Biogeosciences
Discussions

Open Access

EGU

Divergence of dominant factors on soil microbial communities and functions in forest ecosystems along a climatic gradient

Zhiwei Xu [1], Guirui Yu [2,3,*], Xinyu Zhang [2,3,*], Nianpeng He [2,3], Qiufeng Wang [2,3], Shengzhong Wang [1], Xiaofeng Xu[4], Ruili Wang [5], Ning Zhao [6]

1 College of Geographical Sciences, Northeast Normal University, Changchun, 130024, China

2 Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101,China

3 College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, 100190, China

4 Biology Department, San Diego State University, San Diego, CA 92182, USA

5 College of Forestry, Northwest A&F University, Yangling, Shaanxi Province 712100, China

6 Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China

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

E-mail: yugr@igsnrr.ac.cn (G.Y.), zhangxy@igsnrr.ac.cn (X.Z.)

26 **Abstract.** Soil microorganisms play an important role in regulating nutrient cycling in terrestrial

27 ecosystems. Most of the studies conducted thus far have been confined to a single forest biome or

28 have focused on one or two controlling factors, and few have dealt with the integrated effects of

29 climate, vegetation, and soil substrate availability on soil microbial communities and functions

30 among different forests. In this study, we used phospholipid-derived fatty acid (PLFA) analysis to

31 investigate soil microbial community structure, and extracellular enzymatic activities to evaluate

32 the functional potential of soil microbes of different types of forests in three different climatic

33 zones along the North-South transect in eastern China (NSTEC). In general, soil enzyme activities

34 and microbial PLFAs were higher in primary forests than in secondary forests in temperate and

35 warm temperate regions. In the subtropical region, soil enzyme activities were lower in the

36 primary forests than in the secondary forests and microbial PLFAs did not differ significantly

37 between primary and secondary forests. The microbial PLFAs and enzyme activities differed

38 considerably between broadleaved and coniferous forests. Different species of coniferous trees

39 may cause variations in soil microbial PLFAs and enzyme activities. Both climate and forest type

40 had significant effects on soil enzyme activities and microbial communities with a considerable

41 interactive effect. Litter nutrients made an important contribution to variations in the soil

42 microbial communities and enzyme activities in temperate zones, while soil micro-climate and

43 nutrients were the main controls on the soil microbial community structure and enzymatic

44 activities in warm temperate and subtropical zones. Our results indicate that the main controls on

45 soil microbes and functions vary across forest ecosystems in different climatic zones, and that the

46 effects of soil moisture content, soil temperature, and the soil N/P ratio were considerable. This

47 information will add value to modeling of microbial processes and will contribute to carbon

48 cycling in large-scale carbon models.

49

1 Introduction

50

51   There is a growing awareness that above- and below-ground interactions make an essential

52   contribution to ecosystem function (van Dam and Heil, 2011). Variations in soil microbial

53   diversity and community structure have a strong influence on soil organic matter turnover and

54   may impact on the function of a given ecosystem (Baumann et al., 2013). For example,

55   mycorrhizal fungi and nitrogen (N) fixing bacteria are responsible for 80% of all N, and up to 75%

56   of phosphorus (P), that is acquired by plants annually (van der Heijden et al., 2008). Therefore, it

57   is important to study the composition and enzyme activities of soil microbial communities to

58   obtain an improved understanding of the mechanisms that control soil organic carbon dynamics in

59   different forest ecosystems.

60   Vegetation composition may alter soil physicochemical properties by changing the quantity

61   and quality of plant litter, which further influence microbial community composition and function

62   (Ushio et al., 2010; Deng et al., 2015). There is increasing evidence that vegetation types influence

63   the structure and functions of the soil microbial community (Yin et al., 2014; Zheng et al., 2015).

64   Differences in microbial communities, as represented by PLFAs, have also been reported among

65   adjacent maple, beech, hornbeam, lime, and ash forests in Germany (Scheibe et al., 2015) and

66   among forests of four conifer species in coastal British Columbia (Grayston and Prescott, 2005).

67   From a functional perspective, both soil acid phosphatase and b-glucosidase activities were higher

68   in a monsoon evergreen broadleaf forest than in a Masson pine forest (Zheng et al., 2015).

69   However, vegetation type does not always have an effect on the composition of the soil microbial

70   community. Hannam et al. (2006) reported that the microbial community composition of a white

71   spruce-dominated forest differed substantially from that of an aspen-dominated stand, but was

72   similar to that of a mixed stand with equivalent proportions of deciduous and coniferous trees.

73   Most of the studies conducted thus far have been confined to a single forest biome or have focused

74   on one or two controlling factors (Ultra et al., 2013), and few have dealt with the integrated effects

75   of climate, vegetation, and soil substrate availability on soil microbial communities and functions

76   in different forest biomes.

77   Soil microbial communities and enzyme activities can be influenced by an array of factors,

78   such as climate (Xu et al., 2015), vegetation types (Urbanová et al., 2015), plant diversity (Li et al.,

79   2015), and physico-chemical soil properties (Tripathi et al., 2015). The links between the diversity

80   of plant and soil microbial communities and enzyme activities are widely acknowledged (Chung et

81   al., 2007). The composition of the vegetation species can be used to successfully predict the soil

82   microbial community (Mitchell et al., 2010). Soils with different vegetation types develop distinct

83   physico-chemical properties that will have pronounced effects on the structure and function of the

84   soil microbial community (Priha and Smolander, 1997). Soil organic matter is related to the

85   variations in microbial activities and community function (Brockett et al., 2012). Soil pH (Shen et

86   al., 2013), elemental stoichiometric ratios (Högberg et al., 2007), and nutrient status (Lauber et al.,

87   2008) have also been identified as determinants of microbial community structure. However, we

88   still do not know which mechanisms control the variability in the structure and functions of soil

89   microbial communities within different groups of plant species (broadleaved and coniferous trees)

90   on similar soil types within the same climatic region.

91   Forest soil microbial community structures and enzyme activities are influenced by different

92   factors in different climatic zones. For example, Högberg et al. (2007) found that the soil

93   microbial community composition in a boreal forest was strongly influenced by the soil carbon to

94   nitrogen ratio (C/N) and the soil pH. Studies in temperate forests have shown that dehydrogenase

95   and urease were closely related to the mean air temperature, litter production, and nutrient

96   availability (Kang et al., 2009). In addition, Hackl et al. (2005) reported that soil water availability

97   was responsible for variability in the microbial community structure of temperate forests.

98   Precipitation and soil moisture may be important controls on the structure of soil fungal

99   communities of tropical forests (Eaton et al., 2011; McGuire et al., 2012). However, there is a lack

100   of well-defined information about the factors that influence the structure and functions of soil

101   microbial communities in forests with different plant species (broadleaved and coniferous trees)

102   across a range of climates and soils.

103   The North-South Transect of Eastern China (NSTEC) represents a latitudinal and climatic

104   gradient. It is a unique belt in which vegetation ranges from boreal forest to tropical rain forest,

105   depending on the local temperature and precipitation conditions. In this study we examined

106   variations in the soil microbial communities and their functions in forests comprising different

107   species (broadleaved and coniferous trees) in temperate, warm temperate, and tropical forest

108 biomes along the NSTEC. The temperature and precipitation are different in these three climatic

109 zones. We used information about the soil physico-chemical properties, microbial community

110 structure, and hydrolytic enzyme activities involved in C, N, and P transformations to explore how

111 soil microbial communities and enzyme activities differed among different forest types in different

112 climatic zones, and to determine the influence of different environmental variables on the soil

113 microbial communities and enzyme activities in different climatic zones.

114 **2 Materials and methods**

115 **2.1 Study area and soil sampling**

116 We chose three study sites, namely Liangshui in Northeast China, Taiyue Mountain in North

117 China, and Dinghu Mountain in South China, along the North-South Transect in Eastern China

118 (NSTEC) for field measurements and soil sampling (Fig. 1). Both the air temperature and

119 precipitation decrease from south to north along the NSTEC (Table 1).

