European Journal of Soil Biology 88 (2018) 65–72

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: www.elsevier.com/locate/ejsobi

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

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ARTICLE INFO

Handling Editor: C.C. Tebbe

Keywords:
Spatial heterogeneity
PLFA
Enzyme activity
Forest ecosystem
Geostatistics

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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https://doi.org/10.1016/j.ejsobi.2018.07.001
Received 19 December 2017; Received in revised form 21 June 2018; Accepted 4 July 2018

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].

2.4. Soil enzyme activities

The activities of three hydrolytic enzymes—β-1,4-glucosidase (βG), β-1,4-*N*-acetylglucosaminidase (NAG), and acid phosphatase (AP)—were measured using the method described by Saiya-Cork et al. [33]. The βG, NAG, and AP play important roles in C, N, and P cycling in the soil, respectively. The enzyme substrates, which were all based on 4-methylumbelliferone (MUF), were 4-MUF-β-D-glucoside for βG, 4-MUF-*N*-acetyl-β-D-glucosaminide for NAG, and 4-MUF-phosphate for AP, respectively.

Assays were carried out in 96-well microtiter plates, with eight replicate wells for each blank, negative control, and quench standard. Soil suspensions were obtained by fully mixing 1 g fresh soil with 125 mL of 50 mmol L^{-1} acetate buffer (pH 5.0). Then, a subsample of 200 μL soil suspension was added to the 96-well microplate, with eight replicate wells for each sample per assay. Additionally, 50 μL substrate solution was added to each sample well. Microplates were incubated at 20 °C for 4 h in the dark, and 10 μL of 1.0 mol L^{-1} NaOH was then added to each well to stop the reaction. Fluorescence was determined by a microplate fluorometer (Synergy^{H4} BioTek, USA) with 365 nm excitation and 450 nm emission filters. Enzyme activities were calculated as the rate of substrate converted in units of nmol g^{-1} h^{-1}.

2.5. Calculations and statistical analyses

Descriptive statistics and one-sample Kolmogorov–Smirnov (K-S) tests for microbial biomass and enzyme activities were performed using SPSS 22.0 for Windows. Lognormal transformations were applied to data that did not coincide with a normal distribution ($p < 0.05$) to further conduct geostatistical analyses.

Geostatistical analyses were performed to analyze the spatial distribution patterns of soil microbial groups and enzyme activities. Semivariance was calculated to show the spatial dependence of the dataset on soil microbial community and enzyme activities with increasing distances between samples. Cross-variograms were used to determine whether two variables exhibited a spatial connection or common microscale variance [20,34]. The trees in our studied plot were spatially distant and the density of trees was low. Therefore, we did not consider the effects of related tree factors, but mainly focused on the effects of edaphic and root, on the spatial heterogeneity on microbial community and enzyme activities. We assessed parameters that can describe the variogram, including nugget variance (C_0), structural variance (C), sill ($C_0 + C$), range (A), and the proportion of structural variance ($C/C + C_0$). The nugget variance is the semivariance at lag zero, representing the experimental error and field variation that are undetectable at the scale of the minimum sampling space [34]. The sill refers to the maximum sample variance [34,35], and the nugget can never be larger than the sill. The range represents the maximum separation distance over which the spatial dependence of samples is apparent. The proportion of structural variance to sill ranges from 0 to 1. Spatial dependence is strong as the ratio approaches 1, while spatial dependence is weak as this value approaches 0 [34]. The coefficient of determination (R^2) and residual sum of squares (RSS) value were used as indicators for how well the variogram model fit the semivariance data. Finally, the spatial distribution patterns of microbial community and enzyme activities were interpolated by standard Kriging based on the best fitted semivariograms model [35]. The software GS + version 7 was used for semivariance, cross-variogram, and Kriging analyses.

