

Dear Editor,

We deeply appreciate you for giving us an opportunity to improve our manuscript again. We would like to thank all of you and the two reviewers for the thoughtful and valuable suggestions on our manuscript entitled "**Plant functional traits determined the latitudinal variations in soil microbial functions: evidence from a forest transect in China**" [ID: bg-2018-499]. According to the comments and suggestions, we have carefully revised our manuscript. We have followed the formatting requirements as presented in the Guide for Authors. We have uploaded the document of the responses to reviewer and a clean manuscript. Here are the point-to-point responses (color-coded blue) to Editor's and **Reviewer's** comments (color-coded black). The page and line numbers mentioned here refer to our latest revision of our manuscript simultaneously submitted.

Reviewer #1:

Authors significantly improved this manuscript using reviewers' comments to clarify four hypotheses that motivate the work and explain numerous abbreviations. The large number of measurements made on each forest soil sample provides a great deal of correlative insight into factors that affect respiration potential. A few questions remain, which I expect the authors can address through clarification or some minor recalculations.

1) The Biolog and respiration rate experiments measured only aerobic activities. While these may be the most significant contributors to C mineralization, the authors should clarify that anaerobic microbial decomposition mechanisms may be important for intermittently saturated soils (such as CB at the time of sampling – Table 2) and sites with high mean annual precipitation. Such activities may have different effects on organic acid and DOC concentrations.

Response: The SOM decomposition and Biolog analysis were conducted in 2013-2014. We have no samples now to conduct the experiment about anaerobic microbial decomposition. We have added discussion about the anaerobic microbial decomposition mechanisms as “Some forest soils were intermittently saturated (such as CB, Table 2) or high with mean annual precipitation. Under the anaerobic conditions, soil organic decomposition is mediated by a complex suite of microbial processes (Meronigal et al., 2004). The fermentation products including low molecular weight alcohols, fatty acids, and dihydrogen can serve as substrates for anaerobic respiration using a variety of alternative terminal electron acceptors in place of oxygen to mineralize organic carbon to carbon dioxide (CO<sub>2</sub>). Therefore, the soil organic matter decomposition rate might be slow in these anaerobic conditions. These results demonstrate that the reduction of organic matter is a key step of anaerobic decomposition (Keller and Takagi, 2013).” (P13-14, Line 356-364)

2) Were the significance tests of Pearson correlation coefficients (Figs 5-7 & S2) corrected for multiple hypothesis testing? Please explain in methods.

Response: All P values were adjusted using the Bonferroni correction to account for multiple comparisons. We have added the explanation in the methods. (P10, Line 266-267)

3) As explained in the cited Biolog methods paper by Guckert et al. Biolog results must be corrected for inoculum size. Others have used Average Well Color Development (AWCD) to normalize values, but that does not appear to be done here. Did the authors normalize values before integration for Figure 3 using microbial biomass or another indicator of inoculum size?

Response: 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.(P9,Line 238-242)

General comments

1. In previous comment I mentioned that “Please set more appropriate and clearer hypothesis”. And in this version, numbers of hypothesis increased from 3 to 4. I think multiple hypotheses might cause complex or complicated discussion and conclusion even in case two. In this version, authors added “information about the relationships between soil microbial communities and their functions in natural ecosystems is urgently needed” as necessity of this research after presenting 2 conflicting hypotheses. I agree with the opinion of

field data-oriented validation's necessity. But simple hypothesis or aim to conduct 4 testing is required. Moreover, in case conducting 4 testing, relation between these testing must be described. For example, in section 4.2, hypothesis 1 seemed to be described as counter hypothesis of 2. And these hypotheses should be integrated in conclusion. I could not find any comment about latitudinal variations in conclusion.

Response: we have rewritten our hypotheses as “(1) The profiles of soil microbial substrate use vary along a latitudinal gradient, (2) biogeographical patterns of soil microbial substrate use are constrained by climate and plant functional traits, and (3) different soil microbial communities may have substrate use profiles and SOM decomposition rates.” (P6, Line 165-168)

We have rewritten this part as “There was no significant latitudinal pattern in the soil total microbial metabolic intensity (S) in our study, which was inconsistent with hypothesis (1). However, there was significant latitudinal variation in the use of the six different carbon sources. The soil microbial use of carbon sources were relatively higher in tropical and subtropical climatic area than those in temperate climatic area.” (P14, Line 374-377)

We have added description on the latitudinal pattern of soil microbial use of different carbon sources in the conclusion part as “There was no obvious latitudinal pattern for the soil total microbial metabolic intensity. However, soil microbial use of the different carbon sources varied with the latitude except the

amines carbon source. Soil microbial use of carbon sources were relatively higher in tropical climatic areas.” (P18, Line 491-494)

2. Title of this MS include “latitudinal variations”. And I mentioned as “I could not know about latitudinal distribution in soil microbial functions but comparison between forest and forest types”. In this version authors mentioned that “plant traits have more influence on soil microbial functions than climate” and if it is correct, I think title of this MS can be “Plant functional traits determine soil microbial function”. However, in this case, careful discussion must be needed. Does only MAT represent variation of climate? How do authors count indirect effect of climate (at least climate condition regulate forest type)? Etc. For example, in the paper of Tu(2016), their conclusion was not “climate is not main regulator” but “This study suggests that even microbial subcommunities follow general biogeographic patterns”. And they used several indexes reflecting latitudinal gradient.

Response: we have added the description on the latitudinal pattern of soil microbial functions as “There was no significant latitudinal pattern in the soil total microbial metabolic intensity in our study, which was inconsistent with hypothesis (1). However, there was significant latitudinal variation in the use of different carbon sources. The soil microbial use of carbon sources were relatively higher in tropical and subtropical climatic area than those in temperate climatic area.” (P14, Line 374-377)

We have conducted the path analysis relevant environmental variables to soil microbial use of different carbon sources (Table S4-9, supporting information). We found that the MAT and MAP had indirect effect on the soil microbial use of different carbon sources through influencing the soil temperature, soil total nitrogen, and total phosphorus. (P14, Line 381-383)

We have written this part as “Consistent with hypothesis (2), soil microbial functions were similar in closely related to the CWM values of the tree species (Fig. 4). However, only MAP of climatic factors had a moderate effect on the soil microbial function (Fig. 4). Climate may have indirect effect on the latitudinal pattern of different carbon sources use. In our study, MAT and MAP affected the six different carbon sources use indirectly by influencing the soil temperature (ST), soil total nitrogen (TN), and total phosphorus (TP) (Table S4-9). It was reported that climate was the main environmental parameters driving the latitudinal patterns of community-level leaf traits through the regulation of species composition (Wang et al., 2016). Woody plant species with evergreen broadleaves dominated in the tropic regions characterized by hot, humid, and infertile habitats showed low CWM values of leaf N and SLA and high CWM values of LDMC (Table S2).” (P14, Line 378-387)

#### **Technical comments**

1. (P2, L36 37 38) Meaning of intensities are not clear.

Response: They means the soil microbial substrate use intensities. We have added the full meanings of them in the text. (P2, Line 36-38)

2. (P2, L39) What index do authors use as Plant functional traits and climate?

Response: the indexes included in the plant functional traits were leaf dry matter content, leaf C concentrations, and leaf N concentrations. The climate index was the mean annual precipitation. We have added them in the abstract. (P2, Line 39-41)

3. (P3) Abbreviations needs climate conditions.

Response: Done (P3, Line 65-67).

4. (P17, L462) Contribution of silt content is totally common.

Response: we have rewritten this part as “For the first time, we showed that CWM values of plant traits explained variations in soil microbial C substrate use at the regional scale. Additionally, the fine-grained soils with high silt contents were higher in the microbial C substrate use.” (P18, Line 494-498)

Thanks again to you and the two reviewers for the thoughtful and thorough comments.

We hope that our revisions will be satisfactory, and we are very happy to work with you and the reviewers to resolve any remaining problems.

Yours sincerely,

Zhiwei Xu, Guirui Yu, Qiufeng Wang, Xinyu Zhang, Ruili Wang, Ning Zhao,  
Nianpeng He, Ziping Liu

## A list of all relevant changes

1. Affiliations of the first authors (P1,line7-12)
2. Abstract: (P2,line36-41)
3. Abbreviations (P3,line65-67)
4. Introduction (P6,line165-168)
5. Material and methods(2.4(P9,line238-242); 2.6(P10,line266-267;  
line275-278)
6. Result (P11,line281,287-289)
7. Discussion (4.1 (P13-14,line356-364); 4.2(P14,line374-387)
8. Conclusion (P18,line 491-498)
9. References (P20,line 574-575; 594-595; P21,line 673-674)



# 1 **Plant functional traits determine latitudinal variations** 2 **in soil microbial function: evidence from forests in** 3 **China** 4

5 Zhiwei Xu<sup>1,2,3</sup>, Guirui Yu<sup>4,5,\*</sup>, Qiufeng Wang<sup>4,5,\*</sup>, Xinyu Zhang<sup>4,5</sup>, Ruili Wang<sup>6</sup>, Ning Zhao<sup>7</sup>, Nianpeng  
6 He<sup>3,4</sup>, Ziping Liu<sup>1,2,3</sup>

7 1. Key Laboratory of Geographical Processes and Ecological Security of Changbai Mountains,  
8 Ministry of Education; School of Geographical Sciences, Northeast Normal University,  
9 Changchun, 130024, China

10 2. Institute for Peat and Mire Research, Northeast Normal University, Changchun, 130024, China

11 3. Jilin Provincial Key Laboratory for Wetland Ecological Processes and Environmental Change in  
12 the Changbai Mountains, Changchun, 130024, China

13 4. Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic  
14 Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 10010, China.

15 5. College of Resources and Environment, University of Chinese Academy of Sciences, Beijing,  
16 100190, China

17 6. College of Forestry, Northwest A&F University, Yangling, 712100, China

18 7. Key Laboratory of Remote Sensing of Gansu Province, Heihe Remote Sensing Experimental  
19 Research Station, Cold and Arid Regions Environmental and Engineering Research Institute,  
20 Chinese Academy of Sciences, Lanzhou 730000, China

21  
22  
23 \* Corresponding author at: Key Laboratory of Ecosystem Network Observation and Modeling,  
24 Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences,  
25 Beijing 100101, China.No. 11A, Datun Road, Chaoyang District, Beijing, 100101, China. Tel.: +86-  
26 10-64889268; fax: +86 10 64889432.

