Manuscript bg-2018-499-R2   Response to Reviewers

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 (Megonigal 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_2). 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)

Plant functional traits determine latitudinal variations in soil microbial function: evidence from forests in China

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

56   **Abbreviations**

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^+ Gram positive bacteria
63   G^- Gram negative bacteria
64   F/B   Fungi/Bacteria

65   Climate conditions

66   MAT: Mean annual temperature
67   MAP: Mean annual precipitation

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

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 (Balser 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_2SO_4-$HClO_4$ (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 nmol g^{-1}. The four enzymatic activities of β-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 nmol h^{-1} 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-β-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 °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 (30×40 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 DBH≥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
$$H' = \sum_{i=0}^{n} (PilnPi)$$

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, m^2 kg^{-1}), leaf dry matter content (LDMC,

223 the oven-dried mass of a leaf divided by its water-saturated fresh mass, mg g^{-1}), leaf C concentrations

224 (leaf C, g kg^{-1}), and leaf N concentrations (leaf N, 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:

$$\text{CWM} = \sum_{i=1}^{n} pi \times \text{trait}_i$$

228   Where *pi* 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 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, 20, 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):

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

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 ± 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^{-1}, 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^{-1} (Table 2). The average SOM decomposition rates in

302   the subtropical forests ranged from 0.64 to 2.42 μg C g^{-1} d^{-1}, and were significantly lower than the

303   rates in the temperate forests, which ranged from 3.43 to 4.61 μg C g^{-1} d^{-1} (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

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

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

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

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

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

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

The SOM decomposition rates were significantly and positively correlated with total PLFAs (r=0.456, P=0.005), bacteria (r=0.3836, P=0.021), actinomycetes (r=0.500, P=0.002), and G^- bacteria PLFAs (r=0.520, P=0.001) (Fig. 7a, b, d, and f) but were negatively correlated with fungal 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, 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_2). 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

23

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^+ bacteria and actinomycetes communities were

453 associated with the specific activities of BG, AP, and LAP), whereas the relative abundance of the

454 G^- 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 (Balser 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.

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Figures legends

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

Fig.2. Variations in soil microbial substrate use 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.

Fig. 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.

Fig.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

Fig.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^+, gram positive bacteria; G^-, gram negative bacteria; G^+/G^-, Gram-positive bacteria/ Gram-negative bacteria. BG, β-1, 4-glucosidase; NAG, β-1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase. $**P< 0.01$, $*P< 0.05$.

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

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

Supporting information

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

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

Table S3 Soil organic matter (SOM) decomposition rates during the28 days of incubation time (Mean±SE) ($\mu g\ C\ g^{-1}\ d^{-1}$)

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

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[b] (°C)	MAP[b] (mm)	Vegetation types	Soil type
HZ[a]	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

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

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

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

Sampling site	pH	ST (°C)	SMC (%)	Silt (%)	SOC (g kg^{-1})	MBC (mg kg^{-1})	DOC (mg kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})
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

747　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
748　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.

