Linkages between the soil organic matter fractions and the microbial metabolic functional diversity within a broad-leaved Korean pine forest

Jing Tiana, Luke McCormackb, Jingyuan Wanga, Dali Guoa, Qiufeng Wanga, Xinyu Zhanga, Guirui Yua, Evgenia Blagodatskayab, Yakov Kuzyakovb, c

Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences (CAS), 100101 Beijing, China
b Department of Soil Science of Temperate Ecosystems, University of Göttingen, 37077 Göttingen, Germany
c Department of Agricultural Soil Science, University of Göttingen, 37077 Göttingen, Germany

Abstract

Patterns in the spatial distribution of soil microorganisms and the factors that determine them provide important information about the mechanisms regulating diversity and function of terrestrial ecosystems. The spatial heterogeneity of metabolic functional diversity of soil microorganisms was studied across a 30 × 40 m plot and at two soil depths (0–10 cm and 10–20 cm) in a natural, mixed broad-leaved Korean pine (Pinus koraiensis) forest soil in the Changbai Mountains. In addition, we assessed the importance of the quantity and quality (indicated by labile soil organic matter fractions) of soil organic matter in small-scale structuring of soil microbial metabolic functional diversity. Microbial metabolic functional diversity was characterized based on the Biolog profile. The results showed that metabolic activity exhibited moderate spatial dependence, while functional diversity had a much stronger spatial dependence. All soil organic matter fractions including total soil organic matter, dissolved organic matter, particulate organic matter explained 15–27% of the variance in microbial functional diversity in the two soil layers. Among all soil organic matter fractions including total soil organic matter, dissolved organic matter, particulate organic matter explained 15–27% of the variance in microbial functional diversity in the two soil layers. Among all soil organic matter fractions, the labile dissolved organic carbon accounted for the largest amount of variation. Overall, the significant relationship between soil microorganisms and organic matter fractions allows for better understanding the ecological functions governing C cycling and microbial communities in forest ecosystems.

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1. Introduction

Soil microorganisms are important components of terrestrial ecosystems and mediate many ecological processes including carbon (C) and nutrient cycling [1], C sequestration [2,3], litter decomposition and primary productivity [4]. Due to high soil heterogeneity, the spatial distribution of microbial metabolic activity is a key to understand functional variability of soil microhabitats [5]. In addition, information regarding the spatial variability of soil microbial metabolic diversity, and the factors that determine these patterns could lead to more accurate predictions of microbial properties and functions. This in turn would provide important information regarding mechanisms that regulate ecosystem function, including C and nutrient cycling [5,6]. Spatial heterogeneity of soil microbial community structure (e.g. fungal-to-bacterial ratio) and activity (e.g. enzyme activity) can occur at small (e.g., <10 m), local and regional scales [7–9]. However, there is little information regarding the spatial variability of microbial metabolic functional diversity in natural forest ecosystems. Abiotic and biotic factors (such as soil moisture, pH and plant species) play a key role in explaining the spatial variability of microbial communities and activities across a variety of spatial scales [10–13]. For example, plant diversity partially controls microbial diversity and community structure [10,14]. The diversity and richness of soil bacterial communities at continental scales have been largely explained by soil pH [11]. However, most of these studies of spatial structure of soil microbial communities focused on the effects of aboveground vegetation, and soil properties such as...
moisture, pH, and texture. Few studies have investigated the relative importance of belowground substrate resources (e.g., soil organic matter, root biomass) in structuring soil communities [15–17]. These data are particularly lacking in regards to the functional diversity of soil communities [18,19]. Working at a regional scale, Liu et al. [19] demonstrated that root biomass and soil N:P ratio strongly affected microbial diversity. However, the factors influencing soil microorganisms often differ across a gradient of spatial scales [5,20]. Therefore, information on estimating the relative importance of belowground substrate resources in the small-scale structuring of soil microbial metabolic functional diversity is not well understood.

