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

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Abstract. Soil microorganisms play an important role in regulating nutrient cycling in terrestrial ecosystems. Most of the studies conducted thus far have been confined to a single forest biome or have focused on one or two controlling factors, and few have dealt with the integrated effects of climate, vegetation, and soil substrate availability on soil microbial communities and functions among different forests. In this study, we used phospholipid-derived fatty acid (PLFA) analysis to investigate soil microbial community structure, and extracellular enzymatic activities to evaluate the functional potential of soil microbes of different types of forests in three different climatic zones along the North-South transect in eastern China (NSTEC). Both climate and forest type had significant effects on soil enzyme activities and microbial communities with considerable interactive effects. Except soil acid phosphatase (AP), other three enzyme activities were much higher in the warm temperate zone than in the temperate and the subtropical climate zones. The soil total PLFAs and bacteria were much higher in the temperate zone than in the warm temperate and the subtropical zones. The soil β-glucosidase (BG) and N-acetylglucosaminidase (NAG) activities were much highest in the coniferous forest. Except the soil fungi and fungi/bacteria (F/B), the different group of microbial PLFAs were much higher in the conifer broad-leaved mixed forests than in the coniferous forests and the broad-leaved forests. In general, soil enzyme activities and microbial PLFAs were higher in primary forests than in secondary forests in temperate and warm temperate regions. In the subtropical region, soil enzyme activities were lower in the primary forests than in the secondary forests and microbial PLFAs did not differ significantly between primary and secondary forests. Different compositions of the tree species may cause variations in soil microbial communities and enzyme activities. Our results showed that the main controls on soil microbes and functions vary in different climatic zones, and that the effects of soil moisture content, soil temperature, clay content, and the soil N/P ratio were considerable. This information will add value to modeling of microbial processes and will contribute to carbon cycling in large-scale carbon models.

1 Introduction

There is a growing awareness that above- and below-ground interactions make an essential contribution to ecosystem function (van Dam and Heil, 2011). Variations in soil microbial diversity and community structure have a strong influence on soil organic matter turnover and may impact on the function of a given ecosystem (Baumann et al., 2013). For example, mycorrhizal fungi and nitrogen (N) fixing bacteria are responsible for 80% of all N, and up to 75% of phosphorus (P), that is acquired by plants annually (van der Heijden et al., 2008). Therefore, it is important to study the composition and enzyme activities of soil microbial communities to obtain an improved understanding of the mechanisms that control soil organic carbon dynamics in different forest ecosystems.

Vegetation composition may alter soil physicochemical properties by changing the quantity and quality of plant litter, which further influence microbial community composition and function (Ushio et al., 2010). There is increasing evidence that vegetation types influence the structure and functions of the soil microbial community (Zheng et al., 2015). Differences in microbial communities, as represented by PLFAs, have also been reported among adjacent maple, beech, hornbeam, lime, and ash forests in Germany (Scheibe et al., 2015) and among forests of four conifer species in coastal British Columbia (Grayston and Prescott, 2005). From a functional perspective, both soil acid phosphatase and b-glucosidase activities were higher in a monsoon evergreen broadleaf forest than in a Masson pine forest (Zheng et al., 2015). However, vegetation type does not always have an effect on the composition of the soil microbial community. Hannam et al. (2006) reported that the microbial community composition of a white spruce-dominated forest differed substantially from that of an aspen-dominated stand, but was similar to that of a mixed stand with equivalent proportions of deciduous and coniferous trees. Most of the studies conducted thus far have been confined to a single forest biome or have focused on one or two controlling factors (Ultra et al., 2013), and few have dealt with the integrated effects of climate, vegetation, and soil substrate availability on soil microbial communities and functions in different forest biomes.