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

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

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

753 **Figures**
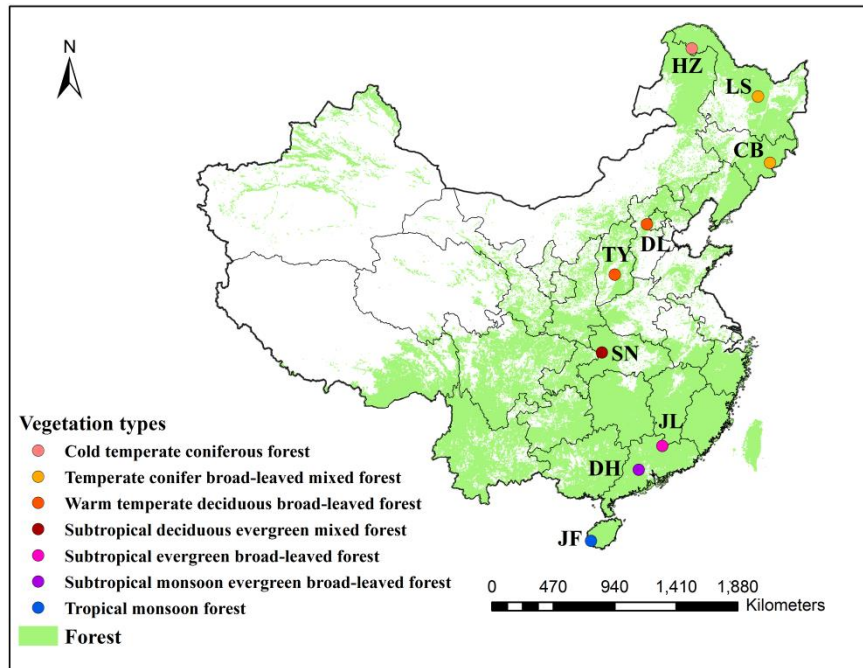
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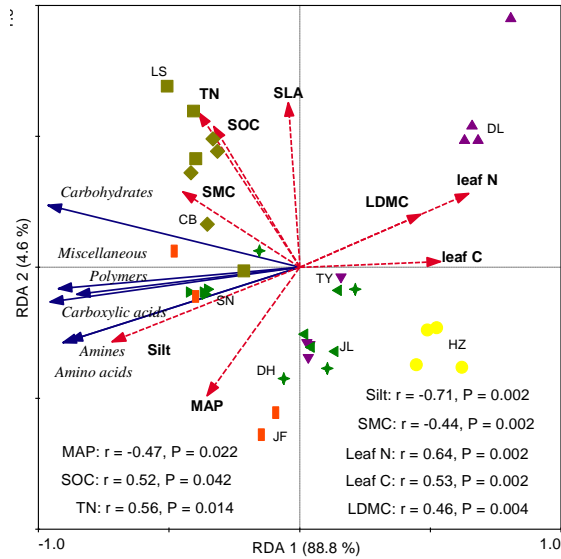757