27 E-mail: [yugr@igsnr.ac.cn](mailto:yugr@igsnr.ac.cn) (G. Y.), [qfwang@igsnr.ac.cn](mailto:qfwang@igsnr.ac.cn) (Q. W.)

28 **Abstract.** Plant functional traits have increasingly been studied as determinants of ecosystem  
29 properties, especially for soil biogeochemical processes. While the relationships between biological  
30 community structures and ecological functions are a central issue in ecological theory, these  
31 relationships remain poorly understood at the large scale. We selected nine forests along the North–  
32 South Transect of Eastern China (NSTEC) to determine how plant functional traits influence the  
33 latitudinal pattern of soil microbial functions, and how soil microbial communities and functions are  
34 linked at the regional scale. We found that there was considerable latitudinal variation in the profiles  
35 of different substrate use along the NSTEC. Specifically, we found that the substrate use by  
36 microorganisms was highest in the temperate forest soils (soil microbial substrate use intensities of  
37 10–12), followed by the subtropical forest soils (soil microbial substrate use intensities of 7–10), and  
38 was least in the coniferous forest soils (soil microbial substrate use intensities of 4–7). The latitudinal  
39 variation in soil microbial function was more closely related to plant functional traits (leaf dry matter  
40 content, leaf C concentrations, and leaf N concentrations,  $P=0.002$ ) than climate (Mean annual  
41 precipitation,  $P=0.022$ ). The soil silt, leaf dry matter, and leaf C and N contents were the main  
42 controls on the biogeographical patterns of microbial substrate use in these forest soils. The soil  
43 microbial community structures and functions were significantly correlated along the NSTEC. Soil  
44 carbohydrate and polymer substrate use were mainly related to soil G<sup>+</sup> bacterial and actinomycic  
45 PLFAs, while the use of amine and miscellaneous substrates were related to soil G<sup>-</sup> bacterial and  
46 fungal PLFAs. The enzyme production varied with changes in the soil microbial communities. The  
47 soil enzyme activities were positively correlated with the bacterial PLFAs but were not correlated  
48 with the fungal PLFAs. The soil organic matter (SOM) decomposition rates were significantly higher  
49 in the temperate forests than in the subtropical and tropical forests, emphasizing the rapid  
50 degradability of high-energy substrates, such as soil microbial biomass carbon, carbohydrates, and  
51 amino acids. The SOM decomposition rates were significantly and negatively related to soil dissolved  
52 organic carbon concentrations, carboxylic acids, polymers, and miscellaneous substrate use. The  
53 relationships between soil PLFAs and microbial substrate use, enzyme activities, and SOM  
54 decomposition rate show that, as the soil microbial community structure changes, soil  
55 biogeochemical processes also change.

56	<b>Abbreviations</b>	
57	NSTEC	North-South Transect of Eastern China
58	AWCD	Average well color development
59	RDA	Redundancy analysis
60		Soil microbial community
61	PLFAs	Phospholipid fatty-acids
62	G <sup>+</sup>	Gram positive bacteria
63	G <sup>-</sup>	Gram negative bacteria
64	F/B	Fungi/Bacteria
65		<b>Climate conditions</b>
66		<b>MAT: Mean annual temperature</b>
67		<b>MAP: Mean annual precipitation</b>
68		Soil enzyme activities
69	BG	β-glucosidase
70	NAG	N-acetylglucosaminidase
71	AP	Acid phosphatase
72	LAP	Leucine aminopeptidase
73		Soil properties
74	SMC	Soil moisture content
75	SOM	Soil organic matter
76	SOC	Soil organic carbon
77	TN	Total Nitrogen
78	DOC	Dissolved organic carbon
79	MBC	Microbial biomass carbon
80	Silt	Soil silt fractions (<53 μm)
81		Plant functional properties:
82	CWM	Community-weighted means
83	SLA	The specific leaf area
84	LDMC	Leaf dry matter content
85	Leaf C	Leaf C concentrations
86	Leaf N	Leaf N concentrations

## 87 **1 Introduction**

88 The catabolic diversity of soil microbial communities is a useful indicator of how microbial functions  
89 adapt to environmental stress. It can be used to test fundamental questions about soil biological  
90 resistance and resilience (Jagadamma et al., 2014; Swallow and Quideau, 2015), and help us  
91 understand the role of microbial communities in different environments (Preston-Mafham et al.,  
92 2002). Biological community structure and function are intimately linked in ecological processes,  
93 and their relationships are a central issue in ecological theory (Talbot et al., 2014). Therefore, a major  
94 goal in ecological research is to identify and understand the mechanisms and relationships that control  
95 the structure and function of microbial communities over large spatial scales.

96 Numerous studies have documented how environmental and anthropogenic perturbations  
97 impact on the structure, diversity (Tu et al., 2016; Zhou et al., 2016), and enzyme activities (Peng  
98 and Wang, 2016; Xu et al., 2017) of soil microbial communities, and have reported that forests in the  
99 same climatic zone develop similar microbial communities. Other researchers have examined spatial  
100 patterns in soil microbial function at different scales. For example, Tian et al. (2015), from their study  
101 of Changbai Mountain, China, found that the soil microbial metabolic activity and functional  
102 diversity were spatially dependent. Others reported that soil microbial activities varied by forest type,  
103 with high local variation and significant separation along regional climate gradients (Brockett et al.,  
104 2012; Cao et al., 2016). Soil microbes from different climatic zones have different affinities for  
105 carbon substrates. For example, microorganisms from boreal pine forest soils used carboxylic acids  
106 more efficiently, but decomposed amino acids much less efficiently, than microorganisms from  
107 temperate forest soils (Klimek et al., 2016). The soil microbial metabolic abilities are also influenced  
108 by the dominant tree species, through the production of chemically-unique litter and root exudates,  
109 and the soil physico-chemical properties (Menyailo et al., 2002). Despite this, because of limitations  
110 in analytical methods, questions still remain about how soil microbial functions vary at the regional  
111 scale.

112 The functional diversity of soil microbial communities is regulated by physico-chemical soil  
113 properties (Gartzia-Bengoetxea et al., 2016), climate (Cao et al., 2016), and the composition of plant  
114 cover (Sherman and Steinberger, 2012). For example, the geographic patterns in soil microbial

115 activities mainly reflect the climate, soil pH, and total phosphorus concentrations over large  
116 geographic scales (Cao et al., 2016). Research has shown that substrate-induced respiration rates  
117 were higher in soil microbial communities that developed under beech and holm oak forests than  
118 under oak and pine forests (Gartzia-Bengoetxea et al., 2016). Plant functional traits have increasingly  
119 been studied as determinants of ecosystem properties, especially for soil biogeochemical processes  
120 (De Vries et al., 2012; Pei et al., 2016). Soil bacteria phospholipid fatty-acids (PLFAs) were found  
121 to be positively correlated with the community-weighted means (CWM) of plant functional traits  
122 (leaf nitrogen (N) concentration) (De Vries et al., 2012). The plant leaf dry matter content and the  
123 leaf carbon (C) to nitrogen (N) ratio both influence the multivariate soil microbial community  
124 structure, and these factors positively promote the abundances of specific microbial functional groups  
125 (Pei et al., 2016). Limited soil resources, particularly in tropical forests, mean that soil  
126 microorganisms may be more reliant on plants than soil for C and nutrients via rhizosphere exudation  
127 or litter production, which varies among plant species (Russell et al., 2007; Raich et al., 2014; Waring  
128 et al., 2015). While soil functional diversity has been used as an indicator of microbial metabolic  
129 potential, there have been few studies of the integrated effects of climate, vegetation, and soil  
130 substrate availability on large-scale soil microbial functional diversity.

131         Although the functional characteristics of soil microorganisms are at least as important as their  
132 patterns of community structure in biogeochemical studies, the links between microbial community  
133 structure and microbial functions are poorly understood. There are two current hypotheses about how  
134 microbes determine ecosystem process rates. In functional redundancy, different microbes perform  
135 the same function and so changes in the microbial community structure do not necessarily lead to a  
136 change in soil function (Balsler and Firestone, 2005; Strickland et al., 2009). For example, Banerjee  
137 et al. (2016) showed that the abundance of different bacterial and fungal groups changed by up to  
138 300-fold under straw- and nutrient-amended treatments but that the decomposition rate remained  
139 similar, indicating possible functional redundancy. The functional redundancy hypothesis has  
140 recently been challenged by a counter-hypothesis, referred to as functional dissimilarity, which  
141 suggests that diversity brings stability, and that every species plays a unique role in ecosystem  
142 function (Fierer et al., 2007; Waldrop and Firestone, 2006). Soil microbial community composition  
143 therefore, combined with environmental variables, may ultimately determine ecosystem process rates.

144 Waldrop and Firestone (2006) showed that gram positive bacteria ( $G^+$ ) were mainly responsible for  
145 the decomposition of pine needles and soil organic matter, but gram negative bacteria ( $G^-$ ) were  
146 mainly responsible for the decomposition of starch and xylose, which are easy to break down.  
147 Philippot et al. (2013), when studying the diversity of denitrifiers, showed that the loss of microbial  
148 diversity could result in decreases of between 4- and 5-fold in denitrification activity. In the  
149 Mediterranean, losses in the mass of decomposing leaf litter from shrub species accelerated as  
150 detritivore assemblages became more functionally dissimilar (Coulis et al., 2015). Research to date  
151 suggests that the different microbial communities will result in variations in soil microbial function  
152 and soil biochemical processes, so information about the relationships between soil microbial  
153 communities and their functions in natural ecosystems is urgently needed.

