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

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ARTICLE INFO

Article history:

Received 21 July 2009

Received in revised form

10 November 2009

Accepted 24 November 2009

Available online 9 December 2009

Keywords:

Plant biomass

Soil water content

Soil N:P ratio

Soil microbial community

functional diversity

Temperate steppe

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.

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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

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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 km² 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 through 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 K₂Cr₂O₇ 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 H₂SO₄ plus HClO₄ (Parkinson and Allen, 1975). Available phosphorus (AP) was extracted with 0.5 mol L⁻¹ NaHCO₃ 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 BiologTM GN₂ 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 GN₂ 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

Fig. 1. Location of study area and sampling sites across Hulunbeir grasslands, Inner Mongolia, China.

permutation procedure of Canoco for Windows 4.5. Regression analysis was used to explore the relationship between the most discriminating soil and vegetation variables with soil microbial properties. Data that were not normally distributed were transformed prior to analysis, using square-root or log transformations.

3. Results

3.1. Variation of soil properties and vegetation characteristics

All the selected soil properties exhibited a high spatial variation across the sampling sites and the value of coefficient of variation (CV) is ranked as followings: soil water content (53.84%) > soil organic carbon (48.7%) > SMBC:SOC (43.77%) > total nitrogen (38.25%) > total phosphorus (38.24%) > soil microbial biomass carbon (35.07%) > available phosphorus (31.59%) > soil N:P ratio (31.12%) > soil C:N ratio (23.35%) > bulk density (15.92%). Vegetation data showed similar high degree of spatial variation across the sampling sites and the CV value is ranked as followings: aboveground plant biomass (81.88%) > root biomass (52.97%) > total plant biomass (52.38%) > Shannon richness index (43.04%) > plant coverage (33.19%) > Shannon diversity index (20.4%) > Shannon evenness index (11.12%).

3.2. Effect of vegetation on soil microbial community functional diversity

RDA on soil microbial data constrained by vegetation data was performed to quantify the effects of vegetation on the variation in

soil microbial community functional diversity (Fig. 2). Eigen values for the first, second, third and fourth axes were 0.218, 0.024, 0.013 and 0.010 respectively and axis 1 explained most of the variation in the soil microbial community functional diversity. The first RDA axis was mainly correlated to aboveground plant biomass ($r=0.667$, $P<0.001$), plant root biomass ($r=0.547$, $P<0.001$), Shannon richness index ($r=0.588$, $P<0.001$), total plant biomass ($r=0.581$, $P<0.001$), plant coverage ($r=0.477$, $P<0.001$) and Shannon diversity index ($r=0.400$, $P<0.002$). All the vegetation data together could explain 28.2% of the soil microbial community functional diversity variation (Monte Carlo permutation test with 999 permutation, $P=0.001$). Variables explaining the largest statistically significant amount of variation were aboveground plant biomass ($P=0.001$) and plant root biomass ($P=0.002$). Aboveground plant biomass and root biomass could explain 21% of the variance in soil microbial community functional diversity (Monte Carlo permutation test with 999 permutation, $P=0.001$). Regression analysis results showed that soil microbial biomass C, catabolic activity (AWCD), catabolic diversity, catabolic richness and catabolic evenness increased linearly with aboveground plant biomass and root biomass (Table 1).

3.3. Effect of soil properties on soil microbial community functional diversity

The correlation structure between soil microbial community functional diversity and soil data is summarized in Fig. 3. The eigen values for the first, second, third and fourth axes were 0.198, 0.028, 0.017, and 0.084, respectively. The variables that were highly

Fig. 2. Biplots diagram of the redundancy analysis (RDA) on soil microbial community (Biolog data (72 h)) constrained by vegetation data. Aboveground plant biomass (AB), root biomass (RB), total biomass (TB), plant coverage (coverage), Shannon–Wiener diversity index (SW), Shannon–Wiener richness index (R), Shannon–Wiener Evenness index (E) are used as quantitative explanatory variables. Axis 1 (21.8%, λ : 0.218, $P=0.001$) and Axis 2 (2.4%, λ : 0.024, $P=0.001$). The total explained variance by all the selected vegetation variables is 28.2% ($P=0.001$). Samples are labeled according to the sampling sites.