Soil organic matter is a heterogeneous mixture that contains numerous compounds with varying levels of degradability and turnover rates [21]. Labile SOM fractions such as dissolved organic carbon (DOC), dissolved organic nitrogen (DON), particulate organic carbon (POC) and particulate organic nitrogen (PON) are the primary energy source for microorganisms and are characterized by rapid turnover. Further, these labile SOM fractions are thought to be related to the quality of SOM [22,23]. A number of studies have linked SOC, as well as other fractions such as DOC, to soil microbial community structure and activity [24–27]. The catabolic diversity of soil microorganisms was reduced by the decrease of SOC content [28]. In addition, bacterial biomass has also been related to small-scale spatial variations in sediment POC [29].

Natural forest ecosystems generally have heterogeneous distribution of aboveground vegetation [30] and root biomass belowground [31,32]. The heterogeneous nature of plant biomass in these ecosystems may create local patchiness of belowground total and labile SOM (e.g., through the quality and quantity of litter inputs and root exudates releases). Presumably, these total and labile SOM sources are key factors that may help explain the spatial heterogeneity of soil microbial functional diversity.

In the current study, we hypothesized that microbial functional diversity is patchily distributed at the small-scale in soil, and the heterogeneity in soil microbial functional diversity is correlated to the SOM source, and particularly to labile SOM hotspots. To test our hypotheses, the spatial variability of soil microbial functional diversity and belowground SOM source were studied in a mixed broad-leaved Korean pine (Pinus koraiensis) forest in a 30 × 40 m plot at 2 soil depths (0–10 cm and 10–20 cm) in the Changbai Mountains, China. We specifically (1) determined the spatial heterogeneity of soil microbial metabolic functional diversity, and (2) assessed the relative importance of the quantity and quality (labile SOM fractions) of organic substrates in the small-scale structuring of soil microbial metabolic functional diversity.

2. Materials and methods

2.1. Site description and sample collection

The soil sampling was conducted in a long-term plot at the Forest Ecosystem Open Research Station of Changbai Mountains in northeast China in August 2013 (128°28′E, 42°24′N). The site is covered by a multi-storied, uneven-aged, multi-species forest that has been relatively undisturbed for ca. 200 years. The area is dominated by Korean pine (P. koraiensis), Tilia amurensis, Acer mono, Fraxinus mandshurica and Quercus mongolica along with 135 other species [33]. The climate for this region is characterized as temperate monsoon with a mean annual temperature of 2.0 °C and an annual precipitation of 700 mm. The soil is dark brown forest soil originating from volcanic ash, and it is classified as a Haplic Luvisol.

Within the long-term plot, a 30 × 40 m sub-plot was established on a relatively flat and homogeneous portion of the study site. The sampling scheme was based on a Latin hypercube design [34], and a total of 51 sampling points were collected (Fig. 1). This design provided a range of separation distances between any two sampling points with the minimum distance being 0.49 m and the maximum being 44 m. Each point was sampled using a 10 cm diameter soil corer to a depth of 20 cm.

Following the collection of soil cores, the samples were immediately separated into the 0–10 cm and 10–20 cm depth increments. These samples were then stored in airtight polypropylene bags, placed in a cooler box at ~4 °C and transported to the laboratory. Once in the lab, roots and rock fragments were carefully removed and the remaining soil samples were divided into several subsamples. The subsamples for soil microbial functional diversity and dissolved organic matter concentration analysis were stored at 4 °C. Those for soil organic matter and particulate organic matter were air dried at room temperature.

2.2. Soil microbial functional diversity analyses

The soil microbial functional diversity was characterized using the Biolog System [35]. This method has been widely used in determining soil microbial community functional diversity under various conditions [36–38]. 31C substrates associated with plant root exudates were used in the Eco-plate. We divided them into six groups (β-Methyl-D-glucoside, D-Xylose, D-Erythritol, D-Mannitol, N-Acetyl-D-galactosamine, D-Cellobiose and α-D-Lactose), six amino acids (L-Arginine, L-Asparagine, L-Phenylalanine, L-Serine, L-Threonine, and Glycyl-l-glutamic acid), nine carboxylic acids (D-Galactonic acid Y-lactone, D-Galacturonic acid, 2-Hydroxy benzoic acid, 4-Hydroxy benzoic acid, Y-Hydroxy butyric acid, Itaconic acid, α-Keto butyric acid, D-Glucosaminic acid and D-Malic acid), two amines (Phenylethylamine and Putrescine), four polymers (TWEEN 40, TWEEN 80, α-Cyclodextrin and Glycogen), and three miscellaneous (Pyruvic acid methyl ester, D,L-α-Glycerol phosphate and Glucose-1-phosphate). Brieﬂy, 10 g of fresh soil was added to 90 ml of sterilized NaCl (0.85%) solution and shaken at 200 rpm min⁻¹ for 30 min. Ten-fold serial dilutions were prepared, and each well of the Biolog Eco-plates was inoculated with 150 μL of the 10⁻² suspension. The plates were incubated at 30 °C for 10 days, and the
color development was read as absorbance every 24 h with an automated plate reader (VMAX, Molecular Devices, Crawley, UK) at a wavelength of 590 nm.