154 The North-South Transect of Eastern China (NSTEC) extends from a cold temperate coniferous  
155 forest in the north to a tropical rainforest in the south, and includes almost all the forest types found  
156 in the Northern Hemisphere (Zhang and Yang, 1995) (Fig. 1 and Table 1). This transect, therefore,  
157 provides the optimal environment for investigating large-scale geographical patterns in microbial  
158 communities and their responses to environmental changes. In this study, we examined spatial  
159 patterns in soil labile C concentrations, soil organic matter (SOM) decomposition rates, and  
160 metabolic activity and functional diversity of microbes in nine forest biomes along the NSTEC. We  
161 assessed how abiotic factors, such as climate, soil physical and chemical properties, and biotic factors,  
162 in the form of community-weighted means (CWM) of plant functional traits, contributed to soil  
163 functional diversity at the regional scale. We also examined the links between soil microbial  
164 community structure (PLFAs) and function (SOM decomposition rate, enzyme activities, and  
165 microbial substrate use). We tested four hypotheses in this study, as follows: (1) The profiles of soil  
166 microbial substrate use vary along a latitudinal gradient, (2) biogeographical patterns of soil  
167 microbial substrate use are constrained by climate and plant functional traits, and (3) different soil  
168 microbial communities may have substrate use profiles and SOM decomposition rates.

## 169 **2 Material and methods**

### 170 **2.1 Study area and soil sampling**

171 We selected nine forest ecosystems along the NSTEC, namely Huzhong (HZ), Liangshui (LS),  
172 Changbai (CB), Dongling (DL), Taiyue (TY), Shennong (SN), Jiulian (JL), Dinghu (DH), and  
173 Jianfeng (JF) (18°44'–51°46'N, 128°53'–108°51'E) (Fig. 1, Table 1). Further information about the  
174 soil types and sites has been documented previously by Xu et al. (2017). Forest soils have been  
175 classified following the U.S. soil taxonomy and are described in Table 1 (Soil Survey Staff, 2010),  
176 where information about the climate and the dominant vegetation at each site is also presented.

177 Soil samples were collected from four random plots at each site in July and August 2013, as  
178 described previously by Xu et al. (2017). Briefly, we established four sampling plots measured 30 ×  
179 40 m and collected soil samples from a depth of between 0 and 10 cm at between 30 and 50 points  
180 in each plot along an S-shape. On return to the laboratory, the fresh soil samples were immediately  
181 sieved through a 2-mm mesh and subdivided into three subsamples. One subsample was stored briefly  
182 at 4 °C until analysis for soil enzyme activities and soil pH. Another was stored briefly at –20 °C  
183 until analysis for PLFAs and Eco-Biolog. The third was air-dried, sieved through a 0.25 mm mesh,  
184 and analyzed for soil nutrients.

## 185 2.2 Soil chemical analyses

186 Soil pH was measured at a soil-to-water ratio of 1:2.5. The soil moisture content (SMC) was  
187 measured gravimetrically on 20 g fresh soil that was oven-dried at 105 °C to constant weight  
188 immediately on arrival at the study sites' laboratories (Liu et al., 2012a). Soil organic carbon (SOC)  
189 and total N (TN) concentrations were determined by dry combustion of ground samples (100-mesh)  
190 in a C/N analyzer (Elementar, Vario Max CN, Germany). Total phosphorus (TP) was determined  
191 with a flow injection auto-analyzer following digestion with H<sub>2</sub>SO<sub>4</sub>-HClO<sub>4</sub> (Huang et al., 2011).  
192 After extraction with distilled water at a soil:distilled water ratio of 1:5, dissolved organic carbon  
193 (DOC) concentrations were determined by Liqui TOC II (Elementar, Liqui TOC II, Germany) (Jones  
194 and Willett, 2006). Soil microbial biomass carbon (MBC) was measured using the chloroform  
195 fumigation and direct extraction technique (Vance et al., 1987). A conversion factor of 2.64 was used  
196 to convert extracted C to biomass C. The silt fractions (<53 μm) of the samples were separated by  
197 wet-sieving and then were freeze-dried in the laboratory, as described by Six et al. (2000). The soil  
198 properties are shown in Table 2. We followed the method described by Bååth et al. (2003) for PLFA

199 analysis and PLFAs are expressed in units of  $\text{nmol g}^{-1}$ . The four enzymatic activities of  $\beta$ -glucosidase  
200 (BG), N-acetylglucosaminidase (NAG), acid phosphatase (AP), and leucine aminopeptidase (LAP)  
201 responsible for soil C, N, and phosphorous cycling, were measured following the procedure outlined  
202 in Saiya-Cork et al. (2002) and are expressed in units of  $\text{nmol h}^{-1} \text{g}^{-1}$ . Information about PLFA and  
203 enzyme activities are presented in Table S1.

204 The Biolog-ECO plates were purchased from Biolog, US. The substrates for BG, NAG, AP, and  
205 LAP were 4-MUB- $\beta$ -D-glucoside, 4-MUB-N-acetyl-b-D-glucosaminide, 4-MUB-phosphate, and L-  
206 Leucine-7-amino-4-methylcoumarin, and were stored at  $-20^\circ\text{C}$ . An MUB standard was used for the  
207 BG, NAG, and AP enzymes and an AMC standard was used for the LAP enzyme. The substrates and  
208 standards were purchased Sigma. Analytical grade reagents were used for the soil nutrient analysis.

### 209 2.3 Vegetation data

210 We established four sampling plots ( $30\times 40$  m) in each forest ecosystem. In each plot, we recorded all  
211 the tree individuals, and measured the height and diameter-at-breast-height (DBH) of each woody  
212 individual with a  $\text{DBH}\geq 2$  cm. The diversity of the tree species in the sampling plots was represented  
213 by  $H'$ , and the diversity ( $H'$ , Shannon-Wiener) of the tree species in the community was calculated  
214 as follows:

$$215 \quad H' = \sum_{i=0}^n (P_i \ln P_i)$$

216 Where  $P_i$  was the importance value of the species  $i$  as a proportion of all species, and  $n$  was the  
217 number of the species.

218 We also calculated the community-weighted means (CWM) values of the tree traits using the  
219 cover of each tree. As described by Xu et al. (2018), we collected litter and sun-exposed and mature  
220 leaves (leaf blades for trees) from between five and ten individuals of each plant species at each site  
221 and determined their TN and TC concentrations. We calculated the specific leaf area (SLA, the one-  
222 sided area of a fresh leaf divided by its oven-dried mass,  $\text{m}^2 \text{kg}^{-1}$ ), leaf dry matter content (LDMC,  
223 the oven-dried mass of a leaf divided by its water-saturated fresh mass,  $\text{mg g}^{-1}$ ), leaf C concentrations  
224 (leaf C,  $\text{g kg}^{-1}$ ), and leaf N concentrations (leaf N,  $\text{g kg}^{-1}$ ) for ten fully expanded leaves of each  
225 sampled individual. To measure the leaf traits at the community level, we calculated the CWM of the



226 tree layer, as follows:

$$227 \quad CWM = \sum_{i=1}^n p_i \times \text{trait}_i$$

228 Where  $p_i$  is the relative contribution of the species  $i$  to the cover of the whole community,  $n$  is the  
229 number of the most abundant species, and  $\text{trait}_i$  is the trait value of species  $i$ , as described by Garnier  
230 *et al.* (2004). The diversity of the tree species and plant functional traits are summarized in Table S2.

#### 231 2.4 Microbial substrate use

232 Microbial functional diversities were determined using a Biolog EcoPlate™ (Biolog Inc., Hayward,  
233 California, USA) as described by Garland and Mills (1991). Briefly, approximately 10 g of fresh soil  
234 was suspended in 100 ml saline solution (0.85% NaCl) and shaken on an orbital shaker for 30 min at  
235 190 rpm. A 150 µl aliquot of supernatant from 1:1 000 dilutions of each soil sample was added to  
236 each well. The plates were incubated at 25°C, and the absorbance at 590 nm was measured using a  
237 microplate reader (GENios Pro™, Tecan Trading AG, Männedorf, Switzerland) every 24 h up to 240  
238 h (0, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h). **To minimize the influence of cell density in  
239 comparisons among samples, results can be analyzed at constant average well color development  
240 (AWCD). The AWCD for each microplate was calculated by subtracting the control well optical  
241 density (OD) from the substrate well OD (blanked substrate wells), setting any resultant blanked  
242 substrate wells with negative values to 0 and taking the mean of the 95 blanked substrate wells.**

243 The Richness ( $R$ ), Shannon-Weiner diversity index ( $H'$ ), Shannon evenness index ( $E$ ), and  
244 Simpson dominance index ( $D$ ) were calculated from the absorption values after EcoPlate™  
245 incubation for 96 h (Gomez *et al.*, 2006). Additionally, the 31 C sources were divided into six groups,  
246 namely carbohydrates, carboxylic acids, amines, amino acids, polymers, and miscellaneous, as  
247 suggested by Zak *et al.* (1994). The average absorbance of all C sources within each group was  
248 computed as the intensity of the single substrate use. The soil total microbial metabolic intensities ( $S$ )  
249 of six carbon sources were estimated by the area underneath  $AWCD$  vs.  $t$ , and were obtained by  
250 integrating the equation against time  $t$  (Guckert *et al.*, 1996):

$$251 \quad S = \sum [(v_i + v_{i-1})/2 \times (t_i + t_{i-1})]$$

252 Where  $v_i$  was the average optical density of the  $i$ th incubation time.

253 2.5 SOM decomposition rate

254 Four replicates from each sampling site with a 60% water-holding capacity were incubated at 20°C.  
255 In brief, 40 g of each fresh soil sample were put into a 150-ml incubation bottle, and the samples  
256 were then adjusted so that their moisture content corresponded to a water-holding capacity of 60%.  
257 During the 4-week incubation period, the soil respiration rates were measured on days 1, 7, 14, 21,  
258 and 28 using an automatic system. The SOM decomposition rates were calculated as described in the  
259 study of Xu et al. (2015).

260 2.6 Statistical analysis

261 One-way analysis of variance (ANOVA) followed by a post hoc Tukey HSD test were used to test  
262 the significance of the differences among the soil properties, C use, functional diversity, and SOM  
263 decomposition rates in the different forest ecosystems. We tested the relationships between labile C,  
264 soil microbial community structure, microbial function, and the SOM decomposition rates with the  
265 Pearson correlation test. Differences were considered significant when  $P < 0.05$ , with the marginal  
266 significance set at  $P < 0.01$ . All P values were adjusted using the Bonferroni correction to account for  
267 multiple comparisons.