3. Results

3.1. Characterization of soil properties root biomass

The average SOC content was 10.4 g kg^{-1}, with the coefficients of variation (CV) of 33.9% (Fig. 1). The average TN content was 0.85 g kg^{-1} with a CV of 30.7%. The average DOC content was 202 mg kg^{-1} while that of POC was 4.9 g kg^{-1}. The variation of pH value (from 5.05 to 6.6, CV of 5.5%) was relatively small compared with the other edaphic factors. The average root biomass was 5.4 g and had the highest variation (CV of 72.0%) among the other factors.

3.2. Characterization of soil microbial community structure and enzyme activities

The mean total PLFAs content was 40.1 nmol g^{-1} with a CV of 42.6% (Fig. 2a). The average abundances of bacteria, G(+) bacteria, and G(−) bacteria accounted for 74.6%, 28.9%, and 23.6% of the total PLFAs, respectively (Fig. 2a). The fungal PLFAs content ranged from 0.08 to 1.46 nmol g^{-1} with a CV of 68.3% (Fig. 2a). The mean βG, NAG, and AP activities were 934, 247, and 1297 g^{-1} h^{-1}, respectively, with CVs of 49.0%, 76.7%, and 43.6%, respectively (Fig. 2b).

3.3. Spatial structure of microbial community and enzyme activities

The parameters of the best-fitted semi-variogram models indicated that the soil microbial abundances and enzyme activities were spatially structured (Table 1). The optimal theoretical variogram models for the total PLFAs and bacteria contents were exponential, while spherical model fits best the abundances of G(−) bacteria, fungi, and actinomycetes. The Gaussian model best fit the G(+) bacterial abundance. The fungal and G(−) bacterial abundances had moderate spatial dependence, while the abundances of total PLFAs, bacteria, G(+) bacteria, and actinomycetes showed strong spatial dependence with nuggets representing 12.6–22.0% of the total variance. Thus, the G(+) bacterial abundance exhibited a stronger spatial dependence than the G(−) bacterial abundance (Table 1).

The best-fitted semi-variogram models for the βG and AP activities were Gaussian, while that for the NAG activity was exponential (Table 1). The βG and AP activities were strongly spatial dependent with nuggets representing 0.3–3.4% of the total variance, while the NAG activity showed a moderate spatial dependence. The ranges for the βG, NAG, and AP activities were 2.9 m, 16 m, and 3.3 m, respectively.

3.4. Kriged estimates of spatial distribution for soil microbial community and enzyme activities

The Kriged maps revealed the spatial variabilities and distributions of microbial community and enzyme activities. Microbial community and enzyme activities had patchy distribution patterns (Fig. 3). The hotspots in the Kriged maps of enzyme activities corresponded poorly with those for microbial abundances. The maps also indicated the lower spatial variability of NAG activity compared to the βG and AP activities.

3.5. Spatial connections among soil microbial community, enzyme activities, and soil and plant factors

The abundances of total PLFAs, bacteria, G(+) bacteria, G(−) bacteria, and fungi had closest spatial connections with DOC, pH, and root biomass (Table 2). The abundance of fungi was also spatially connected to the SOC, TN and POC. Environmental factors (soil and plant parameters) affected enzyme activities (βG, NAG, and AP) closer than the microbial communities (Fig. 4). The activities of βG, NAG, and AP were spatially correlated with the SOC, TN, DOC, pH, and root biomass. The βG activity was spatially connected to the abundances of total PLFAs, bacteria, fungi, and G(+) bacteria, but the NAG and AP activities showed spatial connections only with fungal abundance (Table 2 and Fig. 4).