Soil microbial communities and enzyme activities can be influenced by an array of factors, such as climate (Xu et al., 2015), vegetation types (Urbanová et al., 2015), plant diversity (Li et al.,

2015), and physico-chemical soil properties (Tripathi et al., 2015). The links between the diversity of plant and soil microbial communities and enzyme activities are widely acknowledged (Chung et al., 2007). The composition of the vegetation species can be used to successfully predict the soil microbial community (Mitchell et al., 2010). Soils with different vegetation types develop distinct physico-chemical properties that will have pronounced effects on the structure and function of the soil microbial community (Priha and Smolander, 1997). Soil organic matter is related to the variations in microbial activities and community function (Brockett et al., 2012). Soil pH (Shen et al., 2013), elemental stoichiometric ratios (Högberg et al., 2007), and nutrient status (Lauber et al., 2008) have also been identified as determinants of microbial community structure. However, we still do not know which mechanisms control the variability in the structure and functions of soil microbial communities within different groups of plant species (broadleaved and coniferous trees) on similar soil types within the same climatic region.

Forest soil microbial community structures and enzyme activities are influenced by different factors in different climatic zones. For example, Högberg et al. (2007) found that the soil microbial community composition in a boreal forest was strongly influenced by the soil carbon to nitrogen ratio (C/N) and the soil pH. Studies in temperate forests have shown that dehydrogenase and urease were closely related to the mean air temperature, litter production, and nutrient availability (Kang et al., 2009). In addition, Hackl et al. (2005) reported that soil water availability was responsible for variability in the microbial community structure of temperate forests. Precipitation and soil moisture may be important controls on the structure of soil fungal communities of tropical forests (McGuire et al., 2012). However, there is a lack of well-defined information about the factors that influence the structure and functions of soil microbial communities in forests with different plant species (broadleaved and coniferous trees) across a range of climates and soils.

The North-South Transect of Eastern China (NSTEC) represents a latitudinal and climatic gradient. It is a unique belt in which vegetation ranges from boreal forest to tropical rain forest, depending on the local temperature and precipitation conditions. In this study we examined variations in the soil microbial communities and their functions in forests comprising different species (broadleaved and coniferous trees) in temperate, warm temperate, and tropical forest

biomes along the NSTEC. The temperature and precipitation are different in these three climatic zones. We used information about the soil physico-chemical properties, microbial community structure, and hydrolytic enzyme activities involved in C, N, and P transformations to explore how soil microbial communities and enzyme activities differed among different forest types in different climatic zones, and to determine the influence of different environmental variables on the soil microbial communities and enzyme activities in different climatic zones.

2 Materials and methods

2.1 Study area and soil sampling

We chose three study sites, namely Liangshui in Northeast China, Taiyue Mountain in North China, and Dinghu Mountain in South China, along the North-South Transect in Eastern China (NSTEC) for field measurements and soil sampling. Both the air temperature and precipitation decrease from south to north along the NSTEC (Table 1).

We examined all the representative forest species in each climatic zone. In Liangshui, on the Xiao Xing'an Mountain, we sampled primary conifer broad-leaved mixed forest (PCB), secondary conifer broad-leaved mixed forest (SCBt), and two coniferous plantations, one of which was mainly *Pinus koraiensis* (PK) while the other was *Larix olgensis* (LOt). On Taiyue Mountain, we sampled primary deciduous broad-leaved forest (PDB), secondary deciduous broad-leaved forest (SDB), and two coniferous plantations, one of which was comprised mainly of *Pinus tabulaeformis* (PT) while the other was mainly *Larix olgensis* (LOw). On Dinghu Mountain, we sampled a primary evergreen broadleaved forest (*Castanopsis chinensis*, *Cryptocarya chinensis*, *Cryptocarya concinna*, *Erythrophleum fordii*, and *Cyathea podophylla*), secondary conifer and broadleaf mixed forest (*Pinus massoniana*, *Schima superba*), aconiferous plantation (*Pinus massoniana*), and an evergreen broadleaved plantation (*Erythrophleum fordii*) along a successional stage, hereafter referred to as PEB, SCBs, PM, and EF, respectively. The average temperature of the sampling month was 21.3 ℃, 17.4 ℃, 27.3 ℃ with the relative humidity of 78%, 60-65%, 83.5% in LS, TY, and DH, respectively. The sampling dates are Jul.5 2013, Jul.28 2013, Aug.15 2013 in LS, TY, and DH, respectively. The primary forests are zonal forests that reflect the regional climate and the others are zonal forests that reflect the extreme site conditions. Information about the climate, soil classification (Soil Survey Staff 2010), and soil properties at

each site is provided in Table 1.