268 We used redundancy analysis (RDA) to examine the relationship between the environmental  
269 variables and soil microbial substrate use. The environmental variables were the same as those  
270 described in Xu et al. (2018), including climate, soil properties, litter properties, and plant functional  
271 traits. Before RDA, we conducted forward selection of the environmental variables that were  
272 significantly correlated with variations in the microbial substrate use profile using stepwise  
273 regression and the Monte Carlo Permutation Test. We used CANOCO software 4.5 (Ter Braak and  
274 Smilauer 2002) for the RDA and stepwise regression. The environmental properties, which were  
275 significantly correlated with the microbial substrate use in the RDA, were stressed in the plots. Path  
276 analysis was conducted to examine the direct and indirect effect of biotic and abiotic factors on soil  
277 microbial use of carbon sources. All ANOVA, regression analyses, and path analysis were performed  
278 using SPSS 19.0 for Windows. Data are reported as the mean  $\pm$  SE.

279 **3 Results**

280 3.1 Patterns in the microbial substrate use, soil labile carbon, and SOM decomposition rates

281 There was no obvious latitudinal pattern for the soil total microbial metabolic intensity (Fig.2). Of  
282 the forests along the NSTEC, the C metabolic intensity of soil microbes was lowest in HZ and LS;  
283 the C metabolic intensity of soil microbes differed significantly between JF and the other forests (Fig.  
284 2), which indicates that the color development was significantly higher in the tropical forest soils  
285 than in the subtropical and temperate forest soils and is consistent with the variations in the AWCD  
286 (Fig. S1). The average values of  $R$ ,  $H'$ , and  $D$  were significantly different among the nine forest soils  
287 and were highest in JF, SN, and CB (Table 3). Generally, the soil microbial use of the six different  
288 carbon sources were relatively higher in tropical and subtropical climatic area (low latitude) than  
289 those in temperate climatic area (high latitude) (Fig.3).

290 Across the nine forests, soil microorganisms used the six substrate groups in the same order; the  
291 carboxylic acid substrate was used most, followed by amino acids, carbohydrates, polymers, amines,  
292 and miscellaneous substrates (Fig. 3). Microorganisms in the boreal and temperate forests mainly  
293 metabolized carbohydrates, amino acids, and carboxylic acids, while those from the subtropical and  
294 tropical forests used the substrates in equal proportions. The substrate microbial use ability was  
295 highest in the coniferous broad-leaved mixed forest and tropical forest soils, and lowest in the  
296 coniferous forest soil (Fig. 3).

297 Overall, soil MBC concentrations in the boreal and temperate forests were three to eight times  
298 higher than those of the subtropical and tropical forests. In contrast, the average DOC concentrations  
299 in the tropical and subtropical forest soils, which ranged from 311 to 458 mg kg<sup>-1</sup>, were significantly  
300 higher than the average concentrations in the temperate and boreal forest soils, where the average  
301 concentrations ranged from 204 to 284 mg kg<sup>-1</sup> (Table 2). The average SOM decomposition rates in  
302 the subtropical forests ranged from 0.64 to 2.42 μg C g<sup>-1</sup> d<sup>-1</sup>, and were significantly lower than the  
303 rates in the temperate forests, which ranged from 3.43 to 4.61 μg C g<sup>-1</sup> d<sup>-1</sup> (Table S3).

304 3.2 Effect of environmental properties on soil microbial substrate use

305 Redundancy analysis showed that the variations in soil microbial substrate use were strongly and  
306 positively correlated with the CWM values of LDMC, leaf N, and leaf C, and strongly and negatively

307 correlated with the soil silt content and SMC (Fig. 4). The RDA2 of soil microbial substrate use was  
308 strongly positively correlated with TN and SOC, but negatively correlated with the mean annual  
309 precipitation (MAP) (Fig. 5). RDA1 mainly represented the plant functional traits, soil texture, and  
310 micro-meteorological conditions, while RDA2 represented climate and soil nutrients. Overall, the  
311 soil silt content and the CWM values of plant functional traits were the main predictors of the  
312 latitudinal variation in the soil microbial substrate use along the NSTEC.

### 313 3.3 Relationships between soil microbial substrate use, enzyme activities, and PLFAs

314 Microbial carbohydrate use was positively related with bacterial biomass and actinomycic biomass  
315 (Fig. 5). Microbial polymer use was negatively related with bacterial biomass and actinomycic  
316 biomass. Microbial amines use was negatively related with  $G^-$  bacterial and fungal biomass.  
317 Miscellaneous substrate use was positively related with fungal biomass and  $G^+/G^-$  bacterial biomass  
318 (Fig. 5).

319 The abundance of  $G^-$  bacteria was positively associated first with the specific activities of BG,  
320 whereas actinomycetes and  $G^+$  bacteria were positively associated with BG and LAP. Soil fungi were  
321 negatively associated with BG (Fig. 5).

### 322 3.4 Relationships between SOM decomposition rate, PLFAs, enzyme activity, and microbial 323 metabolic activities

324 The SOM decomposition rates were significantly and positively related to soil MBC concentrations  
325 but significantly and negatively related to soil DOC concentrations (Fig. 6a and b). Except for amino  
326 acid and amine substrates, the SOM decomposition rates were significantly and positively related to  
327 microbial metabolic activities (AWCD) and carbohydrate substrate use (Fig. 6c and d) and negatively  
328 related to carboxylic acid, polymer, and miscellaneous substrate use (Fig. 6e, g, and i).

329 The SOM decomposition rates were significantly and positively correlated with total PLFAs  
330 ( $r=0.456$ ,  $P=0.005$ ), bacteria ( $r=0.3836$ ,  $P=0.021$ ), actinomycetes ( $r=0.500$ ,  $P=0.002$ ), and  $G^-$   
331 bacteria PLFAs ( $r=0.520$ ,  $P=0.001$ ) (Fig. 7a, b, d, and f) but were negatively correlated with fungal  
332 PLFAs ( $r=-0.370$ ,  $P=0.026$ ), F/B ( $r=-0.513$ ,  $P=0.001$ ), and the  $G^+/G^-$  ( $r=-0.496$ ,  $P=0.002$ ) (Fig. 7c,  
333 g, and h). Except for LAP activity, soil enzyme activities were significantly and positively correlated

334 with the SOM decomposition rates ( $P < 0.01$ ) (Fig. 7i, j, and l).

## 335 **4 Discussion**

### 336 4.1 Response of soil labile C and SOM decomposition rates to variations in forest type

337 Soil organic matter is one of the most important C pools in terrestrial ecosystems. The concentrations  
338 of soil DOC in the temperate forests were lower than those in subtropical forests but the soil MBC  
339 concentrations were higher in temperate forests than in subtropical forests. This reflects the results  
340 of previous regional and global studies (Tian et al., 2010; Xu et al., 2013), and shows that the  
341 production/consumption ratio of soil DOC was lower, but that microbial C immobilization was higher,  
342 in the high latitude forests than closer to the tropics (Fang et al., 2014). Soil DOC, as a labile SOM  
343 fraction with a rapid turnover, is one of the primary energy sources for microorganisms. The higher  
344 temperatures and precipitation in subtropical and tropical forests lead to higher turnover rates (Fang  
345 et al., 2014), so soil DOC concentrations were highest in subtropical, and MBC concentrations were  
346 lowest, in tropical forests. However, in temperate forests, more C is assimilated into microbial  
347 biomass, so that less C is lost through chemical and physical processes (Liu et al., 2010). Also,  
348 because the decomposition ability of different microbe groups varies, the differences in the soil  
349 microbial communities in different forest ecosystems may also be responsible for the spatial  
350 variations in the soil DOC and MBC concentrations along the NSTEC (Hagedorn et al., 2008).

351 Heterotrophic soil respiration is sustained by the decomposition of SOM. The SOM  
352 decomposition rates along the NSTEC were greater in temperate forests than in subtropical forests,  
353 which was consistent with the variations in the soil MBC and SOC concentrations. These results  
354 indicate that, as found in other studies, large scale SOM decomposition rates are driven by the  
355 amounts of substrate available (Yu et al., 2010). Changes in the availability of C in SOM may affect  
356 the microbial resource strategies, which may in turn influence the SOM decomposition rate. **Some**  
357 **forest soils were intermittently saturated (such as CB, Table 2) or high with mean annual precipitation.**  
358 **Under the anaerobic conditions, soil organic decomposition is mediated by a complex suite of**  
359 **microbial processes (Megonigal et al., 2004). The fermentation products including low molecular**  
360 **weight alcohols, fatty acids, and dihydrogen can serve as substrates for anaerobic respiration using a**

361 variety of alternative terminal electron acceptors in place of oxygen to mineralize organic carbon to  
362 carbon dioxide (CO<sub>2</sub>). Therefore, the soil organic matter decomposition rate might be slow in these  
363 anaerobic conditions. These results demonstrate that the reduction of organic matter is a key step of  
364 anaerobic decomposition (Keller and Takagi, 2013).

#### 365 4.2 Mechanisms driving latitudinal variations in microbial substrate use

366 The AWCD reflects the sole C source use ability of the soil microbial community (Garland and Mills,  
367 1991). Of the six groups of C substrates, microbial communities in the temperate forests mainly used  
368 carbohydrates, carboxylic acids, and amino acids, which suggests that microorganisms in temperate  
369 forests probably use high-energy substrates that degrade easily (Kunito et al., 2009). The carbon  
370 substrate use was lowest in the boreal coniferous forest (HZ). This shows that, compared with  
371 coniferous species, broadleaved tree species produce root exudates and litter high in water-soluble  
372 sugars, organic acids, and amino acids that are more favorable for microbial activity (Priha et al.  
373 2001).

374 There was no significant latitudinal pattern in the soil total microbial metabolic intensity (S) in  
375 our study, which was inconsistent with hypothesis (1). However, there was significant latitudinal  
376 variation in the use of the six different carbon sources. The soil microbial use of carbon sources were  
377 relatively higher in tropical and subtropical climatic area than those in temperate climatic area.  
378 Consistent with hypothesis (2), soil microbial functions were similar in closely related to the CWM  
379 values of the tree species (Fig. 4). However, only MAP of climatic factors had a moderate effect on  
380 the soil microbial function (Fig. 4). Climate may have indirect effect on the latitudinal pattern of  
381 different carbon sources use. In our study, MAT and MAP affected the six different carbon sources  
382 use indirectly by influencing the soil temperature (ST), soil total nitrogen (TN), and total phosphorus  
383 (TP) (Table S4-9). It was reported that climate was the main environmental parameters driving the  
384 latitudinal patterns of community-level leaf traits through the regulation of species composition  
385 (Wang et al., 2016). Woody plant species with evergreen broadleaves dominated in the tropic regions  
386 characterized by hot, humid, and infertile habitats showed low CWM values of leaf N and SLA and  
387 high CWM values of LDMC (Table S2).