120   We examined all the representative forest species in each climatic zone. In Liangshui, on the

121 Xiao Xing'an Mountain, we sampled primary conifer broad-leaved mixed forest (PCB), secondary

122 conifer broad-leaved mixed forest (SCB), and two coniferous plantations, one of which was

123 mainly *Pinus koraiensis* (PK) while the other was *Larix olgensis* (LO). On Taiyue Mountain, we

124 sampled primary deciduous broad-leaved forest (PDB), secondary deciduous broad-leaved forest

125 (SDB), and two coniferous plantations, one of which was comprised mainly of *Pinus*

126 *tabulaeformis* (PT) while the other was mainly *Larix olgensis* (LO). On Dinghu Mountain, we

127 sampled a primary evergreen broadleaved forest (*Castanopsis chinensis*, *Cryptocarya chinensis*,

128 *Cryptocarya concinna*, *Erythrophleum fordii*, and *Cyathea podophylla*), secondary conifer and

129 broadleaf mixed forest (*Pinus massoniana, Schima superba*), aconiferous plantation (*Pinus*

130 *massoniana*), and an evergreen broadleaved plantation (*Erythrophleum fordii*) along a

131 successional stage, hereafter referred to as PEB, SCB, PM, and EF, respectively. The primary

132 forests are zonal forests that reflect the regional climate and the others are zonal forests that reflect

133 the extreme site conditions. Information about the climate, soil classification (Soil Survey Staff

134 2010), and soil properties at each site is provided in Table 1.

135   Soil samples were collected at nine sampling sites along the NSTEC in July and August 2013.

136 Each site had four independent plots in well-drained areas, which covered an area of 30 m $\times$ 40 m,

137   and were at least 10 m apart. The vegetation composition of the four plots at each site was similar.

138   Samples of mineral soil were collected from a depth of 0–10 cm at between 30 and 50 points in

139   each plot along an S-shape using a custom-made coring device with a diameter of 6 cm. The

140   above-ground standing biomass, dead plant parts, and litter were removed from each sampling

141   point. These samples were pooled together as a composite sample. Visible roots and residues were

142   removed and then the soil fractions of each sample were homogenized.

143   We stored the samples at 4 ℃ in a portable refrigerator during field sampling. Once returned

144   to the laboratory, samples were stored at 4 ℃ before analysis. Soils were analyzed for enzyme

145   activities and PLFAs in September 2013. The fresh soil samples were sieved through a 2-mm

146   mesh and were subdivided into three subsamples. One subsample was stored at 4 ℃ until

147   analyzed for soil enzyme activities and physical and chemical properties. The second was stored at

148   −20 ℃ before analysis for microbial community structures. The third was air dried, and then

149   sieved through a 0.25 mm mesh before SOC, TN, and TP analysis. The soil temperatures were

150   measured *in situ* at the time of sampling. Soil moisture content (SMC) was measured

151   gravimetrically on 20 g fresh soil that was oven-dried at 105 ℃ to constant weight immediately on

152   arrival at the laboratories at the study sites (Liu et al., 2012).

153   **2.2 Soil chemical analyses**

154   Soil pH was measured at a soil-to-water ratio of 1:2.5. Soil total N (TN) concentrations were

155   determined by dry combustion of ground samples (100-mesh) in a C/N analyzer (Elementar, Vario

156   Max CN, Germany). The soil organic carbon (SOC) concentrations were determined by

157   dichromate oxidation and titration with ferrous ammonium sulfate (Huang et al., 2014). The litter

158   total C (litter TC) and total N (litter TN) were determined with the same method that was used for

159   soil TN. Total phosphorus (TP) was determined with a flow injection auto-analyzer following

160   digestion with H_2SO_4-$HClO_4$ (Huang et al., 2011). The soil clay fraction (hereafter referred to as

161   Clay, comprised of particles <53 μm) was separated by wet-sieving and then freeze-dried (Six,

162   Elliott & Paustian 2000).

163   **2.3 Phospholipid fatty-acid and enzyme activity analysis**

164   Samples were analyzed for phospholipid fatty-acids (PLFA) using the method described by Bååth

165   & Anderson (2003). After mild alkaline methanolysis to form fatty acid methyl esters (FAMEs),

166 samples were then dissolved in hexane and analyzed with a DB-5 column in a gas chromatography

167 mass spectroscopy (GCMS) system (Thermo TRACE GC Ultra ISQ). Total amounts of the

168 different PLFA biomarkers were used to represent the different groups of soil micro-organisms

169 (Table S1). Taken together, the combination of bacterial, fungal and actinomycic PLFAs

170 biomarkers represented the total PLFAs of the soil microbial community.

171   The activities of β-glucosidase (BG), N-acetylglucosaminidase (NAG), acid phosphatase

172 (AP), and leucine aminopeptidase (LAP) were measured as outlined by Saiya-Cork, Sinsabaugh &

173 Zak (2002). The microplates were incubated in the dark at 20 ℃ for 4 h. During the incubation,

174 the incubation plates were shaken every hour to ensure the reaction mixtures were homogenous.

175 Fluorescence was measured using a microplate fluorometer with 365-nm excitation and 450-nm

176 emission filters (Synergy[H4] Hybrid Reader, Synergy[H4] BioTek, USA).

177 **2.4 Statistical analysis**

178 One-way analysis of variance (ANOVA) with a post-hoc Tukey HSD test was used to test the

179 differences between the soil and microbial properties in the various forests of the three climatic

180 zones. All data were normality distributed. Two-way analysis was used to test the effect of climate

181 and vegetation on the soil microbial properties. All ANOVA and two-way analysis were

182 performed using SPSS 19.0 for Windows. Figures were generated using the Origin 8.0 package.

183 Data are reported as the mean ±SE.

184   Redundancy analysis (RDA) was used to examine the relationships between the litter factors

185 (litter TC, litter TN, litter C/N), soil biochemical variables (soil temperature (ST), soil moisture

186 content (SMC), pH, C/N, soil carbon to phosphorus ratio (C/P), soil nitrogen to phosphorus ratio

187 (N/P), SOC, TN, TP), soil texture (Clay), and the soil microbial community compositions and

188 enzyme activities. Before redundancy analysis, we conducted forward selection of the

189 environmental variables that were significantly correlated with variations in the microbial

190 communities and enzyme activities using stepwise regression and the Monte Carlo Permutation

191 Test that was similar to the multiple regression analysis. Stepwise regression and RDA were

192 processed using CANOCO software 4.5 (Ter Braak & Smilauer 2002). The vectors of greater

193 magnitude that formed smaller angles with an axis were more strongly correlated with that axis.

194 **3 Results**

3.1 Soil enzyme activities in different vegetation types

195

196 The soil enzyme activities were generally higher in the primary forests than in the secondary

197 forests in temperate and warm temperate climatic zones (Fig. 2). However, in the subtropical

198 climatic zone, soil enzyme activities were higher in the SCB forest than in the PEB forest. The BG,

199 NAG, and AP enzymes in the two soils of the PT and LO in the warm temperate zone were

200 significantly different (Fig. 2(A, B, D)). Climate, a significant influence on the variations of soil

201 enzyme activities ($P<0.0001$), had more influence than forest type. The effects of climate and

202 forest type interactions were only significant for soil NAG ($P<0.0001$) and AP activities ($P=0.035$)

203 (Table 2, Table S2).

3.2 Soil microbial community composition in different vegetation types

204

205 Soil PLFAs were higher in the primary forest in the temperate and warm temperate zones than in

206 the secondary forest. In the temperate zones, soil PLFAs were higher in the PCB forest than in the

207 SCB, PK, and LO (Fig. 3A). In the warm temperate forests, total soil microbial PLFAs were

208 highest in the LO forest (Fig. 3B). In the subtropical zone, total, bacterial, and actinomycic PLFAs

209 were higher in the PEB and SCB forests than in the PM and EF forests (Fig. 3C).

210 Climate had a significant effect on the Total PLFAs, fungi, and G^- ($P<0.0001$), and the forest

211 type had a significant effect on the soil bacteria, fungi, G^+, and G^- PLFAs. With the exception of

212 the soil G^+/G^-, the effect of the combination of climate + forest type on all soil PLFAs was

213 significant, and was stronger than the individual effects of either climate or forest type (Table 2,

214 Table S2). The soil microbial communities in the different forests in the three climate zones were

215 generally unique (Fig. 5).

3.3 Relationships between soil enzyme activities and soil properties

216

217 The litter C/N, litter TN, and SMC ($P=0.002$) were the most important influences on the soil

218 enzyme activity variations in the temperate forests, followed by ST, soil N/P, and soil TP (Fig.

