (43.70%), *Leymus chinensis* (36.03%), *Achnatherum sibiricum* (26.36%), and *Cleistogenes squarrosa* (23.35%). PB and PR were the two dominant plant functional groups, occupying 98% of the quadrats in our plot (Fig. S2). In the plot at this fine scale, the spatial distributions of plant species and plant functional groups were quite heterogeneous (Fig. S2), and a significant negative spatial relationship was found between PB and PR (0–5 m) (Fig. S3).

Two hundred 0.2 × 0.2 m quadrats (four 0.1 × 0.1 m quadrats combined) were randomly chosen as sampling plots in the study plot, and the shoot biomass of each plant species was measured after oven-drying at 65 °C for 48 h. The 0.2 × 0.2 m quadrats were divided into 4 vegetation groups (named VEG groups) according to the ratio of the biomass of perennial rhizome grass (PR) to perennial bunchgrasses (PB) as follows: group I, PR/PB = 0; group II, 0 < PR/PB ≤ 1/6; group III, 1/6 < PR/PB ≤ 1/2; group IV, PR/PB > 1/2.

Soil cores were taken at a depth of 0–15 cm in the 0.2 × 0.2 m quadrats, and root samples in each soil core were separated from the soil using a 2-mm sieve. For each VEG group, 10 soil samples and 10 root samples were chosen for fungal community determination and soil elemental composition measurement. Both root and soil samples were stored at −20 °C before DNA extraction.

### 2.3. Measurement of soil elemental composition

Soil samples were air-dried for 2 weeks to a constant weight and sieved using a 0.154-mm sieve. Soil organic carbon (SOC) was determined using the dichromate oxidation method (Yeomans and Bremner, 1988). Soil was digested using the Kjeldahl acid digestion method (Tan et al., 2013) and analyzed on an Alpkem autoanalyzer to determine the soil total nitrogen (STN) (Kjeltec 2200 Auto Distillation Unit, FOSS, Sweden). Soil total phosphorus (STP) was measured using the molybdenum blue colorimetric method at 880 nm with a UV-2550 spectrophotometer (Shimadzu, Japan) (Chen et al., 2013).

### 2.4. DNA extraction and fungal community identification

Soil DNA was extracted from 0.5 g soil using the MoBio Soil DNA isolation kit (Mo Bio Laboratories, Inc., 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 Co., Ltd., China). Both root and soil DNA were stored at −20 °C until amplification by polymerase chain reaction (PCR).

Fungal Internal transcribed spacer 1 (ITS1) regions were used to determine the fungal community, and PCR amplification (95 °C for 2 min, followed by 30 cycles at 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min) was carried out using the primers ITS1-1737F 5′-barcode-GGAAGTAAAAGTCGTAACAAGG-3′ and ITS2-2043R 5′-GCTGCGTTCTTCATCGATGC-3’. The barcode is an eight-base sequence unique to each sample. PCRs were performed in triplicate in a 20-μl mixture containing 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.4 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase, and 10 ng of template DNA.

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) following the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were pooled at an equimolar volume and paired-end sequenced (2 × 250) on an Illumina MiSeq PE250 sequencing machine adopting the standard protocols (Majorbio Bio-pharm Technology Co., Ltd., China).

Raw FASTQ files were demultiplexed and quality-filtered using QIIME (ver 1.8) with the following criteria: (i) the reads were truncated at any site receiving an average quality score < 20 over a 10-bp sliding window, discarding the truncated reads that were shorter than 50 bp; (ii) exact barcode matching, 2 nucleotide mismatch in primer matching, and reads containing ambiguous characters were removed; and (iii) only sequences that overlapped by longer than 10 bp were assembled according to their overlap sequence. Forward and reverse reads that could not be merged were discarded.

Open-reference OTU picking was carried out with pick_open_reference_otus.py using the uclust method at a 97% similarity cutoff, and singletons and sequences with lengths less than 200 bp were