Soil samples were collected at nine sampling sites along the NSTEC in July and August 2013. Each site had four independent plots in well-drained areas, which covered an area of 30 m × 40 m, and were at least 10 m apart. The vegetation composition of the four plots at each site was similar. Samples of mineral soil were collected from a depth of 0–10 cm at between 30 and 50 points in each plot along an S-shape using a custom-made coring device with a diameter of 6 cm. The above-ground standing biomass, dead plant parts, and litter were removed from each sampling point. These samples were pooled together as a composite sample. Visible roots and residues were removed and then the soil fractions of each sample were homogenized.

We stored the samples at 4 ℃ in a portable refrigerator during field sampling. Once returned to the laboratory, samples were stored at 4 ℃ before analysis. Soils were analyzed for enzyme activities and PLFAs in September 2013. The fresh soil samples were sieved through a 2-mm mesh and were subdivided into three subsamples. One subsample was stored at 4 ℃ until analyzed for soil enzyme activities and physical and chemical properties. The second was stored at –20 ℃ before analysis for microbial community structures. The third was air dried, and then sieved through a 0.25 mm mesh before SOC, TN, and TP analysis. The soil temperatures were measured *in situ* at the time of sampling. Soil moisture content (SMC) was measured gravimetrically on 20 g fresh soil that was oven-dried at 105 ℃ to constant weight immediately on arrival at the laboratories at the study sites (Liu et al., 2012).

2.2 Soil chemical analyses

Soil pH was measured at a soil-to-water ratio of 1:2.5. Soil total N (TN) concentrations were determined by dry combustion of ground samples (100-mesh) in a C/N analyzer (Elementar, Vario Max CN, Germany). The soil organic carbon (SOC) concentrations were determined by dichromate oxidation and titration with ferrous ammonium sulfate (Huang et al., 2014). The litter total C (litter TC) and total N (litter TN) were determined with the same method that was used for soil TN. Total phosphorus (TP) was determined with a flow injection auto-analyzer following digestion with H_2SO_4-$HClO_4$ (Huang et al., 2011). The soil clay fraction (hereafter referred to as Clay, comprised of particles <53 μm) was separated by wet-sieving and then freeze-dried (Six, Elliott & Paustian 2000).

2.3 Phospholipid fatty-acid and enzyme activity analysis

Samples were analyzed for phospholipid fatty-acids (PLFA) using the method described by Bååth & Anderson (2003). After mild alkaline methanolysis to form fatty acid methyl esters (FAMEs), samples were then dissolved in hexane and analyzed with a DB-5 column in a gas chromatography mass spectroscopy (GCMS) system (Thermo TRACE GC Ultra ISQ). Total amounts of the different PLFA biomarkers were used to represent the different groups of soil micro-organisms (Table S1). Taken together, the combination of bacterial, fungal and actinomycic PLFAs biomarkers represented the total PLFAs of the soil microbial community.

The activities of β-glucosidase (BG), N-acetylglucosaminidase (NAG), acid phosphatase (AP), and leucine aminopeptidase (LAP) were measured as outlined by Saiya-Cork, Sinsabaugh & Zak (2002). The microplates were incubated in the dark at 20 ℃ for 4 h. During the incubation, the incubation plates were shaken every hour to ensure the reaction mixtures were homogenous. Fluorescence was measured using a microplate fluorometer with 365-nm excitation and 450-nm emission filters (Synergy^{H4} Hybrid Reader, Synergy^{H4} BioTek, USA).