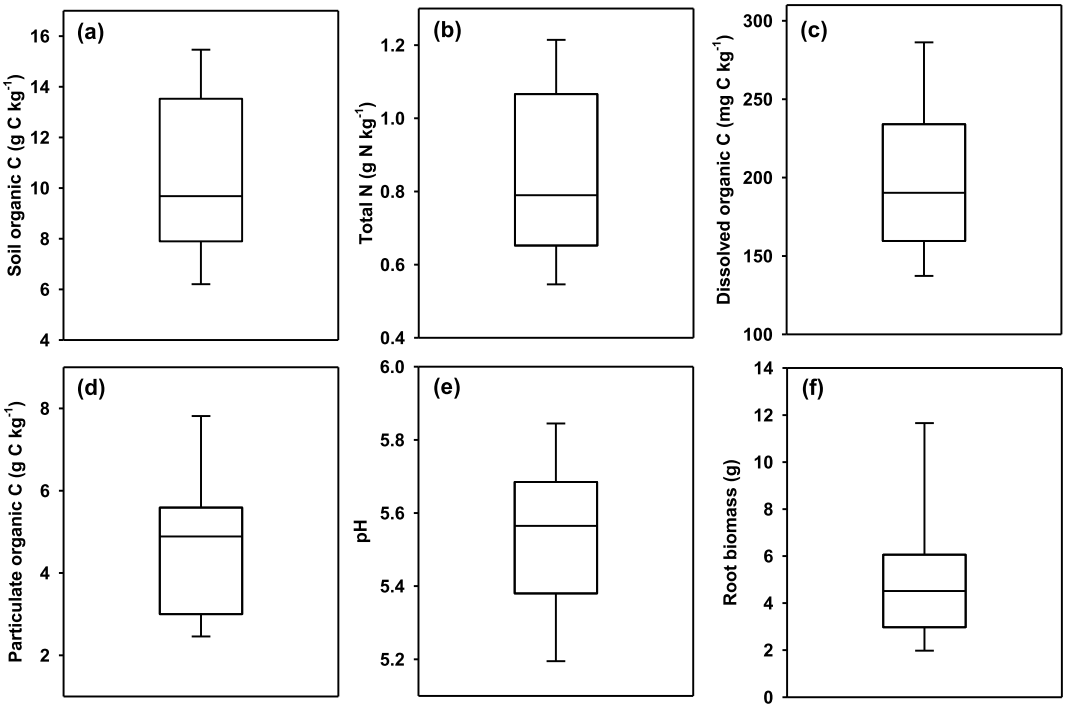

Fig. 1. Boxplots of (a) soil organic C, (b) total N, (c) dissolved organic C, (d) particulate organic C, (e) pH, and (f) root biomass. Boxes illustrate the median and 25th and 75th percentiles. Whiskers represent non-outlier ranges.

