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Shift in soil microbial communities with shrub encroachment in Inner Mongolia grasslands, China

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ABSTRACT

The ongoing expansion of shrub encroachment into grasslands represents a unique form of land cover change. How this process affects soil microbial communities is poorly understood. In this study, we aim to assess the effects of shrub encroachment on soil microbial biomass, abundance and composition by comparing data between shrub patches and neighboring herb patches in shrub-encroached grasslands (SEGs) in Inner Mongolia, China. Fourteen SEG sites from two ecosystem types (typical and desert grasslands) were investigated. The phospholipid fatty acid (PLFA) method was used to analyze the composition and biomass of the soil microbial community. Our results showed that the top-soil microbial biomass and abundances of gram-negative bacteria, arbuscular mycorrhizal fungi, and actinomycetes were significantly higher in shrub patches than in herb patches in both typical and desert grasslands ($P < 0.05$). The fungi to bacteria ratio was significantly higher in shrub patches than in herb patches in desert grassland ($P < 0.05$). The microbial biomass was positively associated with mean annual precipitation, total nitrogen and available phosphorus, and negatively associated with mean annual temperature. Our results also indicated that the variation in microbial composition was largely explained by edaphic factors, followed by climate factors. In conclusion, shrub encroachment in Inner Mongolia grasslands has significantly influenced the structure and abundance of soil microbial communities, which makes the microbial communities toward a fresh organic carbon-based structure. This study highlights the importance of edaphic and climate factors in microbial community shifts in SEGs.

1. Introduction

Shrub encroachment is a form of land use or land cover change That is widespread in arid and semi-arid grasslands ecosystems [1–3]. Shrub encroachment into grasslands has been shown to significantly alter the landscape, the microclimate, and above- and below-ground biological processes [4–6]. The transition from grassland to shrub-encroached grassland (SEG) typically leads to a mosaic landscape, with shrub patches interspersed with herb patches [7,8]. The shrubs in SEGs result in a landscape-scale redistribution of resources, such as the uptake of water or nutrition from the surroundings and deep soils, the capture of soil particles transported by winds, and nitrogen fixation by legume shrubs [9,10]. As a result, a fertile island forms in the shrub patch, with significant differences in plant community structure and soil nutrient cycling compared with herb patches in SEGs [3,6,10]. Studies of the effect of shrub encroachment have mainly focused on vegetation and soil chemical cycles [11–14]. However, although below-ground processes are known to be highly influenced by land cover change [12,14], the effects of shrub encroachment on microbial communities remain unclear [15–17].

Some studies have found that shrub encroachment results in greater soil bacterial and fungal biomass and diversity in shrub patches compared with herb patches [14,18], and the composition of arbuscular mycorrhizal fungi (AMF) communities has been found to respond to shrub encroachment [15,19]. However, most of these studies were conducted on North America grasslands or in special

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ecosystems (e.g., peatland, riparian areas, hill prairie) and were conducted at a limited number of sites. Additionally, the effects of shrub encroachment on soil microbial communities may vary with climate, vegetation and soils [2,16]. Soil microbial communities have been found to be directly or indirectly affected by climate, plant community composition and the cycling of soil C, N and other nutrients [20,21]. For example, climate variables such as the mean annual precipitation (MAP) can affect microbial turnover and distribution by regulating soil moisture and substrate availability. MAP can also regulate plant autotrophic respiration and production to influence litter quality and quantity, which in turn influences carbon availability, thereby affecting microbial community composition [21–23]. Changes in above- and below-ground processes with shrub encroachment have been observed but few studies have assessed how the shrub encroachment along with climate, vegetation and soil properties influence the microbial community. Therefore, more studies of arid or semi-arid grasslands, where shrub encroachment typically occurs are needed, and multi-site studies should be introduced.

In China, more than 5.1 million ha of Inner Mongolia meadow, typical and desert grasslands have been encroached by *Caragana* species [24]. In our previous study, shrub encroachment in Inner Mongolia grassland was found to have strong impacts on plant community structure, and these impacts were mainly controlled by precipitation [7]. However, the relationship between shrub encroachment and the soil microbial community and what factors drive this relationship in Inner Mongolia grasslands remain largely unknown. The objectives of the present study were to assess the microbial community responses to shrub encroachment under different climate, vegetation and soil conditions and to identify the biotic and abiotic factors that explain the variation of microbial communities in Inner Mongolia grasslands in China. Using phospholipid fatty acid (PLFA) analysis, we compared the characteristics of the microbial communities between shrub patches and neighboring herb patches in shrub-encroached grasslands by sampling 14 sites within 2 ecosystem types.