388 A growing number of studies reported that vegetation type, land use, soil nutrients, and soil

389 organic matter quality and quantity can determine large scale patterns of microbial communities (de  
390 Vries et al., 2012; Tu et al., 2016). Plant functional traits that are related to growth may determine a  
391 tree species' ability to contribute to the soil carbon pool via leaf litter inputs. For example, it was  
392 previously reported that plant traits such as the leaf N content, SLA, and LDMC could explain  
393 variations in soil nutrients and litter decomposition rates (Eichenberg et al., 2014; Laughlin, 2011).  
394 Therefore, we examined how these plant traits influenced the soil microbial function by latitude. We  
395 found that changes in the soil microbial C substrate use with latitude were mainly related to the soil  
396 silt contents and the CWMs of LDMC, and leaf C and leaf N concentrations, which indicates that the  
397 quality of nutrients from plants had a major influence on microbial carbon use efficiency (Hypothesis  
398 (2)). Plant species with high a SLA, high leaf N concentrations, and low LDMC can produce  
399 bacterial-dominated soil microbial communities in grasslands (Orwin et al., 2010). Looking beyond  
400 individual traits, related tree species may cultivate microbial communities with similar preferences  
401 for carbon sources through coevolution of plants and microbes (Liu et al., 2012b; Buscot, 2015).

402 As hypothesized, the soil microbial community composition was explained by the CWMs of  
403 plant traits at the regional scale. Carbon substrate use was negatively correlated with the CWM of  
404 leaf N concentrations (Table S2, Fig. S2). Bacterially dominated soil microbial communities develop  
405 from leaf litter comprised of N-rich leaves from fast growing species (De Vries et al., 2012), while  
406 leaves with low N concentrations will promote fungal domination (Orwin et al., 2010; De Vries et  
407 al., 2012). In line with this, fungal biomass decreased, and bacterial biomass increased, as the CWM  
408 leaf N content increased, and is associated with fast-growing, N-exploitative plants (Xu et al., 2018).  
409 Leaf N concentrations are considered as indicators of plant growth and resource uptake (Wright et  
410 al., 2004). The results from this study show that, along the NSTEC, high leaf N restrained microbial  
411 C substrate use (Fig.S2) and was a good indicator of the competition between plants for soil N (Pei  
412 et al., 2016). Soil microbes and nearby plants may have been competing for N in the soil.

413 We also found that the C substrate use was negatively correlated with the CWM of leaf C  
414 concentrations (Table S2, Fig. S2). Plants at high latitudes may have higher leaf C concentrations  
415 than plants at lower latitudes so that they can balance the osmotic pressure of cells and resist freezing  
416 (Millard et al., 2007; Hoch and Körner, 2012). The increased C was most likely in the form of an  
417 increase in non-structural C, including starch, low molecular weight sugars, and storage lipids that

418 are easy to break down. Therefore, soil microorganisms from the temperate forests mainly  
419 metabolized high-energy substrates, such as carbohydrates, carboxylic acids, and amino acids.

420 The LDMC is the ratio of the leaf dry weight to the fresh weight and has been used as a proxy  
421 for the ratio of structural compounds to assimilatory tissue (mesophyll and epidermis, Van Arendonk  
422 and Poorter, 1994). High values of LDMC indicate large amounts of vascular tissue, cellulose,  
423 insoluble sugars, and leaf lignin that are difficult to decompose (Poorter and Bergkotte, 1992); C  
424 substrates such as carbohydrates, carboxylic acid, and amino acid are, however, easy to decompose  
425 (Myers et al., 2001). In line with this, the use of carbohydrate, carboxylic acid, and amino acid  
426 substrates was negatively related to the CWMs of the LDMC (Table S2). Pei et al. (2016) reported  
427 that the LDMC was an important driver of multivariate soil microbial community structure and  $G^-$   
428 bacterial abundance.

429 Soil texture regulates soil biological processes and so affects the soil microbial community  
430 structure (Sessitsch et al., 2001). In the present study, microbial C substrate use was significantly and  
431 positively related to the soil silt content. Soil types and textures varied along the NSTEC. Soil texture  
432 influences how microbes use organic matter, and has a strong influence on soil moisture, nutrient  
433 availability, and retention (Veen and Kuikman, 1990). Fine-textured soils with a higher silt content  
434 are known to be more favorable for bacterial growth than soils with a lower silt content because of  
435 their greater water-holding capacity and nutrient availability, and because they are better protected  
436 from bacterial grazers (Carson et al., 2010). We found that the microbial C substrate use was higher  
437 in LS, CB, SN, and JL than in the other forests, reflecting their fine-grained soils and high silt contents,  
438 which ranged from 60% to 80%.

#### 439 4.3 Links between soil microbial community structure and function

440 The soil microbial community structure and functions were significantly correlated along the NSTEC.  
441 Soil carbohydrate and polymer substrate use were mainly related to soil  $G^+$  bacterial and actinomycic  
442 biomass, but amines and miscellaneous substrates were mainly related to soil  $G^-$  bacterial, fungal  
443 biomass, and the F/B ratio. Soil bacteria mainly decomposed simple carbohydrates, organic acids,  
444 and amino acids, whereas soil fungi mainly decomposed recalcitrant compounds (Myers et al., 2001;  
445 Treonis et al., 2004). Consistent with this, soil bacterial PLFAs were positively correlated with the



446 carbohydrate substrate use and the fungal PLFAs were positively related with miscellaneous substrate  
447 use. The results are similar to those reported by Sterner and Elser (2002), who found that fungi tended  
448 to have higher C/N or C/P ratios while heterotrophic bacteria typically have lower C/N or C/P ratios.

449 Shifts in microbial community composition may influence enzyme production (DeForest et al.,  
450 2012; Waldrop et al., 2000; Brockett et al., 2012). Different microbial groups require different  
451 amounts of nutrients to construct biomass, or have enzymes that differ in their affinity for nutrients.  
452 We found that the relative abundances of the G<sup>+</sup> bacteria and actinomycetes communities were  
453 associated with the specific activities of BG, AP, and LAP), whereas the relative abundance of the  
454 G<sup>-</sup> bacteria was correlated with soil NAG activities involved in chitin degradation. In agreement with  
455 our study, numerous other researchers have reported significant correlations between PLFA profiles  
456 and enzyme activities (Waldrop et al., 2000; DeForest et al., 2012; Brockett et al., 2012; Riah-Anglet  
457 et al., 2015). Soil BG was mainly responsible for cellulose degradation and was involved in breaking  
458 down complex organic compounds (cellobiose) into small molecule substrates (glucose) in favor of  
459 acquiring C through microbial community growth (Waldrop et al., 2000). Soil NAG activities were  
460 weakly and positively related with fungal biomass in the present study, and may have been mainly  
461 produced by fungal populations (Valášková et al., 2007). Fungi are commonly considered as  
462 producers of oxidative enzymes. Therefore, the influence of fungal biomass on variations in enzyme  
463 activities was minimal (Kivlin and Treseder, 2014). The linkages between enzyme activity and  
464 community composition may provide some insight into the microbial mechanisms that drive the  
465 decomposition of macromolecular C compounds. These results suggest that that overall ecosystem  
466 functioning may suffer if soil microbial groups are lost.

467 The quality and amounts of SOM are influenced by the biomass, vegetation coverage, root  
468 distribution, and microbial species (Raich and Schlesinger, 1992). The SOM decomposition rates  
469 were higher in temperate forests than in tropical forests and may reflect the higher soil microbial  
470 biomass (Wang et al., 2016). In line with this, SOM decomposition rates were positively related with  
471 soil MBC concentrations and different groups of PLFAs. The inverse relationships between SOM  
472 decomposition rates and DOC, carboxylic acids, polymers, and miscellaneous along the NSTEC,  
473 indicate a shift in the soil C turnover from open to closed with increases in the soil labile C  
474 concentrations (Fang et al., 2014). Soil DOC and MBC influence SOM decomposition rates indirectly

475 by regulating microbial properties (Boberg et al., 2014; Wei et al., 2014). In our study, SOM  
476 decomposition rates were positively related with bacterial PLFAs but negatively with fungal PLFAs.  
477 Because different communities of microbes have different SOM use efficiencies (Balsler and Wixon,  
478 2009; Lipson et al., 2009; Monson et al., 2006), changes in the microbial community structure may  
479 influence the microbial activities and the decomposition rates of organic matter (Lipson et al., 2009;  
480 Keiblinger et al., 2010). The functional dissimilarity of microbes and fungi may help explain these  
481 results. However, we did not measure some key variables, such as the microbial competition and  
482 interactions, and relationship between the microbial diversity and the decomposition rates. Therefore,  
483 in the future, we will use different experimental techniques that will help us gain an improved  
484 understanding of the mechanisms that drive the relationships between the structure and function of  
485 microbial communities.

## 486 **5 Conclusions**

487 In this study, we examined the patterns in labile C concentrations, SOM decomposition rates,  
488 microbial substrate use, and functional diversity and identified a combination of abiotic and biotic  
489 factors that influenced soil microbial functional diversity at the regional scale. The MBC  
490 concentration and SOM decomposition rates were significantly lower, and the soil DOC  
491 concentrations were higher in the subtropical and tropical forests than in the temperate forests. **There**  
492 **was no obvious latitudinal pattern for the soil total microbial metabolic intensity. However, soil**  
493 **microbial use of the different carbon sources varied with the latitude except the amines carbon source.**  
494 **Soil microbial use of carbon sources were relatively higher in tropical climatic areas. For the first**  
495 **time, we showed that CWM values of plant traits explained variations in soil microbial C substrate**  
496 **use at the regional scale. Additionally, the fine-grained soils with high silt contents were higher in**  
497 **the microbial C substrate use. Climate factors affected the soil microbial uses of carbon sources**  
498 **indirectly by influencing the soil temperature (ST) and soil nutrition.** Soil microbial community  
499 structure and function were strongly related, which suggests that the loss of soil microbial groups  
500 may have consequences for overall ecosystem functioning.