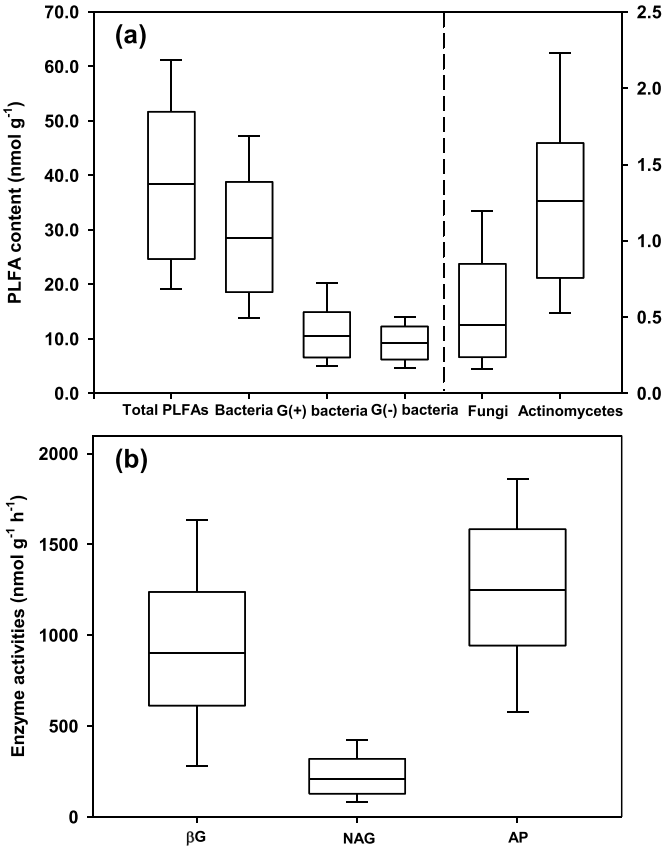

Fig. 2. Boxplots of total PLFA content and PLFAs reflecting (a) microbial groups and (b) enzyme activities. Boxes illustrate the median and 25th and 75th percentiles. Whiskers represent non-outlier ranges.

4. Discussion

4.1. Spatial heterogeneity of microbial community and enzyme activities

The abundances of microbial groups were highly variable and spatially dependent at ranges of 1.0–4.4 m (Table 1, Figs. 2 and 3). The C/C_0 + C ratio was higher than 0.75 for total PLFAs, bacteria, G(+) bacteria, and actinomycetes, indicating a strong spatial dependence of these microbial groups. The spatial autocorrelation of βG and AP activities was stronger than that of microbial abundances, while the NAG activity had a moderate spatial autocorrelation (Table 1). Agreed with previous studies, these results indicated that fungal and bacterial abundances had a high level of spatial variability in forest topsoil [11,12]. Natural forest ecosystems have high levels of spatial heterogeneity both aboveground and belowground as a result of the multilayered vegetation, which contributes to soil heterogeneity via litter input and root distribution [24]. The high spatial heterogeneity of forest topsoils influences the microbial community composition, mainly through soil and litter chemistry, water (re)distribution, and vegetation structure and activity [24,36]. Litter provides substrates and habitats for microorganisms [37]. Roots and rhizosphere denote a large and unique habitat that are specific and rich with organic C and consequently, have high microbial abundance and activities [24,38]. Indeed, high variability of SOC, TN, DOC, POC content and root biomass was observed (Fig. 1). Hence, we speculated that the heterogeneity of the root biomass, rhizosphere resources (e.g. root exudates), and availability of soil nutrients governed the spatial distribution patterns of microbial community and enzyme activities in forest soils.

Fungal abundance had a weak spatial dependence compared with those of all other measured microbial groups, suggesting that fungi are more subject to stochastic noise. This supports a previous work that the fungal community depended on the availability of suitable habitat more than the bacterial community [39]. The high CV of the fungal community also reflected its high variability. One explanation for the high variability of fungi is that fungal hyphae were affected by a large number of environmental factors.

The lower C/C_0 + C ratio of the G(−) bacterial abundance, with a

Table 1
Parameters of the best-fitted semi-variogram models for soil microbial groups and enzyme activities.

	Model	Nugget (C_0)	Sill (C_0+C)	Range (A, m)	C/C_0+C	R^2	RSS
Microbial groups (nmol g^{-1})							
Total PLFAs	Exponential	0.044	0.237	3.33	0.816	0.137	0.034
Bacteria	Exponential	0.054	0.288	3.30	0.814	0.140	0.050
G(+) bacteria	Gaussian	5.239	41.62	1.04	0.874	0.191	0.089
G(−) bacteria	Spherical	0.067	0.253	4.24	0.735	0.236	0.044
Fungi	Spherical	0.019	0.056	4.42	0.662	0.408	0.001
Actinomycetes	Spherical	0.119	0.540	1.30	0.780	0.434	0.244
Enzyme activities (nmol g^{-1} h^{-1})							
βG	Gaussian	0.001	0.354	2.88	0.997	0.674	0.037
NAG	Exponential	0.227	0.473	15.6	0.519	0.508	0.050
AP	Gaussian	0.007	0.190	3.33	0.966	0.721	0.008

range of 4.2 m (Table 1), suggested that G(−) bacteria had a weak spatial dependence and was more sensitive to environmental factors, such as soil nutrients and roots, compared with that of G(+) bacteria, which exhibited a range of 1.0 m. G(+) bacteria are well adapted to soils with low substrate availability [19,40], while G(−) bacteria tend to be more specific to the rhizosphere or resource-rich environments [41]. The C/C_0+C ratio of the NAG activity was lower than those of βG and AP activities, suggesting that the NAG activity had a weak spatial