219 4(A)). In the warm temperate forests, the variations in the soil enzyme activities were significantly

220 and positively correlated with ST and soil pH ($P=0.002$), but were negatively correlated with SMC

221 and soil nutrients (TN and SOC) (Fig. 4(B)). In the subtropical forests, soil enzyme activities were

222 significantly and positively correlated with clay, SMC, soil TN, and TP ($P=0.002$), followed by

223 soil nutrient ratios (Fig. 4(C)). These results indicate that the litter inputs, soil micro-climate, and

224 soil texture were the main drivers of variations in the soil enzyme activities in the temperate, warm

225 temperate, and subtropics, respectively, with ST, pH, SMC, and soil N/P as additional influences.

3.4 Relationships between plfa profiles and measured soil properties

227 In the temperate forests, the variations in the soil microbial community structure were strongly

228 affected by the litter TN, litter TC, litter C/N, soil TP, and ST (P=0.002) (Fig. 5(A)). In the warm

229 temperate forests, the first axis of the RDA plot of the soil microbial community structure was

230 significantly and positively correlated with ST (P=0.002), but was negatively correlated with soil

231 N/P, soil TN, soil C/P, and SOC (P=0.002) (Fig. 5(B)). In subtropical forests, the variations in the

232 soil microbial community structure were significantly and positively correlated with litter TC and

233 ST (P=0.002), but negatively correlated with SMC, soil C/P, soil N/P, and soil C/N (P=0.002),

234 followed by the soil TN and clay contents (Fig. 5(C)). The litter C/N was the main influences on

235 the variations in the soil microbial communities in the temperate, and the soil N/P was the main

236 influences in the warm temperate and subtropical forests. The microbial communities were also

237 influenced by ST, pH, SMC.

4 Discussion

4.1 Response of soil enzyme activities and microbial plfas to variations in forest type

240 As expected, soil enzyme activities differed between the coniferous, deciduous, and broad-leaved

241 forests in the three climatic zones. The PCB in the temperate zone is a conifer broad-leaved mixed

242 forest and has higher inputs of mixed litter than a single species coniferous forest (Zhang et al.,

243 2008). Therefore, all enzyme activities were highest in PCB in the temperate zone. The higher soil

244 enzyme activities in the coniferous forests relative to those in the deciduous broad-leaved forests

245 in the warm temperate zone reflect the high SOC and TN concentrations in the two coniferous

246 forests (Table 1). Extracellular enzymes catalyze the rate-limited steps of decomposition and

247 nutrient cycling (Koch et al., 2007), thereby improving the soil nutrient availability. The soil

248 enzyme activities were highest in the SCB forest, reflecting the higher soil nutrient concentrations

249 in subtropical zones.

250 The soil microbial community structures under the various forest types differed significantly

251 across the three climatic zones. Vegetation transfers substrate material of varying quality to

252 microbes through litter fall. Litter from broadleaved forests typically contains high levels of

253   water-soluble sugar, organic acid, and amino acids (Priha and Smolander, 1997; Priha et al., 2001),

254   and promotes the propagation of bacteria that favor high-nutrient soil. However, fungi are mainly

255   responsible for lignin degradation and are presumably more capable of coping with the

256   degradation of pine litter that contains high amounts of recalcitrant polymeric phenolic

257   compounds such as lignin and tannin than bacteria (Wardle et al., 2003; Hackl et al., 2005).

258   Therefore, the structures and functions of the soil microbial communities that developed in the

259   different types of forest were unique.

260   The variations in the plant functional traits between the different forest types, especially

261   between the deciduous and coniferous forests, will promote the development of different soil

262   microbial communities. Several other studies have described how SLA, LDMC, and leaf N

263   influence soil microbial community structure and function (Orwin et al, 2010; de Vries et al., 2012;

264   Pei et al., 2016). While plant trait data were not available for this study, our results were similar to

265   those from other studies of the nine primary forests along the NSTEC (data have not been

266   published). Despite this, climatic region may have more influence on soil enzyme activities and

267   soil microbial communities than forest type, and other studies have reported how climate

268   influences the large-scale distribution of microorganisms (de Vries et al., 2012; Xu et al., 2017).

269   **4.2 Common influences on soil enzyme activities and microbial communities**

270   Although soil microbial communities and functions varied between the different forests, they were

271   subject to some common influences. For example, our results showed that ST, SMC, soil pH, and

272   soil N/P ratio influenced, but perhaps did not dominate, the responses of the soil microbial

273   community structures and enzyme activities in the different forest types across the three climatic

274   zones.

275   Temperature can influence enzyme activity directly and indirectly by modifying the enzyme

276   kinetics and influencing the proliferation of microbes, respectively (Kang et al., 2009). By

277   changing the quality and quantity of the substrate on which microbes function, soil moisture is an

278   important driver of the overall microbial composition and soil microbial function (Hackl et al.,

279   2005). The responses of soil enzyme activities and microbial communities in the various forest

280   types were all significantly influenced by the SMC in the three climatic zones. Increases in soil

281   moisture can enhance both the release and the diffusion rates of enzymes, substrates, and reaction

282   products (Burns et al., 2013), and our results showed that soil enzyme activities and microbial

283   PLFAs increased as the SMC increased in the warm temperate and subtropical zones. However,

284   water-logged conditions are not suitable for microbes and are not beneficial for the release of soil

285   enzymes (Lucas-Borja et al., 2012), and, similar to other studies, soil enzyme activities and SMC

286   were negatively correlated in the temperate zone forests (Brockett et al., 2012). As the SMC

287   increases, the bacterial PLFAs increase (Myers et al., 2001) and fungal PLFAs decrease (Staddon

288   et al., 1998), which indicates that the soil microbial communities and enzyme activities in the

289   different climatic zones were all influenced by the soil micro-climate. This was also demonstrated

290   by the stronger effect of climate on soil enzyme activities and the combined interaction effect of

291   climate and forest type on soil microbial communities.

292   An increasing number of studies has reported that the soil microbial composition and enzyme

293   activities are largely related to soil pH at continental (Fierer and Jackson, 2006) and global scales

294   (Sinsabaugh et al., 2008). Soil pH directly affects the activities of extracellular enzymes

295   immobilized in the soil matrix, and the effect of soil pH on the soil microbial community and

296   function reflects the influence of vegetation through changes in soil chemistry. Every enzyme has

297   a well-defined optimal soil pH value (Sinsabaugh et al., 2008) that results from different levels of

298   soil enzyme activities under different soil pH conditions. Increases in pH lead to increases in

299   bacterial diversity and cause the bacterial community to shift, so that there are more G^-, and less

300   G^+, bacteria PLFAs (Wu et al., 2009; Shen et al., 2013).

301   Many other studies have reported how different factors determine the response of the soil

302   microbial community and function to variations in forests (Högberg et al., 2007; Kang et al., 2009;

303   Eaton et al., 2011; McGuire et al., 2012). Mostly limited to one climatic zone, these studies were

304   quite diverse and featured a range of microbial methods, sampling times, and environmental

305   properties, which means it is difficult to compare the results. In this study, we collected the

306   samples at the same times and used the same methods to analyze the soil microbial communities

307   and enzyme activities. We found that the different climatic zones shared common factors that

308   influenced the responses of the soil microbial communities and functions to forest variations. Soil

309   microbes have unique roles in C, N, and P cycling that depend on the vegetation type and soil

310   properties (Sugihara et al., 2015; Wu et al., 2015). Our results suggest that the nutrient cycling

311 mechanisms probably vary between different vegetation types and climatic zones; however,

312 further studies are needed to define the patterns and drivers of nutrient cycling.

313 **4.3 Key influences on soil enzyme activities and microbial communities**

314 Our results showed that the most important controls on the responses of soil microbial

315 communities and enzyme activities to vegetation types varied across climatic zones. The litter

316 quality and quantity contribute to the maintenance of soil fertility in forest ecosystems (Wang et al.,

317 2011). In our study, and the C/N ratios were highest, in litter from PCB stands (Table 1), which

318 shows that the soil in the PCB was more N-limited than the other soils because of litter inputs with

319 high C/N ratios (Table 1). Therefore, the microbial N demand was highest in soil in the PCB forest,

320 which resulted in higher NAG and LAP values. Plant litter has a strong influence on soil microbial

321 composition and activity, as the litter decomposition process provides nutrients for microorganism

322 growth through inputs of leaf litter (Attiwill and Adams, 1993), dying roots (Silver and Miya,

323 2001), and root secretion (Grayston et al., 1997). The litter from the mixed forests, represented in

324 our study by PCB, is more diverse than that from the pure forests, and so a wider variety of soil

325 microbes participate in the decomposition process, so that the soil organic matter is richer, and

326 there are more soil microbial PLFAs, than in the other forest types. Fungi typically dominate

327 N-limited environments and the fungal biomass is positively related to the C/N ratio (Nilsson et al.,

328 2012). The F/B ratio was therefore highest in the PCB forest where the litter C/N values were

329 highest.