501 *Data accessibility.* Requests for data and materials should be addressed to N.H. (henp@igsnr.ac.cn) and G.Y.  
502 ([yugr@igsnr.ac.cn](mailto:yugr@igsnr.ac.cn)).

503

504 *Author contributions.* Z.W.X., G.R.Y. and X.Y.Z. planned and designed the research. Z.W.X., N.P.H., R.L.W., and  
505 N.Z. conducted fieldwork. Z.W.X., G.R.Y., X.Y.Z., and Q.F.W wrote the manuscript. All authors contributed  
506 critically to the drafts and gave final approval for publication.

507 *Competing interests.* The authors declare that they have no conflict of interest.

508

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## 705 **Figures legends**

706 **Fig. 1.** Distribution of typical forest ecosystems along the North-South Transect of eastern China (NSTEC). The  
707 abbreviations for the sampling sites from north to south are as follows: HZ, Huzhong; LS, Liangshui; CB, Changbai;  
708 DL, Dongling; TY, Taiyue; SN, Shennong; JL, Julian; DH, Dinghu; JF, Jiangfeng. These abbreviations are used for  
709 the nine forests throughout.

710 **Fig.2.** Variations in soil microbial substrate use during a 240-h incubation for the nine forests. Different colors  
711 represent different forest types: Yellow, coniferous forest; Dark yellow, coniferous broad-leaved mixed forest; Purple,  
712 deciduous broad-leaved forest; Olive, subtropical evergreen broad-leaved forest; Orange, Tropical monsoon forest.  
713 Different lowercase letters indicate significant differences among forests in the same climate zone. The abbreviations  
714 of the sampling sites are given in Table 1.

715 **Fig. 3.** Characteristics of microbial use of (a) carbohydrates, (b) carboxylic acids, (c) amino acids, (d) polymers, (e)  
716 amines, and (f) miscellaneous along the NSTEC. The representatives of different colors were showed in Figure 2.

717 **Fig.4.** Redundancy analysis (RDA) ordination biplot of soil microbial carbon sources use efficiency and  
718 environmental properties. The representatives of different colors were showed in Figure 2. The dotted lines and solid  
719 lines represent the environmental variables and lipid signatures and carbon sources. The abbreviations of the variables  
720 in this figure are as follows: MAP, mean annual precipitation. The vegetation data: LDMC, leaf dry matter weight;  
721 Leaf C, leaf carbon content; Leaf N, leaf nitrogen content; SLA, specific leaf area. Soil properties included SMC, soil  
722 moisture content; Silt, soil silt content; TN, soil total nitrogen; SOC, soil organic carbon. The abbreviations of the  
723 sampling sites were given in Table 1

724 **Fig.5.** The heatmap of the Pearson's correlation coefficients between the use of individual substrates and microbial  
725 PLFAs and soil enzyme activities. Note: The abbreviations of the variables: Actino-, actinomycetes; F/B,  
726 fungi/bacteria; G<sup>+</sup>, gram positive bacteria; G<sup>-</sup>, gram negative bacteria; G<sup>+</sup>/G<sup>-</sup>, Gram-positive bacteria/ Gram-negative  
727 bacteria. BG,  $\beta$ -1, 4-glucosidase; NAG,  $\beta$ -1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid  
728 phosphatase. \*\* $P < 0.01$ , \* $P < 0.05$ .

729 **Fig. 6.** Relationships between soil carbon mineralization rates ( $\mu\text{g C g}^{-1}\text{d}^{-1}$ ) and microbial biomass C (MBC), soil  
730 dissolved organic C (DOC), average well color development (AWCD), and individual substrate use.

731 **Fig. 7.** Relationships between soil carbon mineralization rates ( $\mu\text{g C g}^{-1}\text{d}^{-1}$ ) and different groups of soil microbial  
732 PLFAs (a-h) and enzyme activities (i-l).

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## 735 **Supporting information**

736 **Table S1** Average values of forest soil enzyme activities and different PLFA groups along the NSTEC.

737 **Table S2** Plant diversity and community weighted means of plant functional traits

738 **Table S3** Soil organic matter (SOM) decomposition rates during the 28 days of incubation time (Mean  $\pm$  SE) ( $\mu\text{g C}$   
739  $\text{g}^{-1}\text{d}^{-1}$ )

740 **Fig. S1** Variations in the average well color development (AWCD) values during a 240-h incubation for the nine  
741 forests. The abbreviations of the sampling sites are the same as those in Table 1.

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**Tables**

**Table 1.** The main characteristics of the sampling sites along the North South Transect of East China

Sampling Sites	Longitude (E)	Latitude (N)	Elevation (m)	MAT <sup>b</sup> (°C)	MAP <sup>b</sup> (mm)	Vegetation types	Soil type
HZ <sup>a</sup>	123°01'12"	51°46'48"	850	-3.7	473	Cold temperate coniferous forest	Spodosols
LS	128°53'51"	47°11'06"	401	0.01	648	Temperate conifer broad-leaved mixed forest	Albi-Boric Argosols
CB	128°05'27"	42°24'16"	758	2.8	691	Temperate conifer broad-leaved mixed forest	Albi-Boric Argosols
DL	115°25'24"	39°57'27"	972	6.6	539	Warm temperate deciduous broad-leaved forest	Alfisols
TY	112°04'39"	36°41'43"	1668	6.0	644	Warm temperate deciduous broad-leaved forest	Alfisols
SN	110°29'43"	31°19'15"	1510	8.5	1447	Subtropical deciduous evergreen mixed forest	Inceptisols
JL	114°26'28"	24°35'05"	562	18.2	1770	Subtropical evergreen broad-leaved forest	Ultisols
DH	112°32'14"	23°10'25"	240	21.8	1927	Subtropical monsoon evergreen broad-leaved forest	Ultisols
JF	108°51'26"	18°44'18"	809	23.2	2266	Tropical monsoon forest	Ultisols

744 a:HZ, Huzhong; LS, Liangshui; CB, Changbai; DL, Dongling; TY,Taiyue; SN, Shennong; JL, Jiulian; DH, Dinghu; JF, Jiangfeng.

745 b: MAT, mean annual temperature; MAP, mean annual precipitation.



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**Table 2.** Soil properties of different sampling sites

Sampling site	pH	ST (°C)	SMC (%)	Silt (%)	SOC (g kg <sup>-1</sup> )	MBC (mg kg <sup>-1</sup> )	DOC (mg kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	TP (g kg <sup>-1</sup> )
HZ	6.79±0.02a	10.3±0.15g	45.3±0.90c	56±1.2c	42.29±0.47b	350±6.0a	240±7.6e	2.90±0.16d	0.87±0.02b
LS	6.17±0.02b	15.9±0.02f	46.9±0.76c	64±0.3b	62.08±7.20a	316±0.7a	204±4.9f	4.59±0.29b	0.59±0.02c
CB	6.37±0.04b	16.0±0.06f	102.8±0.25a	76±0.6a	72.38±2.00a	178±8.8b	314±8.6c	6.05±0.17a	1.67±0.08a
DL	6.87±0.02a	17.8±0.14e	32.4±0.30e	6±2.4e	38.83±0.41c	43±0.8e	284±2.6d	3.17±0.04d	0.56±0.01c
TY	6.85±0.05a	16.0±0.12f	36.0±0.23d	49±1.4d	41.34±2.75c	115±4.0c	226±13.8f	2.43±0.15e	0.52±0.01c
SN	6.93±0.01a	18.4±0.12d	50.5±0.63b	74±0.3a	36.13±1.26c	72±13.1e	311±13.2c	3.76±0.05c	0.81±0.01b
JL	5.57±0.19b	25.3±0.01a	39.0±0.89d	68±0.3b	31.55±1.82c	89±19.7d	387±1.9b	2.28±0.09e	0.36±0.01d
DH	5.43±0.03c	24.4±0.04b	37.8±0.38d	50±1.8d	28.47±0.54d	38±0.1e	334±7.7c	1.77±0.02f	0.20±0.01e
JF	6.32±0.01c	22.5±0.07c	38.6±0.12d	49±0.2d	29.38±0.94d	140±1.3c	458±6.6a	1.99±0.02e	0.15±0.01e

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Note: ST=temperature of 0–10 cm soil; SMC=soil moisture content; Silt=soil silt content; SOC=soil organic carbon; MBC=microbial biomass carbon; DOC=dissolved organic carbon; TN=soil total nitrogen; TP=soil total phosphorus. Values were presented as means ± SE (n=4). The abbreviations of the sampling sites were given in the Table 1.

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**Table 3.** Functional diversity of soil microbial communities in forest ecosystems along the NSTEC

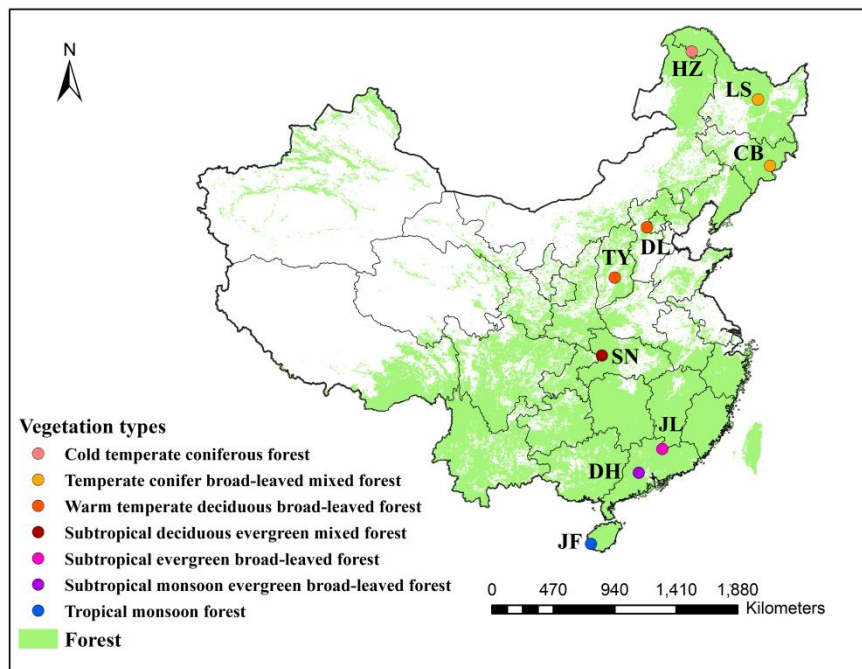
<b>Sampling sites</b>	<b>Richness (R)</b>	<b>Shannon <i>H'</i></b>	<b>Shannon <i>E</i></b>	<b>Simpson <i>D</i></b>
<b>HZ</b>	14.08±0.34d	2.65±0.03d	1.01±0.007b	0.91±0.002c
<b>LS</b>	25.29±0.14b	3.12±0.02b	0.98±0.003c	0.95±0.001a
<b>CB</b>	27.00±0.27a	3.22±0.01a	0.98±0.001c	0.95±0.001a
<b>DL</b>	11.54±0.47e	2.52±0.03e	1.04±0.010a	0.87±0.005d
<b>TY</b>	22.33±0.87c	3.02±0.02c	0.98±0.002c	0.94±0.001a
<b>SN</b>	28.10±0.34a	3.24±0.01a	0.97±0.001c	0.95±0.001a
<b>JL</b>	23.54±0.07c	3.04±0.01c	0.96±0.001c	0.93±0.003b
<b>DH</b>	25.65±0.71b	3.11±0.01b	0.97±0.001c	0.93±0.002b
<b>JF</b>	27.63±0.68a	3.19±0.02a	0.96±0.001c	0.95±0.002a

