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Plant biomass, soil water content and soil N:P ratio regulating soil microbial functional diversity in a temperate steppe: A regional scale study

Zhanfeng Liu [a,b], Bojie Fu [a], Xiaoxuan Zheng [a], Guohua Liu [a,*]

[a] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China
[b] Institute of Ecology, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

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ABSTRACT

Soil microorganisms are influenced by various abiotic and biotic factors at the field plot scale. Little is known, however, about the factors that determine soil microbial community functional diversity at a larger spatial scale. Here we conducted a regional scale study to assess the driving forces governing soil microbial community functional diversity in a temperate steppe of Hulunbeir, Inner Mongolia, northern China. Redundancy analysis and regression analysis were used to examine the relationships between soil microbial community properties and environmental variables. The results showed that the functional diversity of soil microbial communities was correlated with aboveground plant biomass, root biomass, soil water content and soil N: P ratio, suggesting that plant biomass, soil water availability and soil N availability were major determinants of soil microbial community functional diversity. Since plant biomass can indicate resource availability, which is mainly constrained by soil water availability and N availability in temperate steppes, we consider that soil microbial community functional diversity was mainly controlled by resource availability in temperate steppes at a regional scale.

1. Introduction

Soil microbes play key roles in ecosystems and mediate many ecological processes that are central to ecosystem functioning, including nutrient cycling (Balser and Firestone, 2005), litter decomposition (Johnson et al., 2003) and the regulation and maintenance of plant biodiversity (Zak et al., 2003). Further, biotic and environmental factors form the fundamental forces that drive the activity, structure and diversity of soil microbial communities (Ogram et al., 2006). Previous studies have indicated that microbial processes, community diversity and composition are controlled by many factors including plant species (Wardle et al., 2004) and edaphic conditions (Marschner et al., 2001). Analysis of quantitative linkages between soil microbial community structure, function, and biotic and environmental parameters should provide a greater understanding of the factors that control nutrient cycling in ecosystems (Ogram et al., 2006).

Spatial and temporal scales are also important factors that drive the interactions between soil microbial community structure and diversity, and abiotic/biotic factors in the environment. Ecologists have pointed out that spatial scale plays an important role in understanding the nature of relationships between plant and soil microbial community structure and diversity (De Deyn and Van der Putten, 2005). The spatial and temporal scales at which plants and soil organisms interact also differ (De Deyn and Van der Putten, 2005). Microbial abundance and diversity are influenced to different degrees by processes operating at a multitude of scales. At a larger scale, spatial patterns of plant communities, soil characteristics, and landscape properties affect soil microbial community structure and diversity (Ogram et al., 2006). Although a number of studies have examined spatial and temporal variation of soil properties, processes, and the biomass of soil microbial communities (Smith et al., 1994; Morris, 1999), little information is available on the factors that determine soil microbial community composition and functional diversity in natural environments at large spatial scales. For example, recent studies at continental scale indicated that soil pH (Fierer and Jackson, 2006; Lauber et al., 2009), soil temperature, vegetation cover and geographic distance (Yergeau et al., 2007) were major determinants of soil bacterial community structure (Fierer et al., 2009). Furthermore, little is known about whether a common set of factors govern the soil microbial community structure and functional diversity when the spatial scale varies across microcosm, microhabitat, field, landscape, regional, and global levels. Plant community structure is well known to influence microbial community composition, as are soil physical and chemical properties (Brodie et al., 2002). However, these relationships are poorly defined, and knowledge is limited about the scales at which soil microbial interactions and

* Corresponding author. Tel.: +86 10 62849803; fax: +86 10 62923557.
E-mail address: ghliu@rcees.ac.cn (G. Liu).

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associations become important (Mummey and Stahl, 2003). Uncovering the underlying mechanisms fostering microbial diversity in soil will contribute to theories concerning the regulation of biodiversity in general, and to our understanding of the role of soil microbial communities in regulating a myriad of ecosystem processes (Waldrop et al., 2006). These efforts will generate valuable insights about the drivers of soil microbial diversity and how soil microbial community structure and diversity influence nutrient cycling (Ogram et al., 2006).

The temperate steppe, located in arid and semiarid regions of northern China, represents one of the typical Eurasian vegetation types. In previous studies, we sought to gain insight into the relationship between plant species diversity and soil microbial functional diversity across a large spatial scale in Hulunbeir grasslands, Inner Mongolia, Northern China (Liu et al., 2008). In an effort to further assess the driving forces of soil microbial community functional diversity across a large spatial scale, we quantified both soil microbial community functional diversity and its potential driving forces including biotic and abiotic factors across Hulunbeir grassland at a regional scale. We hypothesized that soil microbial functional diversity would exhibit biogeographical patterns at regional scale and these patterns can be predicted by the factors related to vegetation and soil.