2.4 Statistical analysis

One-way analysis of variance (ANOVA) with a post-hoc Tukey HSD test was used to test the differences between the soil and microbial properties in the various forests of the three climatic zones. All data were normality distributed. Two-way analysis was used to test the effect of climate and vegetation on the soil microbial properties. All ANOVA and two-way analysis were performed using SPSS 19.0 for Windows. Figures were generated using the Origin 8.0 package. Data are reported as the mean ± SE.

Redundancy analysis (RDA) was used to examine the relationships between the litter factors (litter TC, litter TN, litter C/N), soil biochemical variables (soil temperature (ST), soil moisture content (SMC), pH, C/N, soil carbon to phosphorus ratio (C/P), soil nitrogen to phosphorus ratio (N/P), SOC, TN, TP), soil texture (Clay), and the soil microbial community compositions and enzyme activities. Before redundancy analysis, we conducted forward selection of the environmental variables that were significantly correlated with variations in the microbial communities and enzyme activities using stepwise regression and the Monte Carlo Permutation Test that was similar to the multiple regression analysis. Stepwise regression and RDA were

processed using CANOCO software 4.5 (Ter Braak & Smilauer 2002). The vectors of greater magnitude that formed smaller angles with an axis were more strongly correlated with that axis.

3 Results

3.1 Soil enzyme activities in different vegetation types

The soil enzyme activities were generally higher in the primary forests than in the secondary forests in temperate and warm temperate climatic zones (Fig. 1). However, in the subtropical climatic zone, soil enzyme activities were higher in the SCBs forest than in the PEB forest. The BG, NAG, and AP enzymes in the two soils of the PT and LOw in the warm temperate zone were significantly different (Fig. 1(A, B, D)). The soil BG and NAG activities were much higher in the coniferous forest than in the conifer broad-leaved mixed forests and the broad-leaved forests (Table S2). The soil AP enzyme activities were highest in the conifer broad-leaved mixed forests and lowest in the coniferous forests (Table S2).

Climate, a significant influence on the variations of soil enzyme activities (P<0.0001), had more influence than forest type. The soil BG, NAG, and LAP activities were much higher in the warm temperate zone than in the temperate and the subtropical climate zones (Table S2). The AP activities were highest in the subtropical climate zone (Table S2). The effects of climate and forest type interactions were only significant for soil NAG (P<0.0001) and AP activities (P=0.035) (Table 2, Table S2). Forests within the same climatic zones had similar soil enzyme activities (Fig. S1).

3.2 Soil microbial community composition in different vegetation types

Soil PLFAs were higher in the primary forest in the temperate and warm temperate zones than in the secondary forest. In the temperate zones, soil PLFAs were higher in the PCB forest than in the SCBt, PK, and LOt (Fig. 2A). In the warm temperate forests, total soil microbial PLFAs were highest in the LOw forest (Fig. 2B). In the subtropical zone, total, bacterial, and actinomycic PLFAs were higher in the PEB and SCBs forests than in the PM and EF forests (Fig. 2C). The forest type had a significant effect on the soil bacteria, fungi, gram-positive bacteria (G^+), and gram-negative bacteria (G^-) PLFAs (Table 2). The soil total PLFAs, bacteria, G^+, G^-, and actinomycete were much higher in the conifer broad-leaved mixed forests than in the coniferous forests and the broad-leaved forests (Table S2). The soil fungi was highest in the broad-leaved

229 forest and lowest in the coniferous forest (Table S2).

230 With the exception of the soil G^+/ G^-, the effects of the combination of climate and forest
231 type on all soil PLFAs were significant, and were stronger than the individual effects of either
232 climate or forest type (Table 2, Table S2). Climate had a significant effect on the total PLFAs,
233 fungi, and G^- (P<0.0001) (Table 2). The soil total PLFAs, bacteria, G^+, and G^- were much higher
234 in the temperate zone than in the warm temperate and the subtropical zones (Table S2). The fungi,
235 F/B, and G^+/G^- were highest in the subtropical zone (Table S2). The soil microbial communities
236 in the different forests in the three climate zones were generally unique (Fig.4, Fig.S2).