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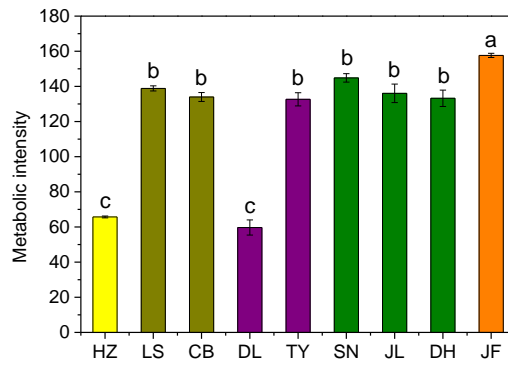
Indices were calculated based on the optical density values after incubation for 96 h. Data are expressed as means±standard errors. Different lowercase letters indicate significant differences among forests. The abbreviations of the sampling sites are the same as those used in Table 1.

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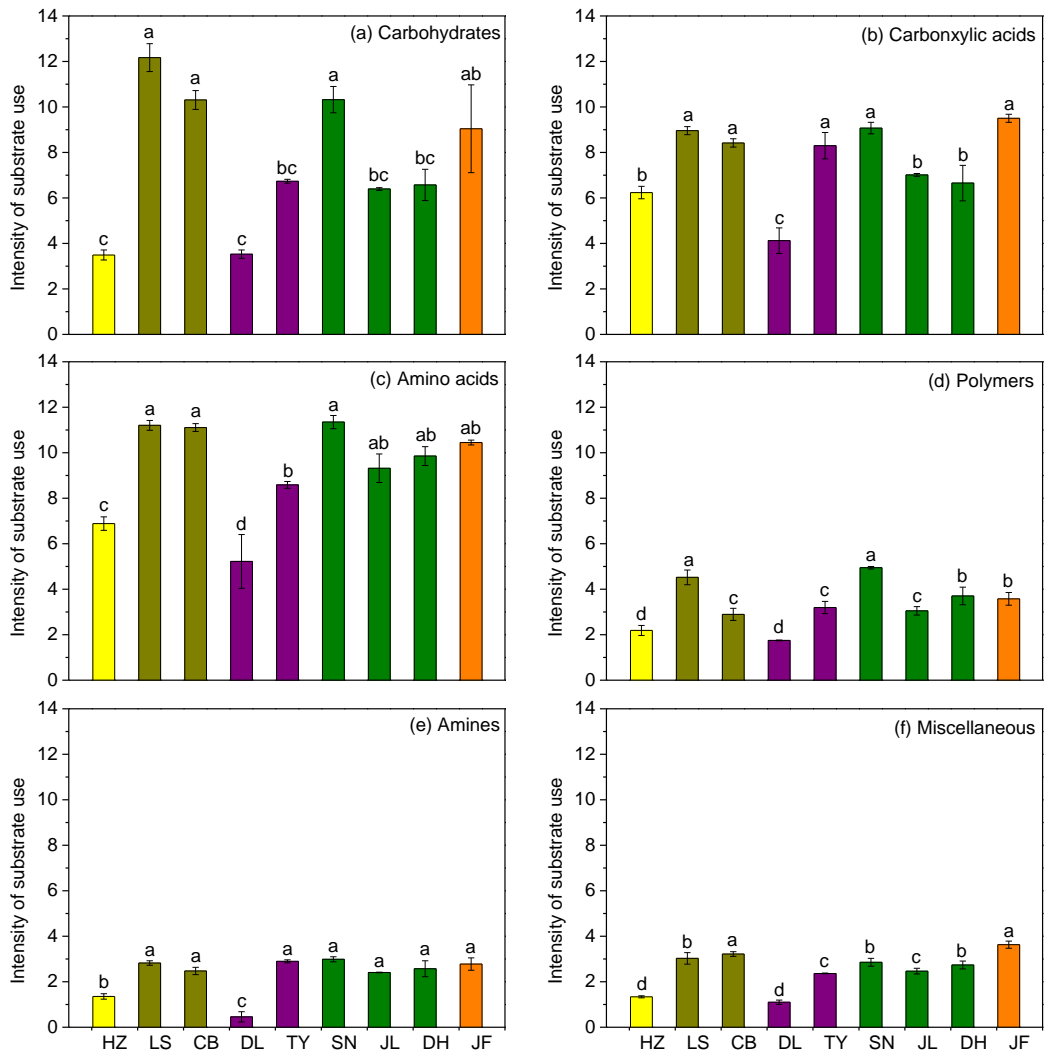


754 **Figure 1.** Distribution of typical forest ecosystems along the North-South Transect of eastern China (NSTEC).  
 755 The abbreviations of sampling sites from north to south are as follows: HZ, Huzhong; LS, Liangshui; CB,  
 756 Changbai; DL, Dongling; TY, Taiyue; SN, Shennong; JL, Jiulian; DH, Dinghu; JF, Jiangfeng.  
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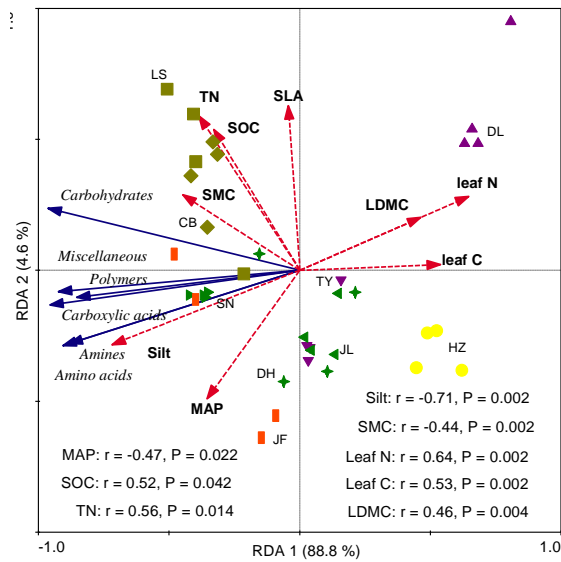
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**Figure 2.** Variations in soil total microbial metabolic intensity during a 240-h incubation for the nine forests. Different colors represent different forest types: Yellow, coniferous forest; Dark yellow, coniferous broad-leaved mixed forest; Purple, deciduous broad-leaved forest; Olive, subtropical evergreen broad-leaved forest; Orange, Tropical monsoon forest. Different lowercase letters indicate significant differences among forests in the same climate zone. The abbreviations of the sampling sites are given in Table 1.



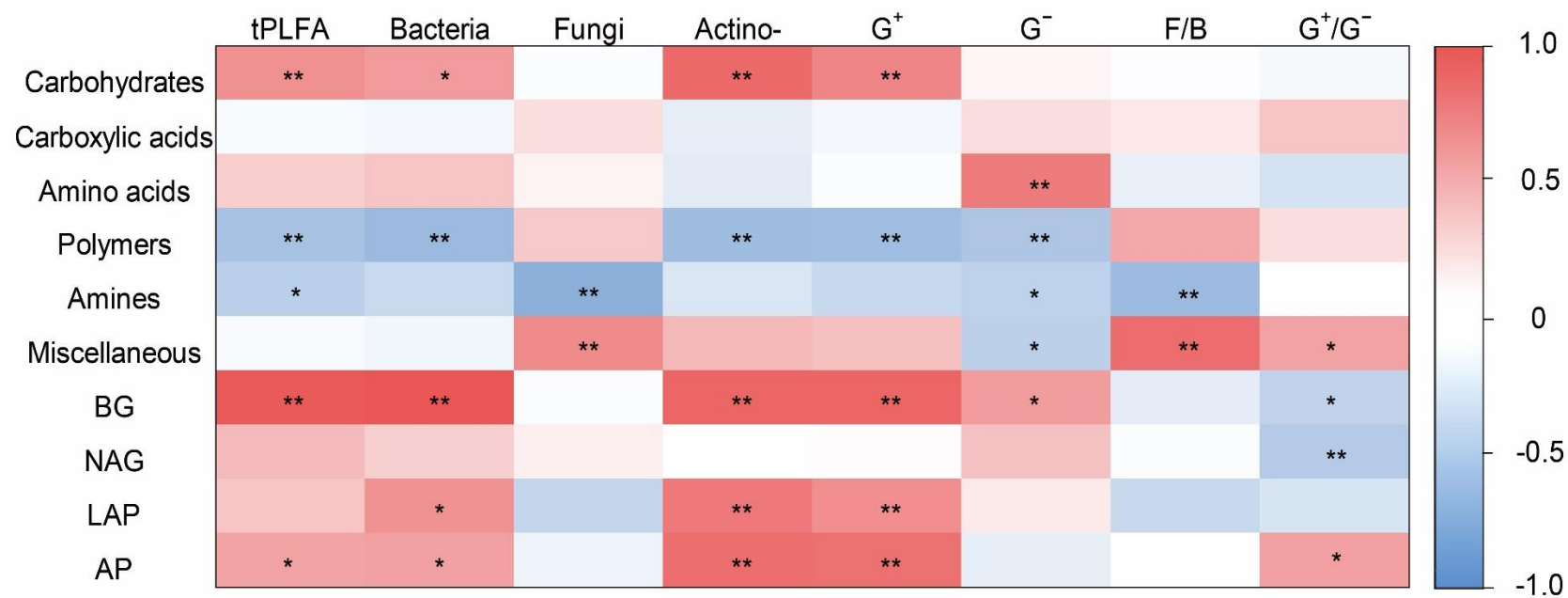
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**Figure 3.** Characteristics of microbial use of (a) carbohydrates, (b) carboxylic acids, (c) amino acids, (d) polymers, (e) amines, and (f) miscellaneous along the NSTEC. The representatives of different colors were showed in Figure 2.