758
759 **Figure 2.** Variations in soil total microbial metabolic intensity during a 240-h incubation for the nine forests.
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763 climate zone. The abbreviations of the sampling sites are given in Table 1.
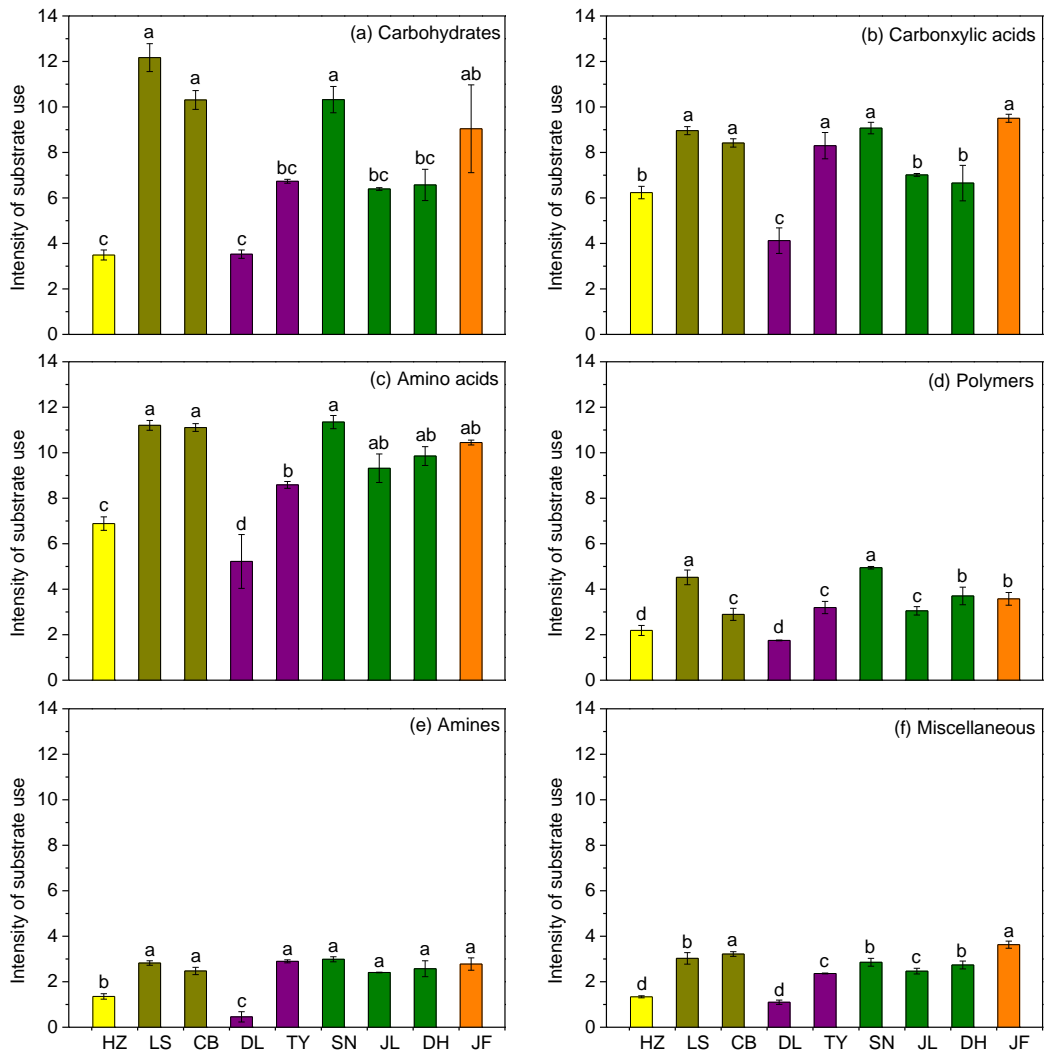
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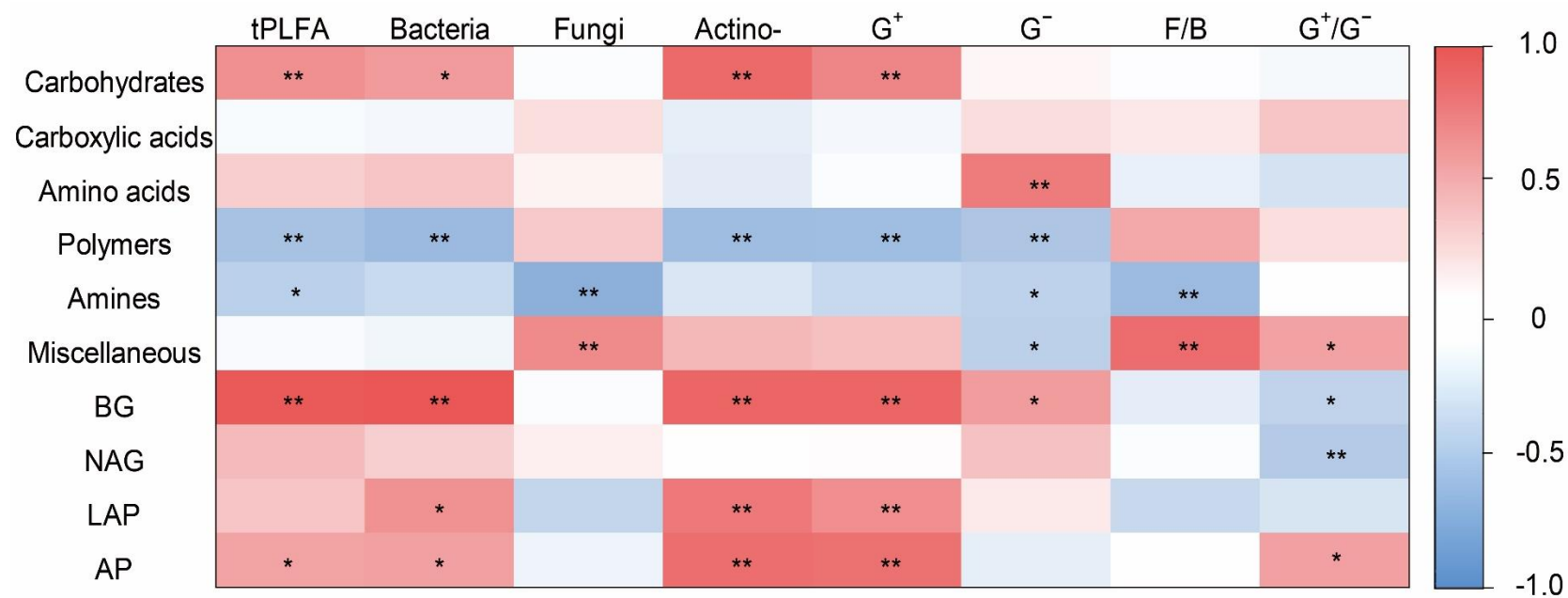