2.3. Soil chemical analyses

The air-dried samples were first passed through a 2 mm sieve, then ball-milled and analyzed for soil organic carbon (SOC) and total nitrogen (TN) by dry combustion using an EA1108 CHN elemental analyzer (Fisons Instruments, Germany).

Dissolved organic C and N (DOC and DON) were measured according to the method recommended by Jones and Willett [40]. The field-moist soil samples (equivalent to 15 g oven-dried soil) were extracted with 60 ml of 0.05 mol L\(^{-1}\) K\(_2\)SO\(_4\) (soil to solution ratio 1:4) for 1 h. The extract was then passed through a 0.45-μm membrane filter and analyzed for DOC and total dissolved N using a Multi 3100 N/C TOC analyzer (Analytik Jena, Germany). Subsamples were analyzed for NH\(_4\) and NO\(_3\) using an autoanalyzer (TRAACS-2000, BRAN + LUBEUBE, Germany); DON was calculated as the difference between the total dissolved N and the combined NH\(_4\) and NO\(_3\).

Particulate organic C and N (POC and PON) were determined using the method described by Cambardella and Elliott [41]. Twenty grams of air-dried soil (<2 mm) were dispersed in 100 ml of sodium hexametaphosphate (\((\text{NaPO}_3)_6\)) (5 g L\(^{-1}\)). The mixture was shaken by hand during the first 10 min, then on a reciprocating shaker (180 rpm min\(^{-1}\)) for 18 h. The soil suspension was poured over a 53 μm sieve using a flow of distilled water. All material remaining on the sieve, defined as the particulate organic pool, was washed into a dry dish, oven-dried at 65 °C, weighed, ball-milled and analyzed for C and N by dry combustion using an EA1108 CHN elemental analyzer (Fisons Instruments, Germany).

2.4. Calculations and statistical analyses

The 72 h absorbance values were used to calculate the average well color development (AWCD), which indicates the microbial metabolic activity, as follows [35]:

\[
\text{AWCD} = \frac{\sum (C_i - r)}{31}
\]

where \(C_i\) is the absorbance from each well and \(r\) is the comparable absorbance of the control well. Negative \((C_i - r)\) values were set to zero.

The 72 h absorbance values were also analyzed to calculate catabolic diversity (Shannon–Weiner diversity index, \(H^\prime\)) [36]. The microbial community functional diversity indicated by the Shannon–Weiner diversity index was calculated as follows:

\[
H^\prime = \sum p_i \ln p_i
\]

where \(p_i = (C_i - r)/\sum (C_i - r)\).

Principal component analysis (PCA) of the 72 h Biolog data (the relative absorbance) was used to identify the most discriminatory effects on the C source metabolic functions. Redundancy analysis (RDA) was used to quantify the effects of soil environmental data on the variation of soil microbial functional diversity. PCA and RDA analyses were conducted using Canoco for Windows 4.5. Statistical tests using Canoco were run using the Monte Carlo permutation procedure.