2. Materials and methods

2.1. Study area

The study area is located at the western part of Daxing'anling Mountains, Hulunbeir (115° 31′ ~ 126°04′E, 47° 05′ ~ 53°20′N), Inner Mongolia, China (Fig. 1). The mean annual precipitation is 339 mm and mean annual temperature is −2.2 °C. The topography was relatively constant in the area and the maximal elevational variation is less than 50 m. The main soil type is chernozem and chestnut soil. The area is characterized by strong climatic gradients (in both precipitation and temperature) that are highly associated with trends in plant community composition and structure. Approximately 8.4×10^4 km^2 of grasslands make this area very suitable for research at a large spatial scale. Floristically distinct plant communities in this region include arid steppe (dominated by *Allium polyrrhizum*), semiarid steppe (dominated by *Serratula centauroides*, *Salsola collina*, *Chenopodium glaucum* and *Carex korshinskyi*) and meadow steppe (dominated by *Aneurolepidium chinense*, *Stipa baicalensis* and *C. korshinskyi*) (Inner Mongolia Federal Investigation Team of CAS, 1985).

2.2. Study design and sample collection

We established twenty sampling sites spanning from west to east and from south to north within the Hulunbeir grasslands (Fig. 1). Vegetation measurements and soil sampling were carried out in August 2006, at the peak of vegetative cover and species richness. At each site three sampling plots (1 m × 1 m) with similarity in plant composition and topography were established at a 15-m interval along a transect. In each plot, all species were identified and measured for cover, height and density. The importance value of each plant species was calculated by the combination of relative cover, height and density (Important value = (relative cover + relative height + relative density)/3). Thus, a floristic data matrix was made for calculating Shannon diversity index (SW), Shannon richness index (R) and Shannon evenness index (E). Aboveground plant biomass (AB) was determined by clipping the plants at ground level, drying at 60 °C for 48 h, and weighing. Root biomass (RB) was determined by collecting 5 cm diameter soil cores, sifting though a 2-mm sieve, washing by water, drying at 60 °C for 48 h, and weighing. Total biomass (TB) was the sum of aboveground plant biomass and root biomass. Soil samples were collected from the top 20 cm in each of the three replicate plots at each site. In each plot, the soil samples were collected from five randomly selected points and mixed into one sample. After carefully removing the surface organic materials and fine roots, each mixed sample was divided into two parts. One part was air-dried for analysis of soil physico-chemical properties and the other was sifted through a 2-mm sieve for microbial assays after transporting to laboratory at 4 °C.

2.3. Soil sample analyses

Soil water content (SWC) of each sample was determined gravimetrically by weighing, after drying in an oven at 105 °C for 12 h. Soil bulk density (BD) was determined from an undisturbed soil core. Soil organic carbon (SOC) was determined with the $K_2Cr_2O_7$ titration method after digestion (Nelson and Sommers, 1975). Total nitrogen (TN) was determined by the semi-micro-Kjeldahl method (Lu, 1999). Total phosphorus (TP) was determined colorimetrically after wet digestion with H_2SO_4 plus $HClO_4$ (Parkinson and Allen, 1975). Available phosphorus (AP) was extracted with 0.5 mol L^{-1} $NaHCO_3$ solution (pH = 8.5) (Olsen et al., 1954).

Soil microbial biomass C (SMBC) was determined by the chloroform fumigation method (Vance et al., 1987), using a $K_c = 0.45$. The soil microbial community functional diversity of culturable bacteria were analyzed using Biolog™ GN_2 plates (Biolog Inc., Hayward, CA) (Garland, 1996). Although these bacteria represent only a small fraction of the taxa in soils, we consider them to be a useful indicator for measuring the relationship of soil microbial community functional diversity with its driving factors. The water content of each soil sample was determined, which ensured that a constant equivalent dry-mass of soil was used for preparation of Biolog inoculum following 7 days incubation at 25 °C. The method used for inoculum preparation was adopted from Zak et al. (1994). To minimize the influence of cell density in comparisons among samples, results can be analyzed at constant average well color development (AWCD). The AWCD for each microplate was calculated by subtracting the control well optical density (OD) from the substrate well OD (blanked substrate wells), setting any resultant blanked substrate wells with negative values to 0 and taking the mean of the 95 blanked substrate wells (Garland, 1996). Biolog data incubated for 72 h were analyzed according to Zak et al. (1994) to give catabolic richness (the number of substrates used), catabolic evenness (the distribution of color development between substrates) and catabolic diversity (Shannon diversity index, a composite measure of richness and evenness).

2.4. Statistical analysis

The whole data set was subdivided into three sets according to the variables: Soil microbial data (72 h Biolog GN_2 data: 95 quantitative variables), Soil data (BD, SWC, SOC, TN, TP, AP, SMBC, C/N, N/P, SMBC/SOC: 10 quantitative variables), Vegetation data (coverage, AB, RB, TB, SW, R, E: 7 quantitative variables). Redundancy analysis (RDA) was applied to quantify and test effects of soil and vegetation data on the soil microbial community functional diversity variation. Partial RDA was also performed to extract the variation in the soil microbial community functional diversity explained by each of the two sets of explanatory variables (Soil data and Vegetation data) and shared by these two data sets (Borcard et al., 1992). The whole process was based on computation made with Canoco for Windows 4.5. The explanatory variables were standardized before the analysis. To avoid overfitting in the regression model due to the large number of explanatory variables, the most discriminating variables for each data set were selected by the 'forward selection' procedure of the program during the analysis. Statistical tests were run using the Monte Carlo