237 **3.3 Relationships between soil enzyme activities and soil properties**

238 The variations in the soil enzyme activities in the 12 forests were significantly and positively
239 correlated with soil nutrient ratios (C/P and N/P), ST, and litter TN (P=0.002), but were negatively
240 correlated with soil pH and TP (P=0.002) (Fig.S1). The litter C/N, litter TN, and SMC (P=0.002)
241 were the most important influences on the soil enzyme activity variations in the temperate forests,
242 followed by ST, soil N/P, and soil TP (Fig. 3(A)). In the warm temperate forests, the variations in
243 the soil enzyme activities were significantly and positively correlated with ST and soil pH
244 (P=0.002), but were negatively correlated with SMC and soil nutrients (TN and SOC) (Fig. 3(B)).
245 In the subtropical forests, soil enzyme activities were significantly and positively correlated with
246 clay, SMC, soil TN, and TP (P=0.002), followed by soil nutrient ratios (Fig. 3(C)). These results
247 indicate that the litter inputs, soil micro-climate, and soil texture were the main drivers of
248 variations in the soil enzyme activities in the temperate, warm temperate, and subtropics,
249 respectively, with ST, pH, SMC, and soil N/P as additional influences.

250 **3.4 Relationships between PLFA profiles and measured soil properties**

251 The variations in the soil microbial communities in the in 12 forests were significantly and
252 positively correlated with ST, clay content, and soil nutrient ratios (C/P and N/P), TN (P=0.002),
253 but were negatively correlated with litter TC (P=0.002) (Fig.S2). In the temperate forests, the
254 variations in the soil microbial community structure were strongly affected by the litter TN, litter
255 TC, litter C/N, soil TP, and ST (P=0.002) (Fig. 4(A)). In the warm temperate forests, the first axis
256 of the RDA plot of the soil microbial community structure was significantly and positively
257 correlated with ST (P=0.002), but was negatively correlated with soil N/P, soil TN, soil C/P, and

SOC (P=0.002) (Fig. 4(B)). In subtropical forests, the variations in the soil microbial community structure were significantly and positively correlated with litter TC and ST (P=0.002), but negatively correlated with SMC, soil C/P, soil N/P, and soil C/N (P=0.002), followed by the soil TN and clay contents (Fig. 4(C)). The litter C/N was the main influences on the variations in the soil microbial communities in the temperate, and the soil N/P was the main influences in the warm temperate and subtropical forests. The microbial communities were also influenced by ST, pH, SMC.

4 Discussion

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

Forests in the same climate zone developed similar microbe functions which confirmed the result that the effect of climate on soil enzyme activities were stronger than the forest type and their interactive effect. However, there were still differences among the enzyme activities in different forest types of the same climate zone. Soil microorganisms are usually considered to be C limited, and the litter inputs with high C/N ratio of PCB in the temperate zone will stimulate microbes to grow and secrete more enzymes (Table 1). Therefore, all enzyme activities were highest in PCB in the temperate zone. The high soil BG enzyme activities in the LOw forest in the warm temperate zone reflect the litter inputs with low C. Because that soil enzyme activities will not continuously increase or decrease as nutrient availability increases or decreases. When the soil nutrients are short in supply, microbes will potentially increase production of nutrient-acquiring enzymes, because they are expected to optimize the allocation of their resource reserves by acquiring the resource that is most limiting (Bloom et al., 1985). (Table 1). The soil enzyme activities were highest in the SCBs forest, reflecting the higher soil nutrient concentrations in subtropical zones.

The interactive effect of climate and forest type were more important than the individual effect of them. Therefore, the soil microbial communities of the 12 forests were separated from each other. Vegetation transfers substrate material of varying quality to microbes through litter fall. Fungi are more suitable for life in environments containing higher C/N ratios and low soil pH (Nilsson et al., 2012). The four broadleaved forests were high in litter C/N ratio (Table 1). Therefore, fungi were dominated in this harsh nutrient environments and highest in broadleaved forests. The litter and soil from conifer broad-leaved mixed forest were high in C, N, and P, and

promotes the propagation of bacteria that favor high-nutrient soil (Priha and Smolander, 1997; Priha et al., 2001). Therefore, the structures and functions of the soil microbial communities that developed in the different types of forest were unique.

