Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China

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ABSTRACT

Fungi play an important regulating role in terrestrial ecosystem functioning. However, their biogeographic distribution patterns along combined gradients of plant communities and environmental variables across regional spatial scales remain poorly understood. This knowledge gap is particularly pronounced in arid and semi-arid grassland ecosystems, which occupy one third of the terrestrial surface of China. Here, a regional-scale field investigation was conducted to collect soil samples from 290 plots at 52 sites along a 4000 km transect in temperate grasslands of northern China to assess the distribution patterns of fungal communities. The high-throughput Illumina sequencing revealed that soil fungal communities were dominated by Ascomycota, Basidiomycota and Zygomycota across the temperate grasslands. Fungal richness increased with plant species richness and mean annual precipitation (MAP). Bray-Cutis dissimilarities in soil fungal community composition between sites were significantly correlated with plant community composition, geographical distance, and abiotic factors including MAP, mean annual temperature, soil pH, and soil organic carbon. Variation partitioning analysis revealed that variation in fungal community composition was mainly explained by plant community composition, followed by geographical distance and abiotic factors. Structural equation models further highlighted the significant role of plant species richness in determining soil fungal richness. Taken together, these results suggest that the regional-scale distribution pattern of soil fungal communities is predominantly mediated by plant community composition, and also by abiotic factors and geographical distance in arid and semi-arid temperate grasslands.

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1. Introduction

Fungi represent one of the most diverse groups of living organisms, and play an essential role in terrestrial ecosystem functioning as plant decomposers (e.g. wood-decaying Basidiomycetes), pathogens and symbionts (e.g. forming mycorrhizal associations) (Zeilinger et al., 2015). There is also evidence that subsets of soil fungi such as arbuscular mycorrhizal fungi have the potential to influence plant productivity and diversity by increasing available nutrient pools, especially in nutrient-poor ecosystems where plant symbionts are largely responsible for the acquisition of limiting nutrients (Van der Heijden et al., 1998, 2008; Klironomos et al., 2011; Bardgett and van der Putten, 2014). Therefore, studying biogeographic patterns of soil fungal communities could provide valuable clues for understanding biogeochemical nutrient cycles, ecosystem processes and services.

Understanding which factors drive observed biogeographical patterns of soil microbial communities is a key objective in microbial ecology (Martiny et al., 2006). Despite their ubiquity in terrestrial ecosystems and importance in ecological functioning,

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the diversity and distribution patterns of soil microbes at regional scales are far less understood than the respective distribution patterns of above-ground macro-organisms such as plants and animals (Martiny et al., 2006). Mounting evidence suggests that soil pH represents a key regulator in shaping the distribution of soil bacterial communities at regional scales (Fierer and Jackson, 2006; Lauber et al., 2009). However, compared with bacteria, little is known about the regional-scale biogeographic distribution patterns of soil fungal communities. Fungi depend more on plant products (Millard and Singh, 2010) and their biotrophic interactions with plants because a large proportion of them act as endophytes, mutualists or pathogens (Wardle, 2006; Gao et al., 2013). Therefore, it is assumed that there would be a strong coupling of plant-fungal distribution patterns at regional scales. Although some site-specific studies reported positive relationships between plant and fungal diversity (Van der Heijden et al., 1998; Hiiesalu et al., 2014), comprehensive empirical evidence from regional scales remain scarce. Furthermore, the range of environmental parameters that are considered in such regional studies is usually limited due to logistic constraints and to the best of our knowledge there have been no field studies that simultaneously considered the relative role of multiple spatial factors, abiotic factors and plant community composition in shaping soil fungal communities at regional scales. This represents a major knowledge gap in our understanding of fungal biogeography.

Biogeographical studies focusing on soil fungal communities have been conducted in European grasslands (Pellissier et al., 2014; Geremia et al., 2016), arctic tundra (Timling et al., 2014) and various forest ecosystems including temperate forests, tropical forests and boreal forests (Wubet et al., 2012; Peay et al., 2013; Taylor et al., 2014). With a focus on temperate grasslands and drylands there have been two recent surveys with a global scope which exemplified how fungal communities are influenced by coarse environmental heterogeneity (Maestre et al., 2015; Prober et al., 2015). These two comprehensive studies have revealed that aridity and plant beta diversity are important predictors of fungal community compositions (beta diversity). Coarse environmental gradients however may lack the resolution required to disentangle confounding effects of different predictors. For instance, latitudinal gradients may be confounded with temperature changes or vice versa. The logistics of the two earlier mentioned global studies may have prevented a sufficiently intense sampling to fully appreciate the importance of individual environmental drivers. Moreover, we can expect that some drivers that are important at a global scale (such as dispersal) will be less important at regional scale and vice versa (e.g. altitude). Therefore, a regional study on Asian grassland ecosystems could represent an excellent compromise between the abovementioned global studies and the existing smaller scale studies.