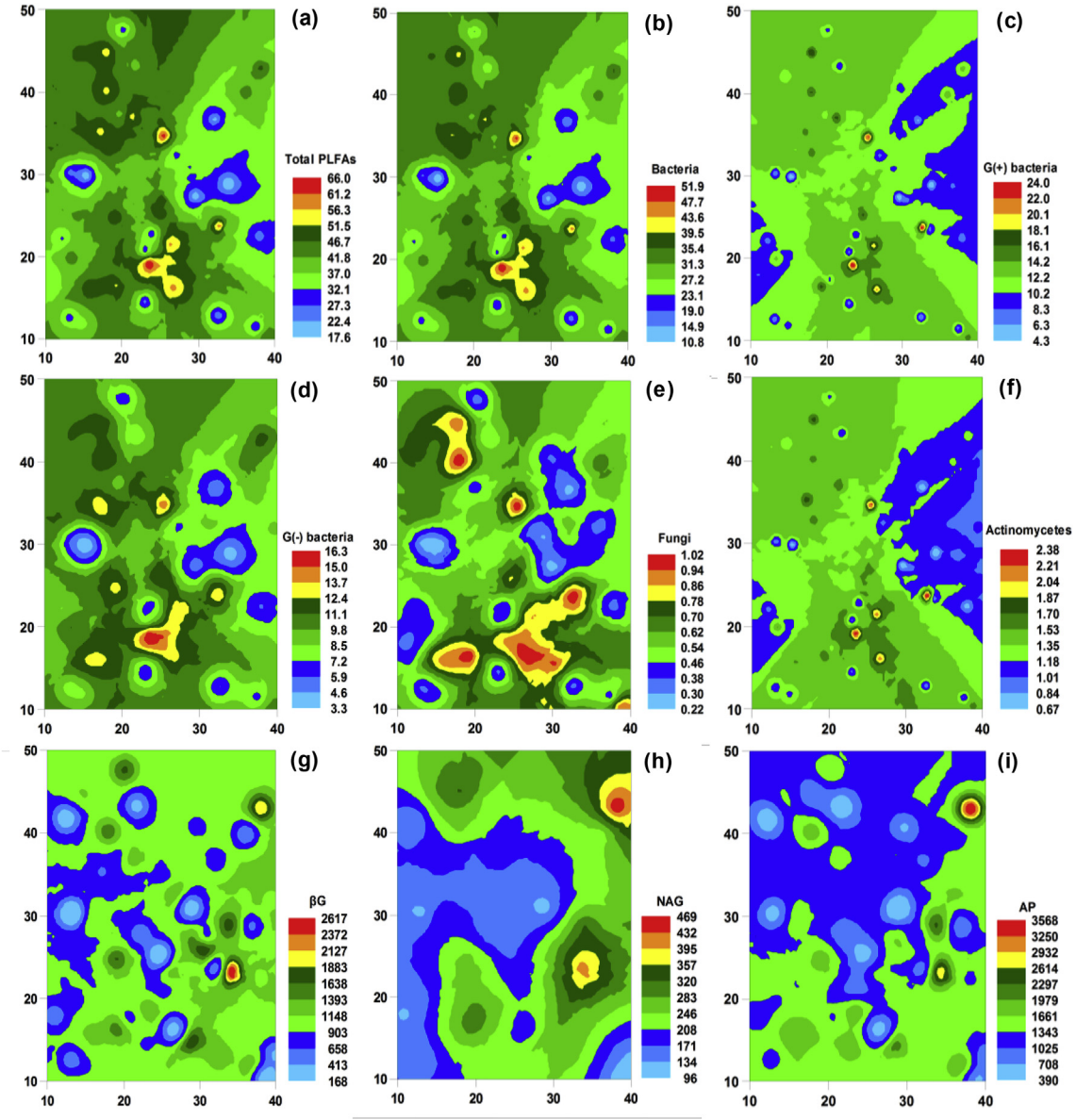

Fig. 3. Kriged maps for the (a) total PLFAs, (b) bacteria, (c) G(+) bacteria, (d) G(−) bacteria, (e) fungi, (f) actinomycetes, (g) βG activity, (h) NAG activity and (i) AP activity. All distances are presented in meters.

Table 2
Parameters of cross-variograms for soil microbial abundances with environmental variables and enzyme activities.

	Model	Nugget (C_0)	Sill (C_0+C)	Range (A, m)	R^2	RSS
Microbial groups × Environmental variables						
Total PLFAs × DOC	Gaussian[a]	0.000	0.010	2.86	0.180	0.004
Total PLFAs × pH	Gaussian	0.000	0.001	3.64	0.112	0.000
Total PLFAs × Root biomass	Gaussian	0.000	0.079	3.15	0.415	0.023
Bacteria × DOC	Gaussian	0.000	0.007	2.82	0.158	0.005
Bacteria × pH	Gaussian	0.000	0.001	3.78	0.114	0.000
Bacteria × Root biomass	Gaussian	0.000	0.085	3.10	0.404	0.028
G(+) bacteria × DOC	Gaussian	0.000	−0.001	1.28	0.392	1.290
G(+) bacteria × pH	Gaussian	0.000	0.200	74.1	0.147	0.020
G(+) bacteria × Root biomass	Gaussian	0.001	0.811	3.36	0.379	6.730
G(−) bacteria × DOC	Gaussian	0.000	0.011	3.22	0.237	0.004
G(−) bacteria × pH	Gaussian	0.000	0.004	3.91	0.116	0.000
G(−) bacteria × Root biomass	Gaussian	0.000	0.087	3.29	0.442	0.023
Fungi × SOC	Exponential	0.000	0.025	40.8	0.293	0.002
Fungi × TN	Gaussian	0.000	0.012	8.21	0.477	0.001
Fungi × DOC	Gaussian	0.000	0.021	3.88	0.249	0.001
Fungi × POC	Gaussian	0.000	0.018	8.19	0.387	0.006
Fungi × pH	Gaussian	0.000	0.002	9.80	0.169	0.000