4.2 Common influences on soil enzyme activities and microbial communities

Many other studies have reported how different factors determine the response of the soil microbial community and function to variations in forests (Högberg et al., 2007; McGuire et al., 2012). Mostly limited to one climatic zone, these studies were quite diverse and featured a range of microbial methods, sampling times, and environmental properties, which means it is difficult to compare the results. In this study, we collected the samples at the same times and used the same methods to analyze the soil microbial communities and enzyme activities. We found that ST, SMC, soil pH, and soil N/P ratio influenced, but perhaps did not dominate, the responses of the soil microbial community structures and enzyme activities in the different forest types across the three climatic zones.

Temperature can influence enzyme activity directly and indirectly by modifying the enzyme kinetics and influencing the proliferation of microbes, respectively (Kang et al., 2009). By changing the quality and quantity of the substrate on which microbes function, soil moisture is an important driver of the overall microbial composition and soil microbial function (Hackl et al., 2005). The responses of soil enzyme activities and microbial communities in the various forest types were all significantly influenced by the SMC in the three climatic zones. Increases in soil moisture can enhance both the release and the diffusion rates of enzymes, substrates, and reaction products (Burns et al., 2013), and our results showed that soil enzyme activities and microbial PLFAs increased as the SMC increased in the warm temperate and subtropical zones. However, water-logged conditions are not suitable for microbes and are not beneficial for the release of soil enzymes (Lucas-Borja et al., 2012), and, similar to other studies, soil enzyme activities and SMC were negatively correlated in the temperate zone forests (Brockett et al., 2012). As the SMC increases, the bacterial PLFAs increase (Myers et al., 2001) and fungal PLFAs decrease (Staddon et al., 1998), which indicates that the soil microbial communities and enzyme activities in the different climatic zones were all influenced by the soil micro-climate. This was also demonstrated by the stronger effect of climate on soil enzyme activities and the combined interaction effect of

316 climate and forest type on soil microbial communities. Other studies have reported that
317 precipitation and mean annual temperature played important roles in explaining on the large-scale
318 distribution of soil microbial community composition and functions (de Vries et al., 2012; Xu et
319 al., 2017).

320 Soil pH directly affects the activities of extracellular enzymes immobilized in the soil matrix,
321 and the effect of soil pH on the soil microbial community and function reflects the influence of
322 vegetation through changes in soil chemistry. Every enzyme has a well-defined optimal soil pH
323 value (Sinsabaugh et al., 2008) that results from different levels of soil enzyme activities under
324 different soil pH conditions. Soil G^+/G^- ratios were highest in the subtropical forest where G^-
325 bacteria PLFAs were least abundant, which may reflect microbial growth strategies. The G^+
326 bacteria are primarily K-strategists that can survive over long periods in the soil under harsh
327 conditions with lower soil pH (Andrews & Hall, 1986). Increased pH causes an increase in
328 bacterial diversity and a shift in the bacterial community to more G^- and fewer G^+ bacteria PLFAs
329 (Wu et al., 2009; Shen et al., 2013).