2. Materials and methods

2.1. Study sites

The study was conducted in Inner Mongolia, China (41.80°-44.91°N, 111.15°-116.69°E; Fig. 1), and included two ecosystem types: typical grassland and desert grassland. Mean annual temperature (MAT) and mean annual precipitation (MAP) ranged from 1.7 to 4.3 °C and from 164.4 to 8356.3 mm, respectively. Both MAP and MAT data at each sampling site were obtained from 119 climate stations across Inner Mongolia (Climate Database, National Meteorological Bureau of Inner Mongolia, China) using mean values from 1975 to 2005. The vegetation was dominated by *Leymus chinensis* and *Stipa krylovii,* and the encroaching shrubs were mainly *Caragana microphylla* in typical grassland but *C. intermedia* and *Amygdalus pedunculata* in desert grassland.The soil type in Inner Mongolia shrub encroached grassland is mainly sandy clay loam, sandy loam, loam sand and sand in both ecosystem types. The soil type was classified according to the food and agriculture organization of the united nations (FAO) soil taxonomy (http://www.fao.org/soils-portal/soil-survey/soil-classification/en/).

2.2. Soil sampling

We conducted soil sampling at 14 shrub-encroached sites from late September to early October 2013, of which 8 sites were typical grassland and 6 sites were desert grassland (Fig. 1). At each site, we established three 20 × 20 m plots. In each plot, we selected one shrub that exhibited normal growth and collected three soil samples beneath the shrub patch, with an additional soil sample collected approximately 2 m away in the herb patch. In total, there were 14 sampling sites, 42 plots and 168 boreholes that were drilled at depths of 0–10 cm.

2.3. Soil preparation and chemical analysis

All of the sampled soils from the field were divided into two sections: one section was air-dried indoors for chemical analysis, and the other was stored at −20 °C for PLFA analysis. Then, all of the samples were sieved (2 mm mesh) to carefully remove fine roots, seeds and plant materials for subsequent analysis. To determine the water content, a subsample of 10 g of cold storage soils were dried at 105 °C for 24 h. We mixed the 3 samples taken form the same shrub patch before chemical analysis. Three samples from the shrub patch and 3 samples from the herb patch at each site were taken for analysis, and the resulting 84 soil samples were then subjected to PLFA analysis.

The total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (Vario EL III, Elementar, Germany), and the inorganic carbon was measured with a carbonate content analyzer (Eijkelkamp 08.53, Netherlands). The soil organic carbon (SOC) content was obtained by subtracting the total carbon from the inorganic carbon, and the soil pH was determined with a pH meter using a soil: water ratio of 1:2.5. The mineral nitrogen (MN, including NH^{4+} and NO^{3-}) was determined with a continuous flow auto-analyzer (AutoAnalyzer 3, Seal Analytical, UK), and the total phosphorus (TP) and available phosphorus (AP) were determined using the molybdenum-blue method [25].

2.4. PLFA analysis

We used the PLFA method to assess microbial community structure [26,27] as described by Frostegard, Tunlid and Baath [28]. Briefly, the soils (8 g dry weight) were treated with methanol, chloroform, and a citric acid solution to extract the phospholipids. The phospholipids were then separated from the glycolipids and neutral lipids using silica columns. The phospholipids were methylated to fatty acid methyl esters (FAME) with mild acid methanolysis and were identified using a MIDI PLFAD1 calibration mix and peak naming table (MIDI, Newark, USA). The content of each PLFA was obtained by comparing peak areas with a 19:0 FAME internal standard.

A total of 117 lipids were identified in all of the soil samples and were used to calculate the total microbial biomass. Several individual PLFAs were used as indicators of key groups of microbes: i14:0, a15:0, i15:0, i16:0, a17:0, i17:0, 16:1 ω7c, 16:1 ω9c, cy17:0 ω7c, i17:1 ω9c, 17:1 ω8c, 18:1 ω7c, cy19:0 ω7c and cy19:0 ω9c for bacterial, including i14:0, a15:0, i15:0, i16:0, a17:0 and i17:0 for gram-positive bacteria (G^+); 16:1 ω7c, 16:1 ω9c, and 18:1 ω7c for gram-negative bacteria (G^-); 16:1 ω5c for arbuscular mycorrhizal fungi (AMF); 18:1 ω9c and 18:2 ω6c for saprophytic fungi (SF); the sum of AMF and SF as fungal biomass; 20:4 ω6c for protozoa; and 10Me16:0, 10Me17:0 and 10Me18:0 for actinomycetes [28–31]. We calculated the ratios of fungi to bacteria (F: B), G^+ to G^-, mono-unsaturated PLFA to branched PLFA (Mu to Br), cy17:0 to 16:1 ω7c and Cy19:0 to 18:1 ω7c to identify the physiological status of microbial communities under different environmental conditions [29,30,32] such as the “fertile island” effect caused by shrub encroachment, or aridity stress for the different ecosystem type.