Fungi × Root biomass	Spherical	0.000	0.045	12.9	0.399	0.004
Microbial groups × Enzyme activities						
Total PLFAs × βG	Spherical	−0.002	−0.017	11.1	0.108	0.016
Bacteria × βG	Spherical	−0.002	−0.019	10.6	0.126	0.019
Fungi × βG	Gaussian	0.000	0.011	11.2	0.106	0.007
G(+) bacteria × βG	Gaussian	0.000	0.018	88.3	0.131	2.400
Fungi × NAG	Gaussian	0.000	0.023	8.50	0.290	0.010
Fungi × AP	Gaussian	0.000	0.008	9.30	0.160	0.002

[a] Only the non-linear models were shown.

dependence and environmental variation affected the enzyme activities involved in soil N cycling. One possible explanation is that the soils in this area had low N availability.

4.2. Spatial connections among microbial groups, enzyme activities, and environmental variables

The abundances of all microbial groups and enzyme activities had strong spatial connections with root biomass (Table 2 and Fig. 4), indicating its critical importance for soil microorganisms. The higher R^2 value in the cross-variograms of the abundances of microbial groups with root biomass (Table 2) indicated their close spatial connection because roots modify the soil environment [24,42]. SOC content is linked to the root C input, including exudation, which changes microbial community composition and activities [43]. High heterogeneity of the root biomass and so, of the rhizosphere resources thus influenced the microbial community structure and activities.