330   The soil N/P ratio was the most important influence on the soil microbial communities and

331 enzyme activities in the warm temperate zone, which is consistent with the results of previous

332 studies (Shen et al., 2013; Högberg et al., 2007). Soil stoichiometric C, N, and P ratios reflect the

333 nutrient limitations of the ecosystems (Sterner and Elser, 2002) and should indicate soil organic

334 matter mineralization and sequestration (Gundersen et al., 1998). Soil microorganisms obtain C, N,

335 and P in such a way that enzyme release corresponds with the soil stoichiometric ratios of C, N,

336 and P. When supplies of N or P are limited, the activities of the enzymes that are responsible for

337 nitrate or phosphate mineralization will be higher. Consistent with this discussion, soil enzyme

338 activities in subtropical forests (DH) responded positively to the soil C/N and N/P ratios.

339   Microbes obtain the nutrients they need to construct biomass by decomposing soil organic

340   matter. Wallenius et al. (2011) found that the soil bacterial biomass was higher in forests where the

341   soil organic matter concentrations were higher than in forests with low soil organic matter

342   concentrations, and Xu et al. (2017) found positive relationships between soil enzyme activities

343   and SOC and TN concentrations along the NSTEC. In line with the resource limitation model, and

344   also confirmed by several other studies (Brockett et al., 2012; Zhang et al., 2013), Schimel and

345   Weintraub (2003) suggested that increases in N and C substrate availability might favor enzyme

346   synthesis. Soil microorganisms however did not grow when the available P concentrations in soil

347   were less than 0.7 mg kg^{-1} and were stimulated by P additions (Zheng et al., 2009). Other studies

348   have reported that P additions stimulated the different PLFA microbial groups in soils (Dong et al.,

349   2015). The positive correlations between both total microbial biomass and microbial composition

350   and available P suggests that microbes may be dependent on the P supply in some forest

351   ecosystems, especially in subtropical forests (DeForest et al., 2012; Zhang et al., 2013; Xu et al.,

352   2017).

353   The soil clay content had most influence on the soil enzyme activities in subtropical forests.

354   Soil texture is a key property that affects the accessibility of organic matter to microbes, and is an

355   important determinant of soil moisture, and nutrient availability and retention (Veen and Kuikman,

356   1990). Consistent with our results, Lagomarsinoa al. (2012) reported that the activities of soil

357   BG, AP, and NAG were higher in silt and clay fractions than in coarser fractions. This may be

358   attributed to the presence of clay-humus-enzyme complexes in the finest soil fractions, and

359   implies that physical protection affects soil enzyme activities. In addition, fine textured soils with

360   higher silt and clay contents are known to be more conducive to bacterial growth than coarser soils

361   because they have a greater water-holding capacity, higher nutrient availability, and offer better

362   protection against bacterial grazers (Carson et al., 2010).

363   **4.4 Implications for ecosystem modeling**

364   There is increasing recognition that, to improve climate models, microbial processes should be

365   simulated (DeLong et al., 2011; Xu et al., 2014). As such, this study has three important

366   implications. First, microbial datasets that have information about enzyme activities and soil

367   microbial properties contribute to improved parameterization of ecosystem models (Xu et al.,

368   2013; 2017). Information about the spatial patterns of, and factors that control, microbial

Biogeosciences
Discussions

369   properties and enzymatic activities can enrich the datasets that are used to parameterize models of

370   microbial processes (Wang et al., 2013; Allison et al., 2010). Secondly, knowledge about

371   microbial community structure and its environmental controls can give a better understanding of

372   how microbes adapt to changing environments, which is the main direction of model development

373   (Schimel and Schaeffer, 2012). Information about edaphic controls on microbial processes is

374   critical for developing new modeling frameworks with improved links with field experimental

375   data (Abramoff et al., 2017). Finally, the information generated in this study about the divergence

376   of the dominant factors that control soil microbial properties across forests is extremely valuable

377   for improving our understanding of soil microbial ecology and forest management.

378   **5 Conclusions**

379   In this study, we characterized the soil microbial communities and enzyme activities and factors

380   that controlled them in various forest types across three different climatic zones. We found that

381   forest types with specific soil conditions supported the development of distinct soil microbial

382   communities with variable functions. The litter TN, soil temperature, and soil clay contents were

383   important predictors of the variance in soil enzyme activities in temperate, warm temperate, and

384   subtropical zones, respectively, while litter and soil nutrient ratios were significant predictors of

385   the variance in soil microbial communities. We also found that SMC, soil temperature, soil pH,

386   and the soil N/P ratio were common drivers of variations in the soil microbial community structure

387   and enzyme activities across the different forest types in the three climatic zones. The data in this

388   study is extremely valuable for improving our understanding of soil microbial ecology and forest

389   management.

396

407   **References**

408   Abramoff, R., Xu, X., Hartman, M., O'Brien, S., Feng, W. T., Davidson, E. A., Finzi, A., Moorhead, D. L., Schimel,
409   J. P., Torn, M. S., and Mayes, M. A.: The millennial model: in search of measurable pools and transformations
410   for modeling soil carbon in the new century, Global Biogeochem. Cy. (In review), 2017.
411   Allison, S. D., Wallenstein, M. D., and Bradford, M. A.: Soil-carbon response to warming dependent on microbial
412   physiology, Nat. Geosci., 3, 336-340, 2010.
413   Attiwill, P.M. & Adams, M.A., 1993. Nutrient cycling in forests. New Phytologist, 124, 561-582.
414   B ååth, E. and Anderson, T.H.: Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and
415   PLFA-based techniques, Soil Biol. Biochem., 35, 955-963, 2003.
416   Baumann, K., Dignac, M-F., Rumpel, C., Bardoux, G, Sarr, A., Steffens, M., and Maron, P. A.: Soil microbial
417   diversity affects soil organic matter decomposition in a silty grassland soil, Biogeochemistry, 114, 201-212,
418   2013.
419   Brockett, B.F.T., Prescott, C.E., and Grayston, S.J.: Soil moisture is the major factor influencing microbial
420   community structure and enzyme activities across seven biogeoclimatic zones in western Canada, Soil Biol.
421   Biochem., 44, 9-20, 2012.
422   Burns, R.G, DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.
423   N., and Zoppini, A.: Soil enzymes in a changing environment: Current knowledge and future directions, Soil
424   Biol. Biochem., 58, 216-234, 2013.
425   Carson, J.K., Gonzalez-Quinones, V., Murphy, D.V., Hinz, C., Shaw, J.A., and Gleeson, D.B.: Low pore
426   connectivity increases bacterial diversity in soil, Appl. Environ. Microbiol., 76, 3936-3942, 2010.
427   Chung, H., Zak, D.R., Reich, P.B., and Ellsworth, D.S.: Plant species richness, elevated CO_2, and atmospheric
428   nitrogen deposition alter soil microbial community composition and function, Global Change Biol., 13,
429   980-989, 2007.
430   van Dam, N.M. and Heil, M.: Multitrophic interactions below and above ground: en route to the next level, J. Ecol.,
431   99, 77-88, 2011.
432   DeLong, E. F., Harwood, C. S., Chisholm, P. W., Karl, D. M., Moran, M. A., Schmidt, T. M., Tiedje, J. M.,
433   Treseder, K. K., and Worden, A. Z.: Incorporating microbial processes into climate models, The American
434   Academy of Microbiology, 2011.
435   Deng, H., Yu, Y.J., Sun, J.E., Zhang, J.B., Cai, Z.C., Guo, G.X., and Zhong, W. H.: Parent materials have stronger
436   effects than land use types on microbial biomass, activity and diversity in red soil in subtropical China,
437   Pedobiologia, 58, 73-79, 2015.
438   Eaton, W.D., Mcdonald, S., Roed, M., Vandecar, K.L., Hauge, J.B., and Barry, D.: A comparison of nutrient
439   dynamics and microbial community characteristics across seasons and soil types in two different oldgrowth
440   forests in Costa Rica, Trop. Ecol., 52, 35-48, 2011.
441   Fierer, N. and Jackson, R.B.: The diversity and biogeography of soil bacterial communities, P. Natl. Acad. Sci.
442   USA, 103, 626-31, 2006.
443   Grayston, S.J. and Prescott, C.E.: Microbial communities in forest floors under four tree species in coastal British
444   Columbia, Soil Biol. Biochem., 37, 1157-1167, 2005.
445   Grayston, S.J., Vaughan, D., and Jones, D.: Rhizosphere carbon flow in trees, in comparison with annual plants:
446   the importance of root exudation and its impact on microbial activity and nutrient availability, Appl. Soil Ecol.,
447   5, 29-56, 1997.
448   Gundersen, P., Callesen, I., and Vries, W.D.: Nitrate leaching in forest ecosystems is related to forest floor C/N
449   ratios, Environ. Pollut., 102, 403-407,1998.
450   Hannam, K., Quideau, S., and Kishchuk, B.: Forest floor microbial communities in relation to stand composition
451   and timber harvesting in northern Alberta, Soil Biol. Biochem., 38, 2565-2575, 2006.

