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Spatial heterogeneity of microbial community and enzyme activities in a broad-leaved Korean pine mixed forest

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ABSTRACT

Soil microbial communities support a great belowground biodiversity, but our knowledge regarding their spatial patterns and underlying driving mechanisms in small scale is very limited, especially for forest ecosystems. The spatial distributions of microbial community and enzyme activities depending on soil environmental factors were studied using geostatistical tools. 55 soil samples were collected across a 30 m × 40 m plot in a broad-leaved Korean pine mixed forest in the Changbai Mountains. Abundances of total and bacterial PLFAs had stronger spatial dependence than fungal PLFAs. Gram-positive bacteria had stronger spatial dependence than Gram-negative bacteria, suggesting that Gram-negative bacteria are more susceptible to stochastic factors. The proportions of structural variance for the activities of β-1,4-glucosidase (βG), β-1,4-*N*-acetylglucosaminidase (NAG) and acid phosphatase (AP) were 0.997, 0.519 and 0.966, respectively, suggesting that βG and AP had high spatial dependence. Cross-variogram analysis showed that root biomass played a critical role in structuring the spatial distributions of total and bacterial PLFAs. Fungi had close spatial connection with total nitrogen (TN), particulate organic carbon and root biomass within the ranges of 8.2–13 m. The βG, NAG and AP activities were closely spatially connected to the soil organic carbon and TN and were all spatially correlated with fungal abundance. Overall, microbial community and enzyme activities were patchily distributed at small spatial scales. Close spatial connections between microbial communities, enzyme activities, and root biomass and soil variables help to understand the main drivers of belowground soil biodiversity in the forest.

1. Introduction

Soil microorganisms play critical roles in biogeochemical processes, such as soil carbon and nitrogen cycling [1–3] and litter decomposition [4]. Patterns of soil microorganisms are strongly connected to the patchy or heterogeneous nature of the soil that occurs at various spatial scales [5]. Therefore, a better understanding of the spatial patterns of microbial community and their driving factors is necessary for understanding the microbial effects on soil biogeochemistry and ecosystem functions.

Spatial scales within individual studies play an important role in understanding microbial distribution [6]. Soil microorganisms follow clear biogeographic trends across a wide variety of landscapes or across a broad range of spatial scales [7–9]. However, the majority of these studies compared samples at regional or continental scales which reflect substantial variability in environmental conditions, and studies have largely neglected the small spatial scale (e.g. < 10 m) variability among soil microorganisms. A major research topic therefore involves identifying the distances at which the patterns in microbial community structure and activities are manifested, particularly the minimum spatial scales at which spatial patterns can be detected [10]. The spatial autocorrelation of the enzyme activities and microbial biomass was demonstrated to occur at similar scales, typically in the range of tens of centimeters, in *Quercus petraea* forest topsoil [11]. Similarly, a high level of spatial heterogeneity was found in bacterial and fungal abundances and enzymatic activities in temperate mountain forest topsoil at

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a small scale (6 m × 6 m) [12]. However, to date, there is still a gap regarding the knowledge of spatial patterns of soil microbial community and the mechanisms for driving these patterns in a small area.

A series of studies have investigated the effects of abiotic and biotic factors on microbial abundance, community structure, and enzyme activities. Nevertheless, the major driving factors are different depending on the spatial scale of each study [2]. Soil pH was observed to play an important role in shaping the bacterial community structure and diversity at continental scale [13,14] and defines extracellular enzyme activities at a global scale [15]. Soil organic carbon (SOC), as well as its labile fractions, is closely related to microbial community structure and activities [16,17]. SOC is a heterogeneous mixture consisting of numerous fractions varying in its degradability and turnover rate [18]. Labile SOC fractions, such as dissolved organic carbon (DOC) and particulate organic carbon (POC) contents, are proposed to be the dominant energy sources for microbes and to serve as early indicators for responses of soil quality to management practices [19]. Therefore, edaphic factors might have important implications for the spatial patterns of microbes and may help to explain microbial distributions across a small area.

Plant community structure is correlated to the microbial community in a 12 m × 12 m area [20] and can affect microbial community by influencing the quality and quantity of litter and root exudates [21,22]. Fine root distribution is closely related to the soil C content and spatial variability of root biomass, which in turn affect microbial biomass and activities [23]. However, whether plant traits, especially the root biomass and architecture, affect the spatial patterns of microbial community and enzyme activities in the small area still remains unclear.

Forest soils, as an important C sink in terrestrial ecosystems, make a considerable contribution to global C cycling. Spatial heterogeneity is one of the important defining features of forest soils [24]. Detailed information regarding soil microbial spatial distribution patterns should be of considerable importance in facilitating our understanding of the functions and services of forest ecosystems. We hypothesized that (1) microbial community and enzyme activities in the soil are patchily distributed at the plot level; and (2) microbial community, enzyme activities, and edaphic and plant factors exhibit spatial connections with each other. To test these hypotheses, we determined the spatial heterogeneities of soil microbial community and enzyme activities in a small area (30 m × 40 m) in a broad-leaved Korean pine (*Pinus koraiensis*) mixed forest in the Changbai Mountains, China. We also assessed the relative importance of edaphic variables (SOC, TN, labile SOC fractions, soil pH) and plant variables (root biomass) in shaping microbial community structure and enzyme activities.