correlated with axis 1 included soil water content ($r=0.606$, $P<0.001$), N:P ratio ($r=0.5921$, $P<0.001$), soil organic carbon ($r=0.535$, $P<0.001$), soil microbial biomass C ($r=0.509$, $P<0.001$), bulk density ($r=-0.488$, $P<0.001$), total nitrogen ($r=0.475$, $P<0.001$), C:N ratio ($r=0.386$, $P<0.005$) and SMBC:

Table 1
Univariate linear regression models ($Y=a+bX$) predicting soil microbial characteristics as a function of selected vegetation and soil variables.

Dependent variables ^a	Variables ^b	a	b	R ²	P value
SMBC	AB	556.79	2.95	0.31	0.0001
	RB	591.58	0.15	0.08	0.0312
	SWC	363.78	32.41	0.59	0.0001
	N:P ratio	352.70	78.01	0.21	0.0003
Catabolic activity (AWCD)	AB	0.18	2.36×10^{-3}	0.42	0.0001
	RB	0.14	1.85×10^{-4}	0.28	0.0001
	SWC	0.13	0.02	0.34	0.0001
	N:P ratio	0.01	0.07	0.33	0.0001
Catabolic diversity	AB	3.53	3.73×10^{-3}	0.25	0.0001
	RB	3.48	2.75×10^{-4}	0.15	0.0032
	SWC	3.40	0.03	0.27	0.0001
	N:P ratio	3.11	0.13	0.30	0.0001
Catabolic richness	AB	26.16	0.23	0.34	0.0001
	RB	23.63	0.02	0.20	0.0004
	SWC	18.89	1.85	0.35	0.0001
	N:P ratio	2.74	7.66	0.37	0.0001
Catabolic evenness	AB	0.83	5.95×10^{-4}	0.26	0.0001
	RB	0.82	4.39×10^{-5}	0.15	0.0026
	SWC	0.81	4.98×10^{-3}	0.29	0.0001
	N:P ratio	0.76	0.02	0.35	0.0001

^a SMBC = soil microbial biomass carbon.
^b AB = aboveground biomass; RB = root biomass; SWC = soil water content; N:P ratio = soil N:P ratio.

Fig. 3. Biplots diagram of the redundancy analysis (RDA) on soil microbial community (Biolog data (72 h)) constrained by soil data. Soil organic carbon (SOC), soil water content (SWC), soil microbial biomass carbon (SMBC), total nitrogen content (TN), total phosphorus content (TP), available phosphorus content (AP), bulk density (BD), soil C: N ratio (C/N), soil N: P ratio (N/P) and microbial quotient (SMBC/SOC) are used as explanatory variables (arrows). Axis 1 (19.1%, λ : 0.191, $P=0.001$) and Axis 2 (3.4%, λ : 0.034, $P=0.001$). The total explained variance by all the selected soil variables is 31.1% ($P=0.001$). Samples are labeled according to the sampling sites.

SOC ($r=-0.314$, $P<0.02$). All the soil variables in combination could explain 31.1% of the variance in soil microbial community functional diversity (Monte Carlo permutation test with 999 permutations, $P=0.001$). The variables explaining the largest statistically significant amount of variation were soil water content ($P=0.001$) and soil N: P ratio ($P=0.002$). Soil water content and soil N: P ratio could explain 20% of the variance in soil microbial community functional diversity (Monte Carlo permutation test with 999 permutation, $P=0.002$). Regression analysis results showed that soil microbial biomass C, catabolic activity (AWCD), catabolic diversity, catabolic richness and catabolic evenness increased linearly with soil water content and soil N:P ratio (Table 1).