Biogeosciences

Open Access

EGU

Discussions

452 Högberg, M.N., Högberg, P., and Myrold, D.D.: Is microbial community composition in boreal forest soils
453   determined by pH, C-to-N ratio, the trees, or all three, Oecologia, 150, 590-601, 2007.
454 Hackl, E., Pfeffer, M., Donat, C., Bachmann, G., and Zechmeister-boltenstern, S.: Composition of the microbial
455   communities in the mineral soil under different types of natural forest, Soil Biol. Biochem., 37, 661-671, 2005.
456 van der Heijden, M.G., Bardgett, R.D., and van Straalen, N.M.: The unseen majority: soil microbes as drivers of
457   plant diversity and productivity in terrestrial ecosystems, Ecol. Lett., 11, 296-310, 2008.
458 Huang, X., Liu, S., Wang, H., Hu, Z., Li, Z., and You, Y.: Changes of soil microbial biomass carbon and
459   community composition through mixing nitrogen-fixing species with Eucalyptus urophylla in subtropical
460   China, Soil Biol. Biochem., 73, 42-48, 2014.
461 Huang, Z., Clinton, P.W., Baisden, W.T., and Davis, M.R.: Long-term nitrogen additions increased surface soil
462   carbon concentration in a forest plantation despite elevated decomposition, Soil Biol. Biochem., 43, 302-307,
463   2011.
464 Kang, H., Kang, S., and Lee, D.: Variations of soil enzyme activities in a temperate forest soil, Ecol. Res., 24,
465   1137-1143, 2009.
466 Koch, O., Tscherko, D., and Kandeler, E.: Temperature sensitivity of microbial respiration, nitrogen mineralization,
467   and potential soil enzyme activities in organic alpine soils, Global Biogeochem. Cy., 21, GB4017,
468   doi:10.1029/2007GB002983, 2007.
469 Lagomarsino, A., Grego, S., and Kandeler, E.: Soil organic carbon distribution drives microbial activity and
470   functional diversity in particle and aggregate-size fractions, Pedobiologia, 55, 101-110, 2012.
471 Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N.: The influence of soil properties on the structure of
472   bacterial and fungal communities across land-use types, Soil Biol. Biochem., 40, 2407-2415, 2008.
473 Li, H., Wang, X., Liang, C., Hao, Z., Zhou, L., Ma, S., Li, X. B., Yang, S., Yao, F., and Jiang, Y.:
474   Aboveground-belowground biodiversity linkages differ in early and late successional temperate forests, Sci
475   Rep-UK, 5, 12234, 2015.
476 Liu, L., Gundersen, P., Zhang, T., and Mo, J. M.: Effects of phosphorus addition on soil microbial biomass and
477   community composition in three forest types in tropical China, Soil Biol. Biochem., 44, 31-38, 2012.
478 Lucas-Borja, M.E., Candel Pérez, D., López Serrano, F.R., Andrés, M., and Bastida, F.: Altitude-related factors but
479   notPinuscommunity exert a dominant role over chemical and microbiological properties of a Mediterranean
480   humid soil, Eur. J. Soil Sci., 63, 541-549, 2012.
481 McGuire, K.L., Fierer, N., Bateman, C., Treseder, K.K., and Turner, B.L.: Fungal community composition in
482   neotropical rain forests: the influence of tree diversity and precipitation, Microb. Ecol., 63, 804-812, 2012.
483 Mitchell, R.J., Hester, A.J., Campbell, C.D., Chapman, S.J., Cameron, C.M., Hewison, R.L., and Potts, J. M.: Is
484   vegetation composition or soil chemistry the best predictor of the soil microbial community, Plant Soil, 333,
485   417-430, 2010.
486 Myers, R.T., Zak, D.R., White, D.C., and Peacock, A.: Landscape-level patterns of microbial community
487   composition and substrate use in upland forest ecosystems, Soil Sci. Soc. Am. J., 65, 359-367, 2001.
488 Nilsson, L.O., Wallander, H., and Gundersen, P.: Changes in microbial activities and biomasses over a forest floor
489   gradient in C-to-N ratio, Plant Soil, 355, 75-86, 2012.
490 Priha, O., Grayston, S.J., Hiukka, R., Pennanen, T., and Smolander, A.: Microbial community structure and
491   characteristics of the organic matter in soils under Pinus sylvestris, Picea abies and Betula pendula at two forest
492   sites, Biol. Fert. Soils, 33, 17-24, 2001.
493 Priha, O. and Smolander, A.: Microbial biomass and activity in soil and litter under Pinus sylvestris, Picea abies
494   and Betula pendula at originally similar field afforestation sites, Biol. Fert. Soils, 24, 45-51, 1997.
495 Saiya-Cork, K.R., Sinsabaugh, R.L., and Zak, D.R.: The effects of long term nitrogen deposition on extracellular
496   enzyme activity in an Acer saccharum forest soil, Soil Biol. Biochem., 34, 1309-1315, 2002.
497 Scheibe, A., Steffens, C., Seven, J., Jacob, A., Hertel, D., Leuschner, C., and Gleixner, G.: Effects of tree identity
498   dominate over tree diversity on the soil microbial community structure, Soil Biol. Biochem., 81, 219-227,
499   2015.
500 Schimel, J. P. and Schaeffer, S. M.: Microbial control over carbon cycling in soil, Front. Microbiol., 3, 1-11, 2012.
501 Schimel, J.P. and Weintraub, M.N.: The implications of exoenzyme activity on microbial carbon and nitrogen
502   limitation in soil: a theoretical model, Soil Biol. Biochem., 35, 549-563, 2003.
503 Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W. J., and Chu, H. Y.: Soil pH drives the spatial
504   distribution of bacterial communities along elevation on Changbai Mountain, Soil Biol. Biochem., 57, 204-211,
505   2013.
506 Silver, W.L. and Miya, R.K.: Global patterns in root decomposition: comparisons of climate and litter quality
507   effects, Oecologia, 129, 407-419, 2001.
508 Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C. Contosta, A.R., Cusack,
509   D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K.i, Keeler, B. L., Powers, J. S., Stursova,
510   Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M. D., Zak, D. R., and Zeglin, L. H.: Stoichiometry of soil
511   enzyme activity at global scale, Ecol. Lett., 11, 1252-1264, 2008.
512 Six, J., Elliott, E.T., and Paustian, K.: Soil structure and soil organic matter: II. A normalized stability index and the
513   effect of mineralogy, Soil Sci. Soc. Am. J., 64, 1042-1049, 2000.
514 Soil Survey Staff: Keys to Soil Taxonomy, 11th editions. Natural resources conservation service, Washington, DC,
515   2010.
516 Staddon, W.J., Trevors, J.T., Duchesne, L.C., and Colombo, C.A.: Soil microbial diversity and community
517   structure across a climatic gradient in western Canada, Biodiversity & Conservation, 7, 1081-1092, 1998.
518 Sterner, R. W. and Elser, J. J.: Ecological stoichiometry: the biology of elements from molecules to the biosphere,