2. Materials and methods

2.1. Study site and sampling

Soil samples were collected from an original broad-leaved Korean pine mixed forest within the Forest Ecosystem Open Research Station of Changbai Mountains in northeast China (128° 28 ′ E, 42° 24 ′ N). This region is characterized by a temperate monsoon climate, with a mean annual temperature of 2.0 °C and a mean annual precipitation of 700 mm. The soil of this region is dark brown forest soil, which originated from volcanic ash, and it is classified as a Haplic Andosol. The main tree species in our studies plot were *Pinus koraiensis*, *Tiliaamurensis*, *Acer mono*, *Acer barbinerve*, *Corylus mandshurica*, and *Acer pseudosieboldianum*, etc.

For studying the spatial heterogeneity of the microbial community and enzyme activities, 55 soil cores were collected from a relatively flat and homogeneous 30 m × 40 m plot at a soil depth of 0–10 cm within the original broad-leaved Korean pine mixed forest plot in August 2013. The sampling scheme followed the Latin hypercube design [25], and the detailed soil sampling method has been described byTian et al. [26]. The minimum and maximum separation distances between any two soil sampling points were 0.49 m and 44 m respectively, which were not technically fixed, but represent tradeoffs of our plot area, particularly for the max distance. Given the measured attributes (e.g. the roots, soil attributes), the minimum distance in our study seemed a fair tradeoff to get coverage across the whole plot with both longer distances and shorter distances. The soil water content of the collected samples ranged from 42% to 55%. The samples were stored in airtight polypropylene bags and placed in a cooler box at about 4 °C during sampling for transport to the laboratory. Visible roots, rock fragments, and residues were carefully removed by hand. Each soil sample was divided into several subsamples. Those for enzyme activity and DOC concentration analyses were stored at 4 °C for no more than one week. Subsamples for microbial community analysis were stored at −80 °C. Those for Soil organic matter (SOM) and POC analyses were air dried at room temperature. We also sampled fine roots (< 2 mm) from 5 to 10 individuals of each plant species within each site.

2.2. Soil chemical analyses

The SOC and total nitrogen (TN) contents were measured by dry combustion with a Vario Max CN elemental analyzer (Elementar, Germany). DOC was determined based on the method detailed by Jones and Willett [27]. Fifteen grams of dry-weight-equivalent fresh soil was extracted with 60 mL of 0.05 mol L^{-1} K_2SO_4 (soil/solution ratio 1:4) for an hour. Then, the extract was passed through a 0.45-mm membrane filter to obtain the liquid for analyzing the DOC using a Multi 3100 N/C TOC analyzer (Analytik Jena, Germany).

POC was measured by the method reported by Cambardella and Elliott [28]. Twenty grams of air-dried soil (< 2 mm) was dispersed in 100 mL of 5 g L^{-1} sodium hexametaphosphate [$(NaPO_3)_6$]. The mixture was shaken first by hand for 10 min and then on a reciprocating shaker (180 rpm min^{-1}) for 18 h. The soil suspension was poured over a 53-μm sieve, and all substances remaining on the sieve were accepted as particulate organic matter (POM), washed into a dry dish with a small quantity of deionized water, oven-dried at 65 °C, and weighed. The oven-dried soil was subsequently ball-milled and used for analyzing C by dry combustion in a Vario Max CN elemental analyzer (Elementar, Germany).

Soil pH was determined by pH meter after shaking the soil in deionized water suspensions (soil/water ratio of 1:2.5 w/v) for 30 min. Roots were oven-dried at 65 °C to a constant weight after being washed off the soil onto a 2-mm sieve and then weighed. The root biomass used in our study was the oven-dried root weight (g) of each sampling point.

2.3. Soil microbial community analyses

Analysis of phospholipid fatty acid (PLFA), modified from the method detailed by Frostegård et al. [29], was carried out to assess the soil microbial community. Fatty acids were extracted from 8 g of dry-weight-equivalent fresh soil using a one-phase extraction mixture containing chloroform: methanol: phosphate buffer (1:2:0.5). Amounts of fatty acid methyl esters (FAMEs) were analyzed using a Thermo ISQ gas chromatography mass spectroscopy (GC-MS) system (TRACE GC Ultra ISQ), with He as a carrier gas. To identify the individual compounds, relative retention times of them were compared with the commercially available 37 FAMEs (FAME 37 47885-U, Supelco, Inc.) and a mixture of 26 bacterial FAMEs (BAME 26 47080-U, Supelco, Inc.). Concentrations of the individual compounds were quantified by comparing their peaks to an internal standard (nonadecanoic acid methyl ester 19:0). The PFLAs of five microbial groups were distinguished as follows: bacteria (14:0, 15:0, i15:0, a15:0, 17:0, 16:0, i16:0, i17:0, 18:0, 16:1ω7c, cy17:0, cy19:0), Gram-positive [G(+)] bacteria (i15:0, a15:0, i16:0, i17:0), Gram-negative [G(−)] bacteria (16:1ω7c, cy17:0, cy19:0), fungi (18:2ω6,9c) and actinomycetes (10Me 16:0, 10Me 18:0) [30–32].