Geostatistical analyses were used to visualize the spatial distribution of the soil microbial data. Semivariograms were used to determine the relatedness of soil microbial data with increasing distance between samples. Each variogram provides information of nugget variance (\(C_0\)), structural variance (\(C\)), sill (\(C + C_0\)), range (\(R\)) and the proportion of structural variance (\(C/(C + C_0)\)). The nugget refers to the undetectable experimental error and field variation within the minimum sampling space, while sill represents total spatial variation [42]. The proportion of structural variance (\(C/(C + C_0)\)) varies from 0 to 1, with 0 indicating no measurable spatial structure and 1 indicating that all variance is caused by spatial structure [7,43]. The range indicates the distance at which data are no longer spatially autocorrelated [7,43]. The experimental variogram can be fit with spherical, Gaussian, exponential or linear models [44]. The resulting \(r^2\) values indicate how well the model semivariogram fits the experimental semivariogram. Finally, spatial maps were generated using standard kriging based on the best-fit model semivariograms (spatial interpolation). Semivariograms and kriging were performed with the geostatistical software GS+ version 9.

3. Results

3.1. Spatial variability of soil microbial metabolic functional diversity

Across the plot, metabolic activity (indicated as AWCD) varied between 0.51–1.57 at 0–10 cm and 0.51–0.54 at 10–20 cm soil depth (Table 1). The average value for the Shannon–Weiner diversity index was 2.59 in the upper soil layer, whereas it was 2.65 in the lower soil layer. Both the metabolic activity and diversity index showed higher variability (CV %) in the upper 10–20 cm than in the lower 10–20 cm soil layer. Principal component analyses (PCA) showed that PC1 and PC2 accounted for 58.7 and 11.4% of the total variance at the 0–10 cm soil depth, while 48.6 and 10.8% in 10–20 cm soil depth, respectively (Fig. 2). In both soil layers, PC1 was mainly driven by strong positive correlations with the availability of most of the different C substrates including carbohydrates, carboxylic acids and amino acids \((R^2 > 0.6, \text{Fig. 2})\).

3.2. Spatial variability of soil organic matter and labile C and N fractions

The SOC content varied between 54–184 g kg\(^{-1}\) in the upper layer and 14–161 g kg\(^{-1}\) in the lower soil layer with a CV of 30.9% and 55.3%, respectively (Fig. 3). The pattern of variability for the TN was similar to the SOC, but the concentration was 14 times lower than the SOC (Fig. 3). The average values for the DOC were 198 and 109 mg C kg\(^{-1}\) in the 0–10 and 10–20 cm soil layers, while the DON averaged 23.5 and 13.4 mg N kg\(^{-1}\) in 0–10 and 10–20 cm soil layers, respectively. The POC concentration accounted for 4.48% of the SOC in upper soil layer, whereas it accounted for 4.05% in the lower soil layer (Fig. 3). At the same time, average PON only accounted for 2.04% of TN averaged across two soil layers with concentrations was 0.17 g kg\(^{-1}\) in upper and 0.08 g kg\(^{-1}\) in lower soil layer (Fig. 3).

<table>
<thead>
<tr>
<th>Depth</th>
<th>Mean (g kg(^{-1}))</th>
<th>Min (g kg(^{-1}))</th>
<th>Max (g kg(^{-1}))</th>
<th>SE</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10 cm</td>
<td>54.0</td>
<td>1.00</td>
<td>1.57</td>
<td>0.03</td>
<td>28.8</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>184.0</td>
<td>0.53</td>
<td>0.51</td>
<td>0.001</td>
<td>1.18</td>
</tr>
<tr>
<td>0–10 cm</td>
<td>14.0</td>
<td>2.59</td>
<td>2.19</td>
<td>0.036</td>
<td>11.7</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>161.0</td>
<td>2.65</td>
<td>2.63</td>
<td>0.002</td>
<td>0.60</td>
</tr>
</tbody>
</table>

SE: standard error, CV: coefficient of variations.
3.3. Spatial structure for soil microbial metabolic functional diversity

Results obtained from the variograms and fitted models suggested that the soil microbial functional diversity was spatially structured (Table 2). The optimal theoretical models for metabolic activity (AWCD) and functional diversity (Shannon-Weaver) were exponential and spherical, respectively. The metabolic activity showed moderate spatial dependence with nuggets representing exponential and spherical, respectively. The metabolic activity was 0.42 for metabolic activity, whereas it was <0.25 for metabolic functional diversity. These results suggest that the microbial metabolic activity, especially diversity showed strong spatial dependence. The higher coefficient of variance and higher sill value in the upper soil layer indicates that soil microbial metabolic activity and functional diversity at the soil surface were strongly affected by environmental variability. At the same time, conditions in the 10–20 cm layer were likely more stable which may be responsible for the roughly equal estimates of diversity among the two layers despite greater metabolic activity being measured from the upper soil layer (Fig. 4).