519   PrincetonUniversity Press, 2002.
520   Sugihara, S., Shibata, M., Mvondo Ze, A. D., Araki, S., and Funakawa, S.: Effects of vegetation on soil microbial
521   C, N, and P dynamics in a tropical forest and savanna of Central Africa, Appl. Soil Ecol., 87, 91-98, 2015.
522   Ter Braak, C.J.F., and Smilauer, P.: CANOCO Reference manual and CanoDraw for Windows User's guide:
523   Software for Canonical Community Ordination (Version 4.5), 2002.
524   Tietema, A.: Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a
525   European itrogen deposition gradient, Forest Ecol. Manag., 101, 29-36, 1998.
526   Tripathi, B.M., Kim, M., Tateno, R., Kim, W., Wang, J., Lai-Hoe, A, Shukor, N.A., Rahim, R.A., Go, R., and
527   Adams, J. M.: Soil pH and biome are both key determinants of soil archaeal community structure, Soil Biol.
528   Biochem., 88, 1-8, 2015.
529   Ultra, V. U., Han, S.-H., and Kim, D.-H.: Soil properties and microbial functional structure in the rhizosphere of
530   Pinus densiflora (S. and Z.) exposed to elevated atmospheric temperature and carbon dioxide, J. Forest Res., 18,
531   149-158, 2013.
532   Urbanová, M., Šnajdr, J., and Baldrian, P.: Composition of fungal and bacterial communities in forest litter and soil
533   is largely determined by dominant trees, Soil Biol. Biochem, 84, 53-64, 2015.
534   Ushio, M., Kitayama, K., and Balser, T.C.: Tree species effects on soil enzyme activities through effects on soil
535   physicochemical and microbial properties in a tropical montane forest on Mt. Kinabalu, Borneo, Pedobiologia,
536   53, 227-233, 2010.
537   Veen, J.A.V. and Kuikman, P.J.: Soil structural aspects of decomposition of organic-matter by micro-organism.
538   Biogeochemistry, 11, 213-233, 1990.
539   Wang, C., Han, G., Jia, Y., Feng, X., Guo, P., and Tian, X.: Response of litter decomposition and related soil
540   enzyme activities to different forms of nitrogen fertilization in a subtropical forest, Ecol. Res., 26, 505-513,
541   2011.
542   Wang, G., Post, W. M., and Mayes, M. A.: Development of microbial-enzyme-mediated decomposition model
543   parameters through steady-state and dynamic analyses, Ecol. Appl., 23, 255-272, 2013.
544   Wardle, D.A., Nilsson, M.-C., Zackrisson, O., and Gallet, C.: Determinants of litter mixing effects in a Swedish
545   boreal forest, Soil Biol. Biochem., 35, 827-835, 2003.
546   Wu, H., Xu, X.K., Duan, C.T., Li, T.S., and Cheng, W.G: Effect of vegetation type, wetting intensity, and nitrogen
547   supply on external carbon stimulated heterotrophic respiration and microbial biomass carbon in forest soils, Sci.
548   China Earth Sci., 58, 1446-1456, 2015.
549   Wu, Y., Ma, B., Zhou, L., Wang, H., Xu, J., Kemmitt, S., and Brookes, P. C.: Changes in the soil microbial
550   community structure with latitude in eastern China, based on phospholipid fatty acid analysis, Appl. Soil Ecol.,
551   43, 234-240, 2009.
552   Xu, X., Schimel, J. P., Janssens, I. A., Song, X., Song, C., Yu, G, Sinsabaugh, R. L., Tang, D., Zhang, X., and
553   Thornton, P. E.: Global Pattern and Controls of Soil Microbial Metabolic Quotient, Ecol. Monog., DOI:
554   10.1002/ecm.1258, 2017.
555   Xu, X., Schimel, J. P., Thornton, P. E., Song, X., Yuan, F., and Goswami, S.: Substrate and environmental controls
556   on microbial assimilation of soil organic carbon: a framework for Earth system models, Ecol. Lett., 17,
557   547-555, 2014.
558   Xu, X., Thornton, P. E., and Post, W. M.: A global analysis of soil microbial biomass carbon, nitrogen and
559   phosphorus in terrestrial ecosystems, Global Ecol. Biogeogr., 22, 737-749, 2013.
560   Xu, Z., Yu, G., Zhang, X., Ge, J., He, N., Wang, Q., and Wang, D.: The variations in soil microbial communities,
561   enzyme activities and their relationships with soil organic matter decomposition along the northern slope of
562   Changbai Mountain, Appl. Soil Ecol., 86, 19-29, 2015.
563   Xu, Z.W., Yu, G.R., Zhang, X.Y., He, N.P., Wang, Q.F., Wang, S.Z.,Wang, R.L., Zhao, N., Jia, Y.L., and Wang, C.Y.:
564   Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China
565   (NSTEC), Soil Biol. Biochem.,104, 152-16, 2017.
566   Yin, R., Deng, H., Wang, H.-L., and Zhang, B.: Vegetation type affects soil enzyme activities and microbial
567   functional diversity following re-vegetation of a severely eroded red soil in sub-tropical China, Catena, 115,
568   96-103, 2014.
569   Zhang, X. P., Wang, X. P., Zhu, B., Zong, Z. J., Peng, C. H., and Fang, J.Y.: Litter fall production in relation to
570   environmental factors in northeast China's forests, J. Plant Ecol. (Chinese Version), 32, 1031-1040, 2008.
571   Zheng, M., Huang, J., Chen, H., Wang, H., and Mo, J.: Responses of soil acid phosphatase and beta-glucosidase to
572   nitrogen and phosphorus addition in two subtropical forests in southern China, Eur. J. Soil Biol., 68, 77-84,
573   2015.

Figure captions

Figure 1. Distribution of typical forest ecosystems along the North-South Transect of Eastern China (NSTEC). The names of the sampling sites from north to south were abbreviated as followed: LS = Liangshui; TY = Taiyue; DH = Dinghu.

Figure 2. Soil enzyme activities under different forest types in different climatic zones (A. Liangshui; B. Taiyue; C. Dinghu). Different lowercase letters indicate significant differences between forests in the same climatic zone. The abbreviations of the sampling sites are shown in Table 1.

Figure 3. The PLFA contents, Fungi:Bacteria ratios, and G^+/G^- for different forest types in different climatic zones (A. Liangshui; B. Taiyue; C. Dinghu). Different lowercase letters indicate significant differences among forests in the same climatic zone. F/B, fungi/bacteria; G^+/G^-, Gram-positive bacteria/ Gram-negative bacteria. The abbreviations of the sampling sites are shown in Table 1.

Figure 4. Redundancy analysis (RDA) ordination biplot of soil enzyme activities and environmental properties for the different forest types in different climatic zones (A. Liangshui; B. Taiyue; C. Dinghu). Only the environmental variables that were significantly correlated with RDA1 are shown. The dotted lines and solid lines represent the environmental variables and enzyme activities. The variables in this table were abbreviated as follows: TC(litter) = litter total carbon; TN(litter) = litter total nitrogen; C/N(litter) = litter total carbon/nitrogen; ST = soil temperature; SMC = soil moisture content; Clay = soil clay content; SOC = soil organic carbon; TN = soil total nitrogen; TP = soil total phosphorus; C/N = soil carbon/nitrogen; C/P = soil carbon/phosphorus, and N/P = soil nitrogen/phosphorus.

Figure 5. Redundancy analysis (RDA) ordination biplot of soil microbial community structure and environmental properties for different forest types in different climatic zones (A. Liangshui; B. Taiyue; C. Dinghu). Only the environmental variables that were significantly correlated with RDA1 are shown. The dotted lines and solid lines represent the environmental variables and lipid signatures. The abbreviations of the variables included in this figure are shown in Figure 4.

Supporting Information

Table S1. The PLFA biomarkers used to represent the different groups of soil micro-organisms (Frostegård *et al.*1996).

Table S2. Average values of soil enzyme activities and microbial PLFAs in the three different climatic zones and three different forest types, respectively.