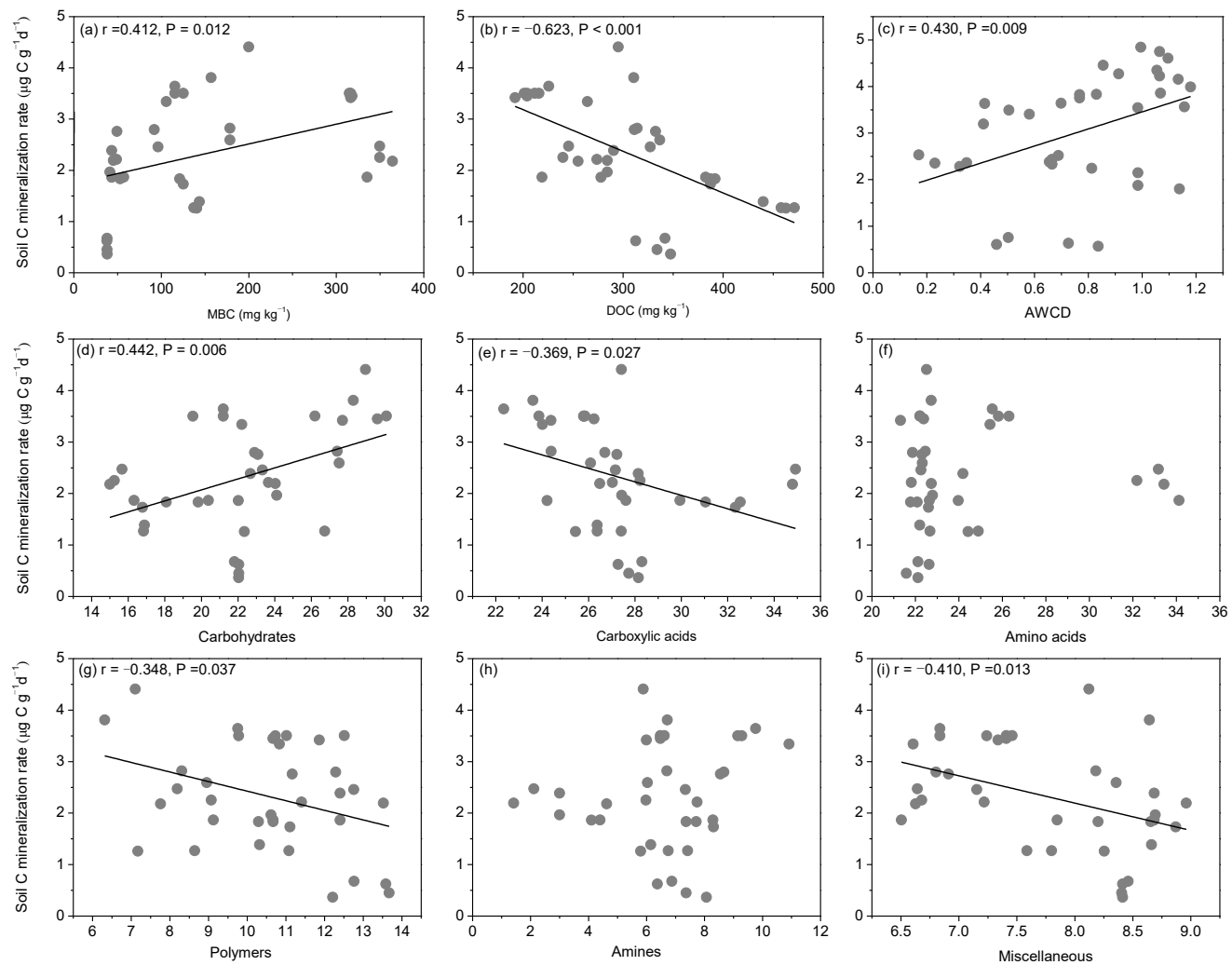


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**Figure 4.** Redundancy analysis (RDA) ordination biplot of soil microbial carbon sources use efficiency and environmental properties. The representatives of different colors were showed in Figure 2. The dotted lines and solid lines represent the environmental variables and lipid signatures and carbon sources. The abbreviations of the variables in this figure are as follows: MAP, mean annual precipitation. The vegetation data: LDMC, leaf dry matter weight; Leaf C, leaf carbon content; Leaf N, leaf nitrogen content; SLA, specific leaf area. Soil properties included SMC, soil moisture content; Silt, soil silt content; TN, soil total nitrogen; SOC, soil organic carbon. The abbreviations of the sampling sites were given in Table 1.

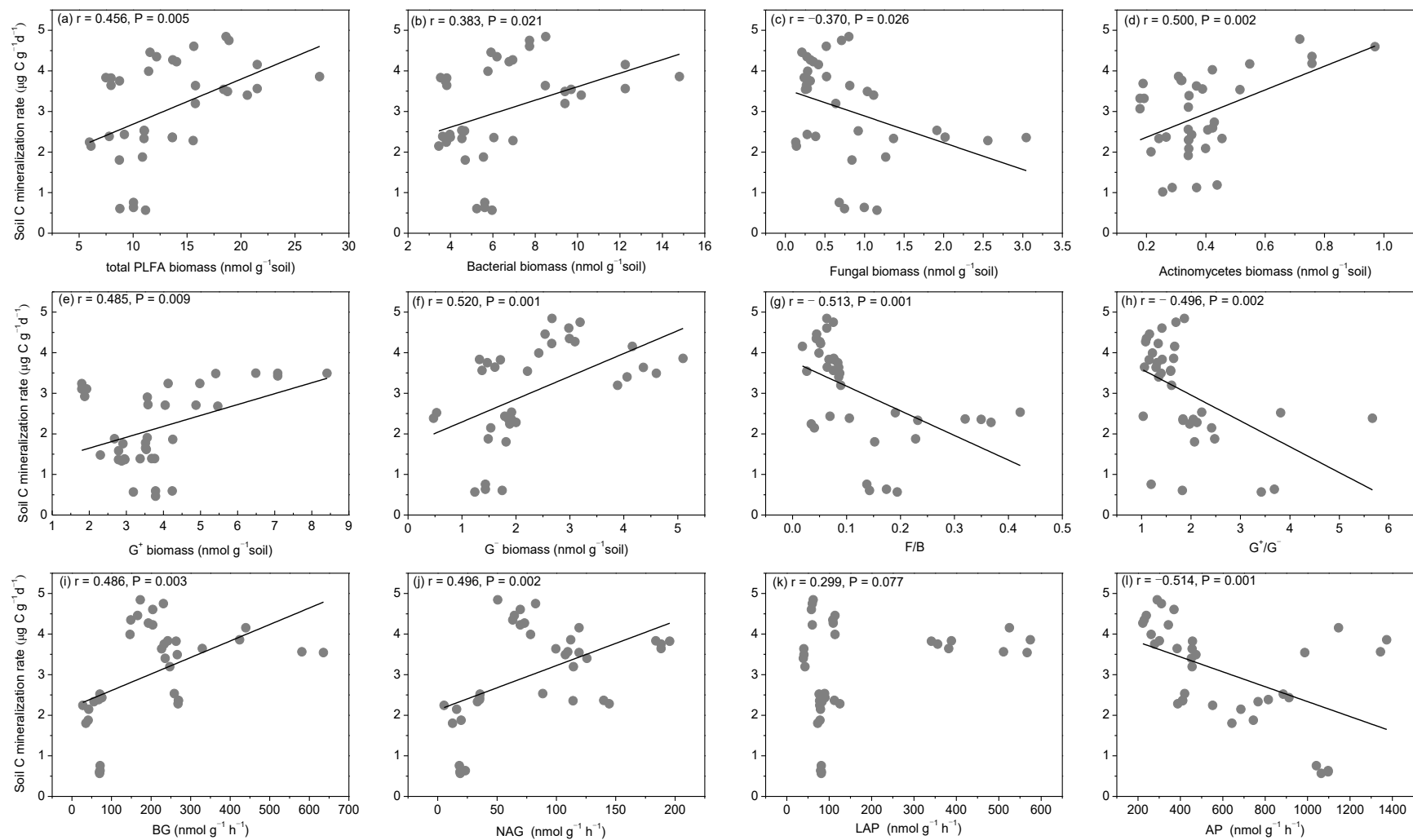


**Figure 5.** The heatmap of the Pearson's correlation coefficients between the use of individual substrates and microbial PLFAs and soil enzyme activities. Note: The abbreviations of the variables: Actino-, actinomycetes; F/B, fungi/bacteria; G<sup>+</sup>, gram positive bacteria; G<sup>-</sup>, gram negative bacteria; G<sup>+</sup>/G<sup>-</sup>, Gram-positive bacteria/ Gram-negative bacteria. BG,  $\beta$ -1, 4-glucosidase; NAG,  $\beta$ -1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase. \*\* $P < 0.01$ , \* $P < 0.05$ .



**Figure 6.** Relationships between soil carbon mineralization rates ( $\mu\text{g C g}^{-1} \text{d}^{-1}$ ) and microbial biomass C (MBC), soil dissolved organic C (DOC), average well color development (AWCD), and use of individual substrates.





**Figure 7.** Relationships between soil carbon mineralization rates ( $\mu\text{g C g}^{-1} \text{d}^{-1}$ ) and different groups of soil microbial PLFAs (a-h) and enzyme activities (i-l).