3.4. Effect of soil organic matter and labile C and N fractions on soil microbial metabolic functional diversity

The correlations between soil microbial metabolic functional diversity and SOM fractions data are shown in Fig. 5. The RDA plots indicated that PC1 and PC2 accounted for 21.3% and 2.10% of the total variance in the upper soil layer. All the SOM fractions variables combined explained 27% of the variance in the soil microbial functional diversity (Monte Carlo permutation test) in the 0–10 cm soil layer. The variables explaining the largest statistically significantly amount of variation were the DOC (17%), and then followed by the SOC (9%) and PON (2%).

PC1 and PC2 accounted for 10.3 and 1.80% of the total variance in the 10–20 cm soil layer, respectively. The SOM fractions that were most strongly correlated with the axis included the DOC \((p = 0.008)\) and DON \((p = 0.022)\). The combined SOM fractions explained 15% of the variance in soil microbial functional diversity (Monte Carlo permutation test). The variables explaining the largest amount of variation were the DOC, DON and PON, which together accounted for 13% of the variance in soil microbial functional diversity.

4. Discussion

Soil microorganisms are actively involved in soil biochemical processes, including organic matter decomposition, nutrient mineralization and cycling. Appreciating patterns of microbial functional diversity and the factors that determine these patterns could lead to more accurate predictions of microbial properties and functions within an ecosystem [5,6]. In this study, we investigated whether spatial patterns of microbial functional diversity was related to total SOM and labile C and N fractions across two soil depths in a diverse temperate forest. Community level physiological profiles (CLPP) obtained by Biolog Eco-plate were used to indicate the soil microbial functional diversity in the current study. However, caution is required in interpreting the results due to the arbitrary concentrations and selection of carbon sources used in the plates which do not reflect the natural environment [39]. Despite the disadvantages, this method has been widely used in determining soil microbial community functional diversity under various conditions and provides a ready means to compare microbial activity across space and under a range of ecosystem types [36–38].

As expected, the soil microbial functional diversity showed high variability and spatial autocorrelation at scales of 8–51 m (Table 1, Fig. 2, Table 2 and Fig. 4). The nugget to sill ratio was 0.42 for metabolic activity, whereas it was <0.25 for metabolic functional diversity. These results suggest that the microbial metabolic activity, especially diversity showed strong spatial dependence. The higher coefficient of variance and higher sill value in the upper soil layer indicates that soil microbial metabolic activity and functional diversity at the soil surface were strongly affected by environmental variability. At the same time, conditions in the 10–20 cm layer were likely more stable which may be responsible for the roughly equal estimates of diversity among the two layers despite greater metabolic activity being measured from the upper layer soils (Table 1).

The availability of different organic resources is regarded as one of the most important environmental factors in influencing soil microbial biomass, activity and structure [45]. Previous studies have reported the effect of leaf litter, total SOC and nutrient availability on microbial diversity [46–48]. Others have also found that decreases in SOC reserves following land-use change broadly reduced the metabolic diversity of soil microbial communities [28]. In this study, the combined SOM fractions explained 27% of the variance in soil microbial metabolic functional diversity in the upper soil layer while 15% in lower layer (Fig. 5). This supported our hypothesis that SOM resource plays an important role in determining soil microbial metabolic functional diversity. However, the unexplained variation was also high, which may be caused by additionally environmental factors including plant species, soil pH, soil moisture and other soil characteristics [10–13]. Additionally, a number of studies have documented that changes of soil microbial community and activities could be regulated by C and N availability.
through certain SOM pools [25,27,49]. We also observed high spatial variability of SOM fractions (Fig. 3), this may result in changes of soil community structure at different spatial scales. Therefore, we can not rule out variation in different microbial community structure as a potential cause for the differences in substrate utilization patterns. This also perhaps helps to explain the lower contribution of SOM to the variance of soil microbial metabolic functional diversity in the lower soil layer. As corresponding to the findings that the distribution of microbial community was largely attributed to the decline of SOC content with increasing soil depth [50].