Tables

Table 1. Stand characteristics and soil properties under different forest types in the three climatic zones

Areas[a]	XiaoXing'an Mountain (LS)				Taiyue Mountain (TY)				Dinghu Mountain (DH)			
Latitude(°)	47.19				36.70				23.17			
Longitude(°)	128.90				112.08				112.54			
Climatic zone	Temperate				Warm temperate				Subtropical			
MAT (°C)	0.3				6.2				20.9			
MAP (mm)	676				662				1927			
Altitude (m)	401				1668				240			
Soil type	Cryumbrept				Eutrochrepts				oxisol			
Vegetation type[b]	PCB	SCB	PK	LO	PDB	SDB	PT	LO	PEB	SCB	PM	EF
pH	6.17a	5.68b	6.01a	6.28a	6.85c	7.70a	7.20b	6.78c	5.43a	5.38a	5.21b	5.07b
ST (°C)	15.87a	15.11b	15.33b	16.13a	16.00b	24.04a	16.37a	15.33b	24.40b	24.59b	25.34a	25.39a
SMC (%)	46.94c	69.97a	50.7b	57.95c	36.01a	22.66c	27.89b	34.87a	37.84b	44.76a	26.67b	30.20b
Clay (%)	63.98a	55.92b	64.57a	64.30a	49.39a	52.13a	35.69b	53.90a	49.74b	76.05a	45.05d	52.31c
SOC (g kg⁻¹)	62.08a	75.23a	61.47a	57.10a	41.34a	17.87b	42.72a	42.15a	28.47b	40.03a	26.83c	37.99b
TN (g kg⁻¹)	4.59a	4.57a	4.01a	4.54a	2.43b	1.41c	3.09a	2.79a	1.77b	2.55a	1.26c	1.83b
TP (g kg⁻¹)	0.59b	0.78a	0.83a	0.94a	0.52b	0.51b	0.56a	0.52b	0.20c	0.26a	0.23b	0.22b
Litter C/N	43.11a	24.03c	31.96b	25.54c	48.56b	37.82c	53.16a	30.82d	28.67a	27.06a	30.31a	29.85a

[a] PCB, SCB, PK, and LO represent primary conifer broad-leaved mixed forest, secondary conifer broad-leaved mixed forest, Korean pine forest and Larix olgensis forest, respectively. PDB, SDB, PT, and LO represent primary deciduous broad-leaved forest, secondary deciduous broad-leaved forest, Pinus tabulaeformis forest and Larix olgensis forest, respectively. PEB, SCB, PM, and EF represent primary evergreen broadleaved forest, secondary conifer and broadleaf mixed forest, Pinus massoniana forest and Erythrophleum fordii forest, respectively. MAT and MAP indicate mean annual air temperature and mean annual precipitation, respectively; ST, soil temperature; SMC, soil moisture content; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; Clay, soil clay content; litter C/N, total carbon/total nitrogen of litter.

Biogeosciences
Discussions
Open Access

Table 2. The effect of forest types and climate on the soil enzyme activities and PLFAs

Treatment		Climate		Forest type		Climate × Forest type	
		F	P	F	P	F	P
Enzyme activity	BG	**30.487**	**<0.0001**	**6.852**	**0.003**	3.105	0.056
	NAG	**32.793**	**<0.0001**	5.183	0.10	3.635	**0.035**
	LAP	**171.864**	**<0.0001**	**16.364**	**<0.0001**	1.813	0.176
	AP	**95.070**	**<0.0001**	**48.117**	**<0.0001**	**22.446**	**<0.0001**
PLFAs	tPLFA	**7.764**	**0.001**	2.697	0.079	**8.666**	**0.001**
	Bacteria	2.796	0.073	**4.921**	**0.012**	**8.357**	**0.001**
	Fungi	**8.002**	**0.001**	**21.255**	**<0.0001**	**25.023**	**<0.0001**
	Actinomycetes	0.533	0.591	2.979	0.062	**3.500**	**0.040**
	F/B	**3.731**	**0.032**	**15.502**	**<0.0001**	**6.378**	**0.004**
	G+	0.603	0.552	**3.395**	**0.043**	**5.934**	**0.005**
	G−	**12.503**	**<0.0001**	**6.890**	**0.003**	**11.106**	**<0.0001**
	G+/ G−	1.662	0.202	0.069	0.933	2.257	0.117

The abbreviations of the variables included in this table are shown in Figure 2 and 3.
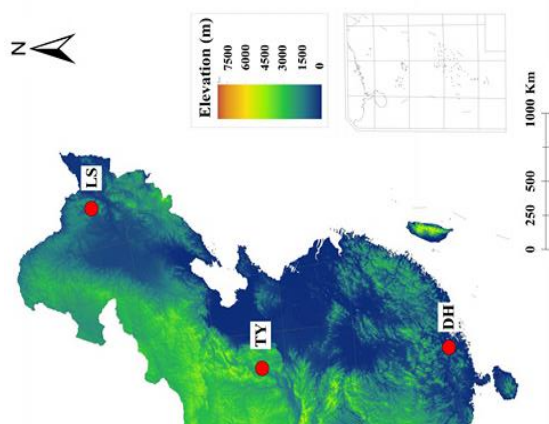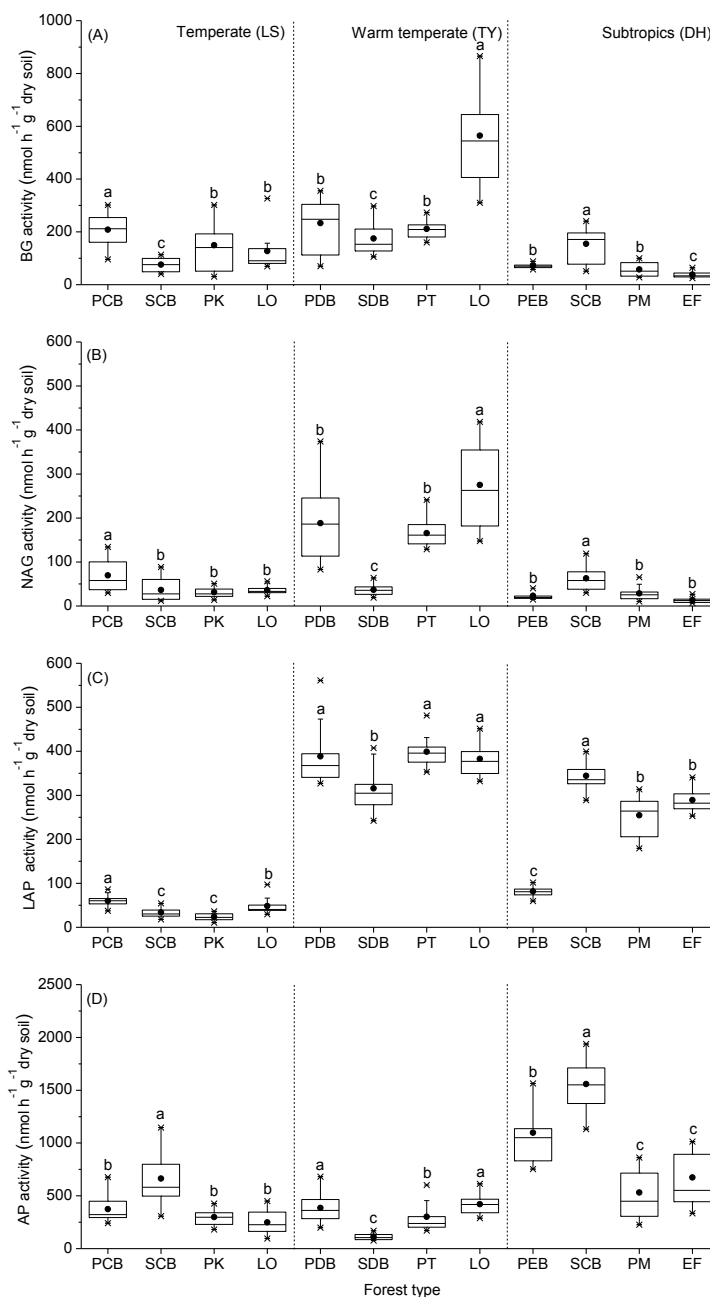
20

610

611

Figure 1. Distribution of typical forest ecosystems along the North-South Transect of Eastern China (NSTEC). The names of the sampling sites from north to south were abbreviated as followed:

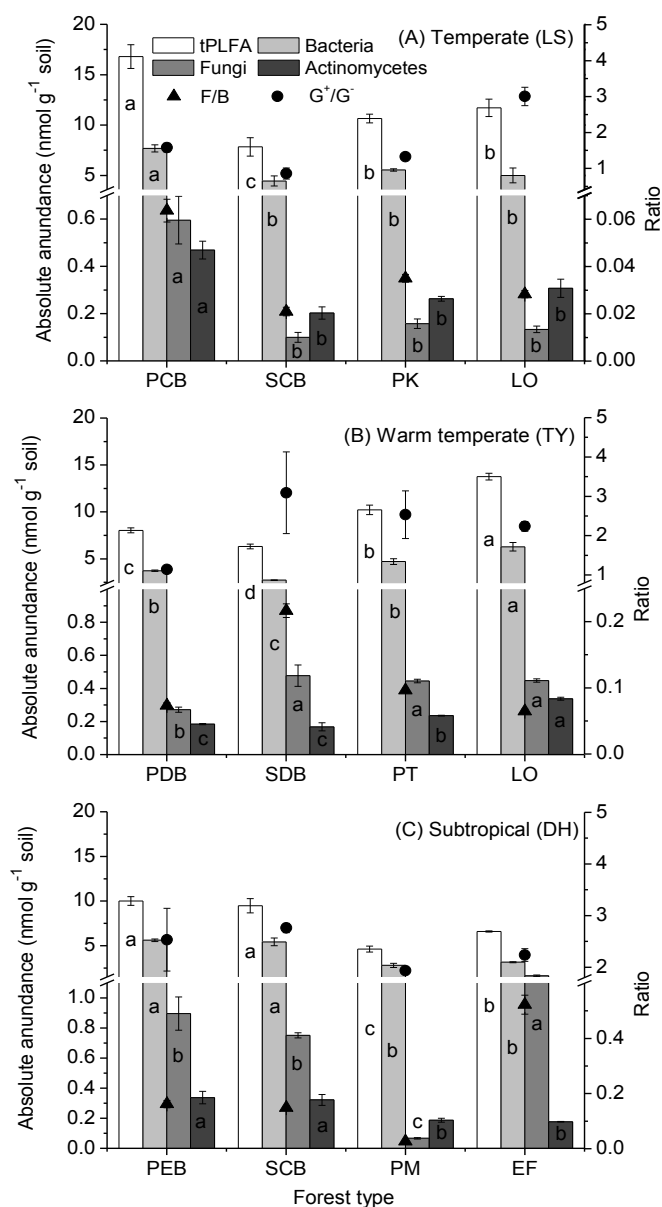LS = Liangshui; TY = Taiyue; DH = Dinghu.

612

613

614

615

Figure 2. Soil enzyme activities under different forest types in different climatic zones (A. Liangshui; B. Taiyue; C.

617 Dinghu). Different lowercase letters indicate significant differences between forests in the same climatic zone. The

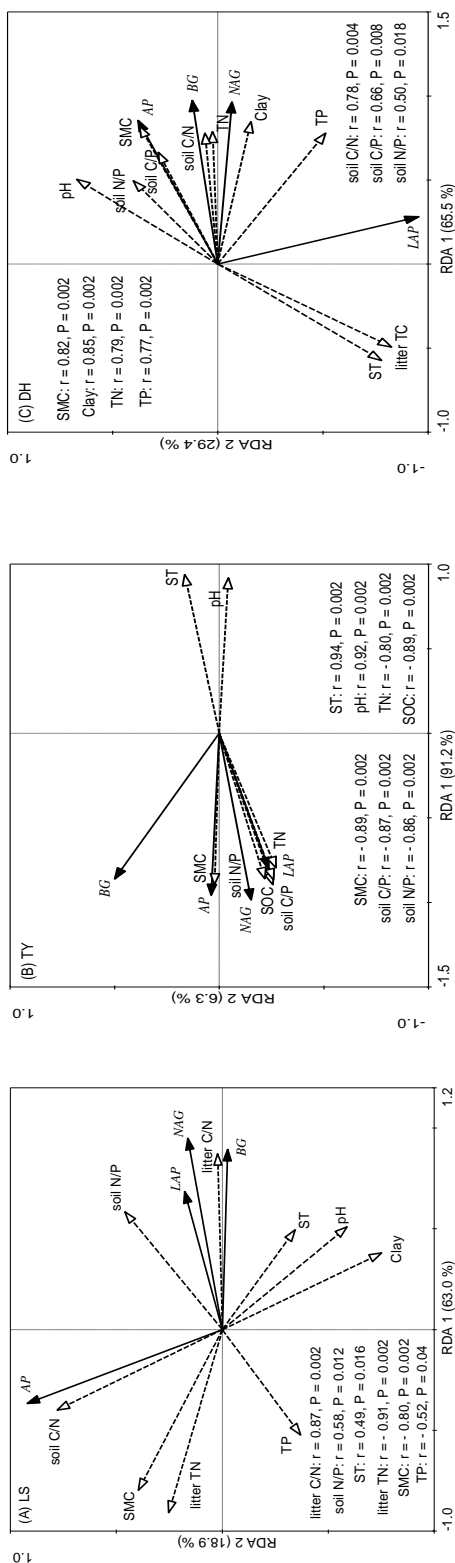618 abbreviations of the sampling sites are shown in Table 1.

619

Figure 3. The PLFA contents, Fungi:Bacteria ratios, and G⁺/G⁻ for different forest types in different climatic zones
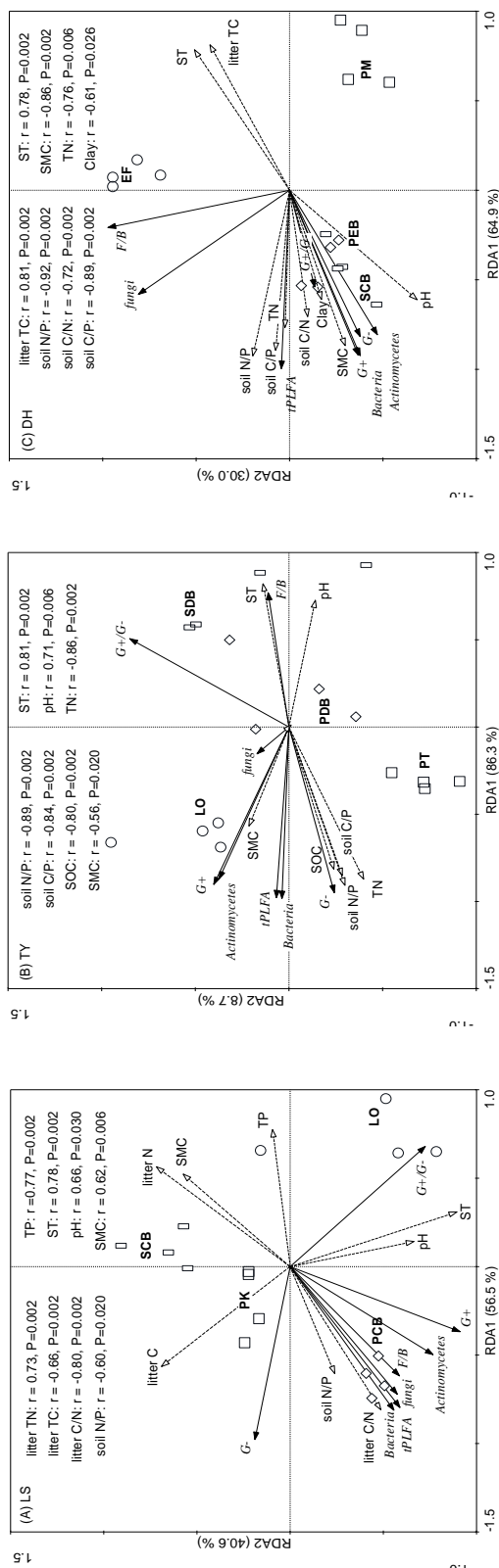(A. Liangshui; B. Taiyue; C. Dinghu). Different lowercase letters indicate significant differences among forests in
the same climatic zone. F/B, fungi/bacteria; G⁺/G⁻, Gram-positive bacteria/ Gram-negative bacteria. The
abbreviations of the sampling sites are shown in Table 1.

620

621

622

623

624

Figure 4. Redundancy analysis (RDA) ordination biplot of soil enzyme activities and environmental properties for the different forest types in different climatic zones (A. Liangshui; B. Taiyue;

C. Dinghu). Only the environmental variables that were significantly correlated with RDA1 are shown. The dotted lines and solid lines represent the environmental variables and enzyme

activities. The variables in this table were abbreviated as follows: TC(litter) = litter total carbon; TN(litter) = litter total nitrogen; C/N(litter) = litter total carbon/nitrogen; ST = soil temperature;

SMC = soil moisture content; Clay = soil clay content; SOC = soil organic carbon; TN = soil total nitrogen; TP = soil total phosphorus; C/N = soil carbon/nitrogen; C/P = soil carbon/phosphorus,

and N/P = soil nitrogen/phosphorus.

24

Figure 5. Redundancy analysis (RDA) ordination biplot of soil microbial community structure and environmental properties for different forest types in different climatic zones (A. Liangshui; B. Taiyue; C. Dinghu). Only the environmental variables that were significantly correlated with RDA1 are shown. The dotted lines and solid lines represent the environmental variables and lipid signatures. The abbreviations of the variables included in this figure are shown in Figure 4.