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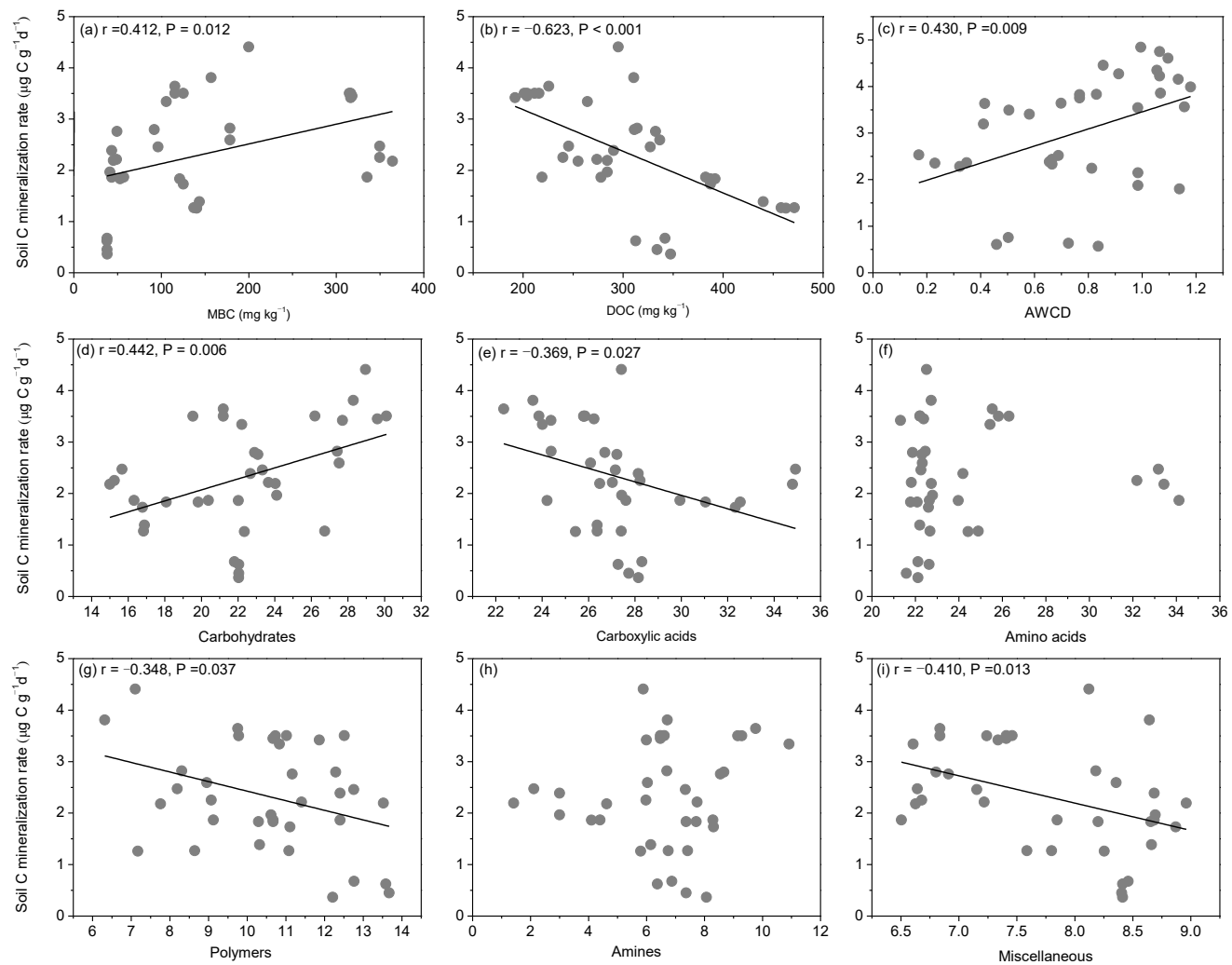
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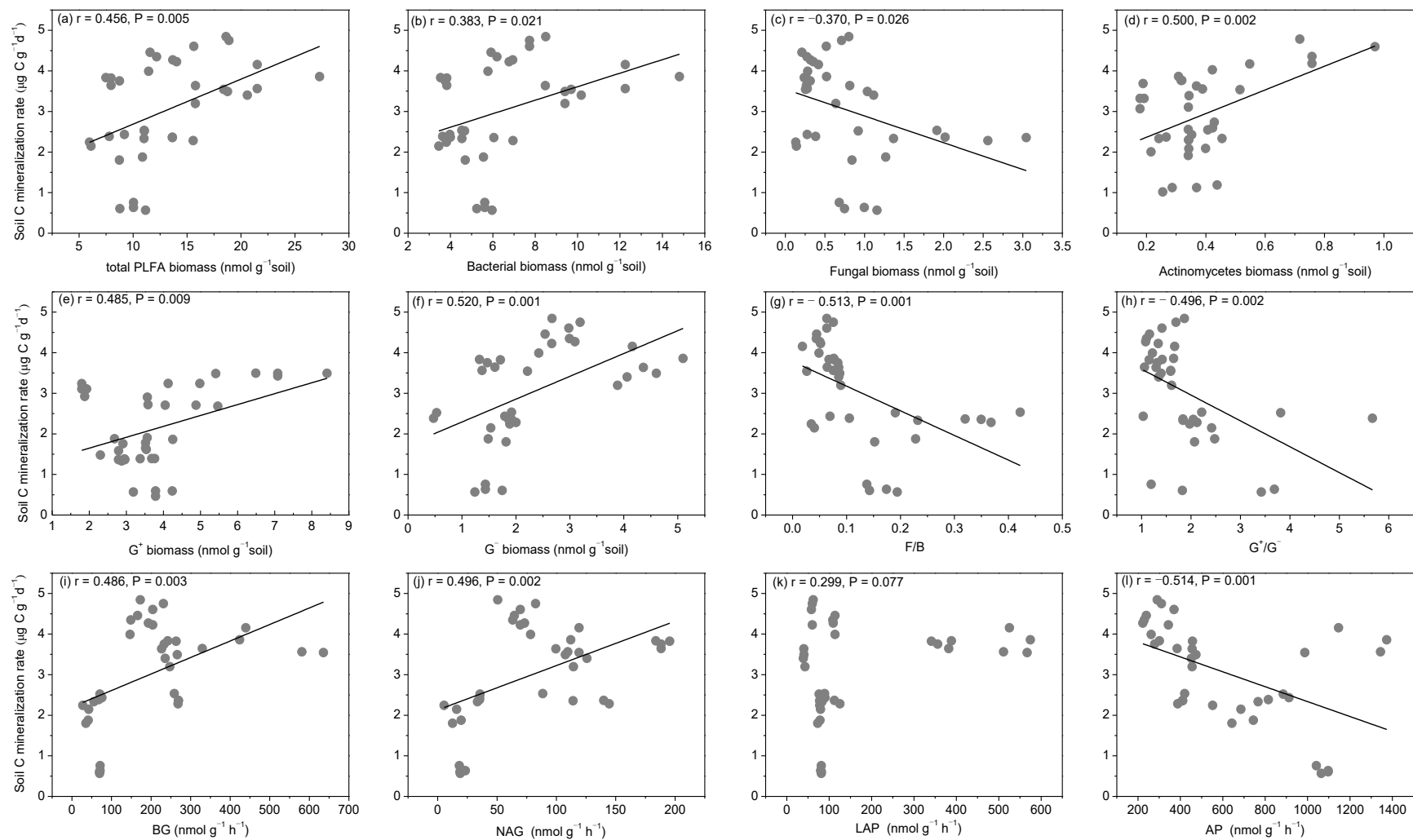
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