3.4. Contribution of soil and vegetation to soil microbial community functional diversity variation

Partial RDA was used to extract the variation in soil microbial community functional diversity by each of the two sets of explanatory variables (soil data and vegetation data) without the effect of the other, as well as the variation shared by these two data sets. The variation of soil microbial community functional diversity explained by each data set (Soil data and Vegetation data) without the second was also significant (soil data: $P<0.05$; vegetation data: $P=0.006$). For each set, percentages of variation presented in Fig. 4 are those without the shared variation. Soil data and vegetation data explained 17.5% and 14.9% of the variation of soil microbial community functional diversity respectively. The variation shared by soil and vegetation data was 13.6%. 54% of variation in soil microbial community functional diversity could not be explained by the measured variables.

Fig. 4. Variance partitioning with partial RDA of soil microbial community (72 h Biolog data) according to vegetation and soil data. Monte Carlo permutation test was performed on each set without the effect of the other by permuting samples freely (999 permutations).

4. Discussion

In this study, community level physiological profiles (CLPP), obtained by BiologTM GN₂ microplate, were used to characterize the functional diversity of soil microbial communities. CLPP is a measurement of potential activity of culturable, aerobic and fast growing bacteria (Smalla et al., 1998). However, caution is required in interpreting the CLPP results since the potential catabolic diversity measured here may not accurately reflect activity under the field conditions. Despite methodological uncertainty, GN₂ plateTM breathprint reflects diversity of carbon-oxidation pathways and therefore functional diversity of soil microbial communities.

Since soil microbes ultimately depend on autotrophs for their supply of C, plant diversity and community composition is likely to determine critically both the biomass and diversity of soil microbial communities (Johnson et al., 2003). Many studies have explored the effect of plants on soil microbial community properties at local scales, however the results are equivocal. Some studies in grassland ecosystems demonstrated that plant diversity can significantly influence the activity and community composition of soil microbes (Stephan et al., 2000; Loranger-Merciris et al., 2006). However, many other studies showed that plant community composition does not have any appreciable effect on soil microbial biomass and community composition (Spehn et al., 2000; Zak et al., 2003). In our study, although there were obvious relationships between soil microbial community functional diversity and plant diversity, plant diversity itself is not a strong predictor of soil microbial community functional diversity. Our data indicated that among the biotic factors, aboveground plant biomass and root biomass explained the largest statistically significant amount of soil microbial community functional diversity variation, suggesting that plant biomass was a major biotic factor affecting the functional diversity of soil microbial community in this study. Similarly, Zak et al. (2003) pointed out that changes in microbial abundance and composition were more related to differences in plant productivity rather than plant diversity based on a long-term field manipulation experiment in Minnesota grassland ecosystem. Another study demonstrated that there was no clear relationship between soil bacterial diversity and plant diversity (Fierer and Jackson, 2006). Waldrop et al. (2006) suggested that variability in resource availability explained the lack of a relationship between plant and fungal diversity based on plot scale data. Since increased plant species richness was associated

with an increase of plant productivity across the spatial gradient in our study, variation in plant productivity across a larger spatial scale would likely affect the effects of plant diversity on soil microbial community functional diversity.

The importance of soil moisture in regulating microbial activity and diversity is well known (Griffiths et al., 2003; Waldrop and Firestone, 2006). Our data indicated that soil water content was a major abiotic factor influencing the functional diversity of soil microbial communities in temperate steppe at a regional scale. Our results were consistent with the studies by Williams and Rice (2007). In arid and semiarid ecosystems, soil water condition was an important factor limiting plant growth, and variation in mean annual precipitation could significantly affect aboveground net primary production and plant species richness across the Inner Mongolia steppe region in northern China (Bai et al., 2008). Therefore, the influence of soil water content on soil microbial community functional diversity can be partially explained by the greater plant above- and belowground productivity as a result of soil water availability.