Besides root biomass, SOM and its labile pools also played important role in the spatial distributions of microorganisms. The abundances of all microbial groups were spatially connected to the DOC (Table 2), confirming that DOC is the primary energy source for microorganisms and can affect their activity and abundance [16,19,26]. Litterfall and root exudation in forest soils make notable contributions to DOC formation [26]. The βG, NAG, and AP activities had closer spatial connections to SOC and TN, as evidenced by the higher R^2 of their cross-variogram models (Fig. 4a–f) supporting prior studies reporting the close correlation between SOM and enzyme activities [15]. SOM can alter the porosity, aeration, and aggregate formation as well as provides substrates for microorganisms [44,45]. Likewise, the better fit of the cross-variogram model for fungal abundance with TN and POC at ranges of 8.2 m (Table 2) suggested that TN and POC strongly influence fungal abundance.

Soil pH is a major factor influencing microbial community structure and enzyme activities at continental and global scales [13–15]. Compared with microbial groups, the βG, NAG, and AP activities showed strong spatial connections with pH, revealing that pH was also an important factor for the spatial distribution of microbial activities in a small area. The better fit of the cross-variogram model for βG and NAG activities with pH (Fig. 4j and k) suggested that βG and NAG activities might be more spatially correlated with pH than AP activity. Soil pH affects vegetation composition, which also influences the microbial community [46]. The activities of extracellular enzymes and cell membrane stability can be influenced by pH, consequently affecting the uptake of C and nutrients by microbes [15,47]. Microbial groups have distinct optimal pH ranges for their growth [48]. The optimum pH range, at which each enzyme activity is most efficient, differs among enzymes with the same functions. Thus, pH affects both microorganisms and the enzymes produced by them.

The βG, NAG, and AP activities were all spatially connected to fungal abundance (Table 2). Correlation between the fungal biomass and these three enzyme activities was also confirmed in a *Quercus petraea* forest soil [11]. Fungi are responsible for the production of lignocellulose-degrading enzymes in soils [11,49]. However, relationships between fungal biomass and enzyme activities are not inevitable, as metabolically inactive fungal biomass is also present in soil [11,50]. Overall, our study indicated close spatial connections between microbial communities, enzyme activities, and root biomass and soil variables.

5. Conclusions

Soil microbial community and enzyme activities had high variability and spatial heterogeneity and were patchily distributed within a 30 m × 40 m plot. The total PLFAs, bacteria and G(+) bacteria had strong spatial dependence within ranges of 1.0–3.3 m. Cross-variogram analysis demonstrated that the fungal community had a weak spatial dependence and was more influenced by TN, POC, and root biomass at the ranges of 8.2–13 m. The spatial distributions of total PLFAs, bacteria, G(+) bacteria, and G(−) bacteria were primarily connected to root biomass, while SOC and TN had a better cross-variogram model fit with enzyme activities. The close spatial connection between pH and βG/NAG indicated that pH is another key factor influencing βG and NAG activities. These results clearly showed the close relationships among soil microorganisms and their regulating factors in forest