The temperate grasslands in northern China represent typical arid and semi-arid ecosystems, occupying one third of the land surface of China. The habitats in this region have been described to be exceptionally fragile and are predicted to suffer from a projected higher frequency of extreme climatic phenomena such as increased drought and precipitation (Ni and Zhang, 2000; Wang et al., 2015). It was suggested that the total area of arid and semi-arid ecosystems will expand as a result of global warming (Dai, 2013) and simultaneously precipitation events will become less regular (Easterling et al., 2000). These changes could induce substantial changes on the growth rates and community assembly structures of soil fungi (Maestre et al., 2015; Vargas-Gastélum et al., 2015). Therefore, it is highly desirable to improve our understanding of the ecology and functioning of soil fungi in arid and semi-arid ecosystems.

Here, we present a regional-scale field study spanning 52 temperate grassland sites in northern China to examine the distribution patterns and driving factors of soil fungal communities using high-throughput sequencing technologies. The studied region is characterized by a decreasing precipitation gradient from the east to the west, and encompasses a wide variety of climatic, edaphic and vegetation conditions (Chen et al., 2014a; Wang et al., 2015). We tested the following hypotheses: (1) plant species richness and community composition are positively related to soil fungal richness and community composition in temperate grasslands in agreement with earlier studies that have demonstrated that plant community structure represents a major environmental filter for fungi (Prober et al., 2015); and (2) after accounting for plant dependencies, climatic predictors, and MAP in particular, would explain a considerable proportion of cross-community variability as has been shown in other regional and global studies (Pellissier et al., 2014; Tedersoo et al., 2014).

2. Materials and methods

2.1. Study sites, field investigation and soil sampling

The study sites span a latitudinal gradient of $36.36°N - 48.54°N$ and a longitudinal gradient of $83.84°E - 125.00°E$, and cover seven provinces in North China including Heilongjiang, Inner Mongolia, Hebei, Shanxi, Gansu, Qinghai, and Xinjiang (Fig. 1; Table S1). Four major grassland types can be identified in this region including meadow steppe, typical steppe, desert steppe and desert. The mean annual precipitation (MAP) ranges from 75 mm to 557 mm, and the mean annual temperature (MAT) from $-1.5 °C$ to $9.6 °C$.

A field sampling campaign was conducted during the growing season from July to August in 2012. A total of 312 soil samples were collected from 52 sites along a 4000 km transect, which covered all the major climate zones and grassland types, and were at least 1 km away from main roads to avoid influences of anthropogenic activities (Table S1). At each site, spatial geographic coordinates (longitude and latitude) and elevation were recorded using a GPS device (eTrex Venture, Garmin, USA). All the sites were surveyed using a standardized sampling protocol. Briefly, six random 1 m × 1 m plots were arranged within an area of 10 m × 10 m for each site. The plot-scale measures of plant species composition included the species richness (SR), Shannon-Wiener diversity, and the number of each plant species within the 1 m² plot. We used the Angiosperm Phylogeny Group (APG) III system (Chase and Reveal, 2009) to identify plants taxonomically. Five soil cores (3 cm in diameter and 10 cm in depth) were collected randomly from each plot, stored in polyethylene bags and transported on ice to the laboratory. After thorough homogenization and removal of roots and stones, the composite soils were sieved through a 2-mm mesh and divided into two portions. One portion was stored at 4 °C for characterization of soil physical and chemical properties, and another portion was stored at $-80 °C$ prior to DNA extraction and molecular analysis.

2.2. Soil physicochemical characterization and climate data collection

Soil moisture content was measured gravimetrically by oven-drying the fresh soil samples at 105 °C for 24 h. Soil ammonium and nitrate were extracted with 2 M KCl (a soil to water ratio of 1:5) and measured using a continuous flow analyzer (SAN++, Skakar, Breda, Holland). Soil pH was determined in a soil suspension with a soil: water ratio of 1: 2.5 (w/v) using a Delta 320 pH-meter (Mettler-Toledo Instruments Co., Shanghai, China). Total carbon (TC) and total nitrogen (TN) were determined on an element analyzer (Vario EL III, Elementar, Germany). Soil organic carbon (SOC) was measured using a $K_2Cr_2O_7$ oxidation method as described in