Dryland ecosystems have long been considered to have a highly heterogeneous distribution of nutrients and soil nutrient availability may affect the biomass, activity and composition of soil microbial communities (Housman et al., 2007). Therefore, we expected the availability of soil C, N, P to play an important role in determining soil microbial community functional diversity across a large spatial scale, given the importance of available energy in structuring soil microbial communities (Brodie et al., 2002). However, we found that soil organic carbon, total nitrogen, total phosphorus, available phosphorus, soil microbial biomass C, soil C:N ratio, SMBC:SOC ratio were not strong predictors of soil microbial community functional diversity. Only soil N:P was found to be an important factor affecting the functional diversity of soil microbial communities in our study. Moreover, soil N:P ratio, as indicator of soil nutrient limitation, can well reflect floristic variation as well (Fanelli et al., 2008). At a large scale, abiotic factors set limits on biotic components. Consequently, soil N:P ratio can affect soil microbial community functional diversity through its impact on plant productivity and composition at regional scale in N-limited environments.

Several hypotheses including productivity–diversity hypothesis (Tilman et al., 1996), plant diversity hypothesis (Hooper et al., 2000), spatial isolation in low organic matter soils and resource heterogeneity (Zhou et al., 2002) have been offered to explain patterns of microbial diversity in soil. However, those hypotheses and related testing researches are mostly limited to small-scale experiments in controlled conditions; it is unclear how to scale-up these relationships to regional and global scales (De Deyn and Van der Putten, 2005). Due to differences in design among the aforementioned studies and our research, we were not able to explicitly test among these varying hypotheses. However, our results at a regional scale could be explained partially by productivity–diversity hypothesis which proposes that the availability of growth-limiting resources limits the diversity of biotic communities. Higher levels of plant detritus production should increase the supply of limiting resources, thereby increasing the size of microbial community with concomitant increase in diversity (Hooper et al., 2000). Although plant detritus production was not identified in our study, a significant positive relationship was found between plant biomass with soil organic carbon and soil microbial biomass carbon. Furthermore, plant productivity in temperate steppes was mainly constrained by the availability of soil water and nitrogen. Therefore, we argued that plant biomass could well indicate plant detritus production and resources availability in soil. Our results showed that plant biomass, soil water content and soil N:P ratio were significantly correlated with the soil microbial community

functional diversity, suggesting the soil microbial community functional diversity is spatially structured in response to the distribution of the plant productivity and the availability of soil water and nitrogen. In other words, resource availability plays an important role in regulating soil microbial community functional diversity at a regional scale in Hulunbeir grasslands.

Although our study suggested that the selected biotic and abiotic factors can well explain the variation of soil microbial community functional diversity, the amount of unexplained variation was high and 54% of microbial variation can not be explained by those variables. This is usually interpreted as variation caused by unmeasured environmental variables including soil pH and soil temperature, complex spatial relationships and stochasticity in biological processes (Borcard et al., 1992).

This work provided an examination of the main driving forces affecting soil microbial functional diversity in a semiarid steppe at a regional scale. The present study also provided a baseline assessment of the soil microbial community functional diversity of the temperate semiarid steppe ecosystem for the future assessments of effects of human perturbation and climate change. How soil microbial communities respond to changes in vegetation and climate will be important for understanding the potential impact of alteration in plant distribution, plant productivity, patterns of precipitation and temperature caused by global changes.

Acknowledgements

We thank Dr. Shenglei Fu for critical review of the earlier version of this work. We are grateful to Yugang Zhang, Jiaming Zhao, Ruidong Wang, Kui Li and Xiaoyu Bai from the Institute of Environmental Science of Inner Mongolia for their assistance in the field investigation. This work was funded by National Basic Research Program of China (No. 2009CB421104), the Innovation Research Group supported by the National Sciences Foundation of China (No. 40621061), Guangdong Natural Science Foundation (9451065005003254) and the Knowledge Innovation Program of South China Botanical Garden, Chinese Academy of Sciences.

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