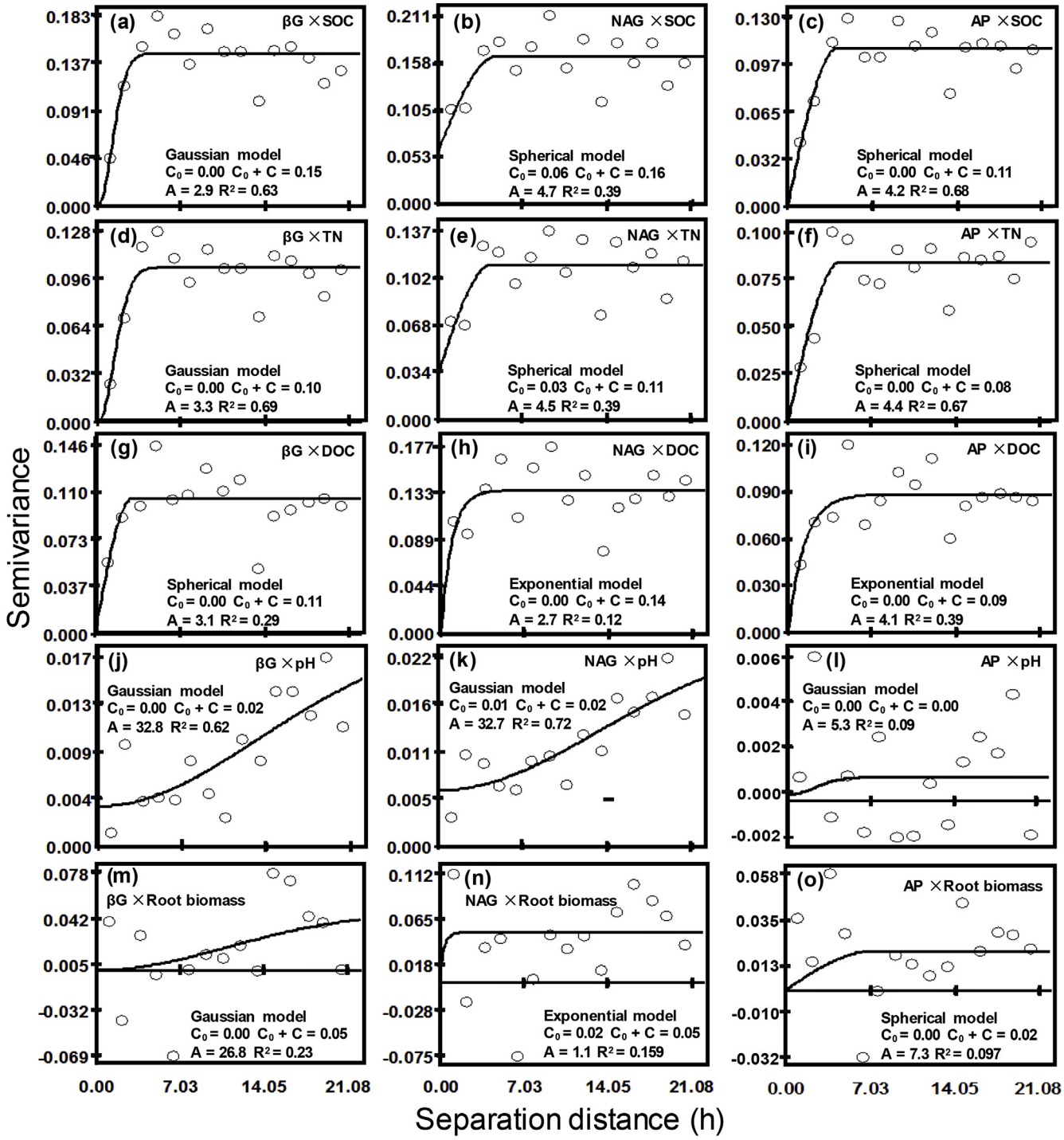

Fig. 4. Cross-variograms for the enzyme activities and soil and plant factors. Only non-linear models are shown. (a)–(c) was cross-variograms for enzyme activities (βG, NAG, AP) with SOC; (d)–(f) was for enzyme activities with TN; (g)–(i) was for enzyme activities with DOC; (j)–(l) was for enzyme activities with pH; (m)–(o) was for enzyme activities with root biomass, respectively.

ecosystems.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant no. 41571130041; 31770560; 31400460), National Key R&D Program of China (2017YFA0604803), and the Youth Innovation Research Team Project (grant no. LENOM2016Q0004). Contribution of YK was supported by the Russian Science Foundation (project No. 18-14-00362).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejsobi.2018.07.001.

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