In addition to total SOM, separate fractions of labile SOM can also be important in determining microbial metabolic function and diversity. Among all the SOM fractions, the DOC accounted for the largest amount of variation microbial activity in both soil layers, especially it accounted for 17% variation in upper soil layer (Fig. 5). This finding agrees with previous studies showing that DOC is the primary energy source for microorganisms and affects their activity in the soil [25,27]. Churchland et al. [17] also reported that even in disturbed sites, DOC was the main source of C that influenced the composition of the microbial community and soil respiration rates up to ten meters from a forested to clear-cut site. Another study found that the addition of N changed the amount and biodegradability of soil DOC through stimulating microbial metabolic activity and preferentially utilizing organic acids based on Biolog analyses [51]. The previous studies and the results of this study indicated that changes in the soil microbial community, activity and functional diversity may be regulated by C and N availability through labile SOM pools. Litterfall, root turnover and exudation were interpreted as providing significant contributions to the DOC production in the forest soil, as it consisted mainly of simple sugars and non humic-bound polysaccharides [52,53]. Presumably, the highly heterogeneous nature of aboveground vegetation composition [30], belowground root biomass [31] and rhizosphere resources such as root exudation [16] in forest ecosystems should induce high variability of DOC in the forest soils as was observed in this study.

![Fig. 3. Contents of soil organic matter, dissolved organic matter and particulate organic matter in the 0–10 cm and 10–20 cm soil depths.](image-url)
Finally, the labile plant C entering the DOC fraction through root and mycorrhizal turnover and root exudation could exert a large influence on soil microbial metabolic functional diversity as was found in this study. However, as mentioned above, the selection of C sources used in the Eco-plates does not fully represent the natural environment, e.g. due to the lack of more recalcitrant compounds, therefore, the comprehensively description of the influence of soil organic matter fractions in explaining the microbial functional diversity remains to be determined in future.

### Table 2
Parameters of the best-fitted semi-variogram for metabolic function of soil microbial communities in two soil depths.

<table>
<thead>
<tr>
<th>Model</th>
<th>Nugget ($C_0$)</th>
<th>Sill ($C + C_0$)</th>
<th>Range</th>
<th>$C/C + C_0$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic activity (AWCD/2h)</td>
<td>exponential</td>
<td>0.067</td>
<td>0.135</td>
<td>51.00</td>
<td>0.500</td>
</tr>
<tr>
<td>10–20 cm exponential</td>
<td>0.00002</td>
<td>0.00006</td>
<td>11.27</td>
<td>0.655</td>
<td>0.808</td>
</tr>
<tr>
<td>Metabolic diversity ($H'$)</td>
<td>0–10 cm spherical</td>
<td>0.0002</td>
<td>0.072</td>
<td>8.82</td>
<td>0.997</td>
</tr>
<tr>
<td>10–20 cm spherical</td>
<td>0</td>
<td>0.00040</td>
<td>28.70</td>
<td>0.997</td>
<td>0.973</td>
</tr>
</tbody>
</table>

Units for nugget, sill, and range are in meters.

*Fig. 4.* Kriging maps for metabolic activity (top) and Shannon-Weaver diversity (bottom) in the 0–10 cm (the left column) and 10–20 cm (the right column) soil depths.

5. Conclusions

This study showed that the soil microbial metabolic functional diversity had high variability and strong spatial autocorrelation at the sub-plot level. The findings also demonstrated that SOM fractions, particularly the labile DOC fraction, can explain a significant portion of the variation of soil microbial functional diversity, especially in the soil surface. The importance of the SOM fractions in structuring soil microbial functional diversity provides insight into the close relationship between soil microorganisms and SOM, which may help us to better understand ecological processes.
including C cycling in forest ecosystems. It remains necessary to quantify the relative importance of other environmental factors including plant species, soil pH and soil moisture as well as other biological processes including soil microbial community in structuring soil microbial functional diversity in future.

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