330 **4.3 Key influences on soil enzyme activities and microbial communities**

331 Our results showed that the most important controls on the responses of soil microbial
332 communities and enzyme activities to vegetation types varied across climatic zones. The litter
333 quality and quantity contribute to the maintenance of soil fertility in forest ecosystems (Wang et al.,
334 2011). In our study, and the C/N ratios were highest, in litter from PCB stands (Table 1), which
335 shows that the soil in the PCB was more N-limited than the other soils because of litter inputs with
336 high C/N ratios (Table 1). Therefore, the microbial N demand was highest in soil in the PCB forest,
337 which resulted in higher NAG and LAP values. Plant litter has a strong influence on soil microbial
338 composition and activity, as the litter decomposition process provides nutrients for microorganism
339 growth through inputs of leaf litter (Attiwill and Adams, 1993), dying roots (Silver and Miya,
340 2001), and root secretion (Grayston et al., 1997). The litter from the mixed forests, represented in
341 our study by PCB, is more diverse than that from the pure forests, and so a wider variety of soil
342 microbes participate in the decomposition process, so that the soil organic matter is richer, and
343 there are more soil microbial PLFAs, than in the other forest types. Fungi typically dominate
344 N-limited environments and the fungal biomass is positively related to the C/N ratio (Nilsson et al.,

345 2012). The fungi/bacteria ratio (F/B ratio) was therefore highest in the PCB forest where the litter
346 C/N values were highest.

347 Microbes obtain the nutrients they need to construct biomass by decomposing soil organic
348 matter. Wallenius et al. (2011) found that the soil bacterial biomass was higher in forests where the
349 soil organic matter concentrations were higher than in forests with low soil organic matter
350 concentrations, and Xu et al. (2017) found positive relationships between soil enzyme activities
351 and SOC and TN concentrations along the NSTEC. In line with the resource limitation model, and
352 also confirmed by several other studies (Brockett et al., 2012), Schimel and Weintraub (2003)
353 suggested that increases in N and C substrate availability might favor enzyme synthesis. Soil
354 microorganisms however did not grow when the available P concentrations in soil were less than
355 0.7 mg kg^{-1} and were stimulated by P additions (Zheng et al., 2009). Other studies have reported
356 that P additions stimulated the different PLFA microbial groups in soils (Dong et al., 2015). The
357 soil TN and TP were lower in the warm temperate and subtropical zone than in the temperate zone
358 in our study (Table 1), and these two kinds of nutrients were more likely limiting factors in warm
359 temperate and subtropical forest (DeForest et al., 2012; Xu et al., 2017). Therefore, soil TN and
360 TP are more important in warm temperate and subtropical forests than in temperate forests.

361 The soil N/P ratio was the most important influence on the soil microbial communities and
362 enzyme activities in the warm temperate and subtropical zone, which is consistent with the results
363 of previous studies (Shen et al., 2013; Högberg et al., 2007). Soil stoichiometric C, N, and P ratios
364 reflect the nutrient limitations of the ecosystems (Sterner and Elser, 2002) and should indicate soil
365 organic matter mineralization and sequestration (Gundersen et al., 1998). Soil microorganisms
366 obtain C, N, and P in such a way that enzyme release corresponds with the soil stoichiometric
367 ratios of C, N, and P. When supplies of N or P are limited, the activities of the enzymes that are
368 responsible for nitrate or phosphate mineralization will be higher. Consistent with this discussion,
369 soil enzyme activities in subtropical forests (DH) responded positively to the soil C/N and N/P
370 ratios.

371 Soil texture is a key property that affects the accessibility of organic matter to microbes, and
372 is an important determinant of soil moisture, and nutrient availability and retention (Veen and
373 Kuikman, 1990). Consistent with our results, Lagomarsinoa et al. (2012) reported that the

activities of soil BG, AP, and NAG were higher in silt and clay fractions than in coarser fractions. This may be attributed to the presence of clay-humus-enzyme complexes in the finest soil fractions, and implies that physical protection affects soil enzyme activities. In addition, fine textured soils with higher silt and clay contents are known to be more conducive to bacterial growth than coarser soils because they have a greater water-holding capacity, higher nutrient availability, and offer better protection against bacterial grazers (Carson et al., 2010). Therefore, soil enzyme activities and microbial PLFAs were highest in the SCBs forest with finely texture. Except SCBt in the temperate zone and PT in the warm temperate zone, the soil clay content were not significant different among other three forest types. However, the soil clay contents of the four forest types in the subtropical zone were significant different from each other and important for variations in microbial communities and functions (Table 1).

4.4 Implications for ecosystem modeling

There is increasing recognition that, to improve climate models, microbial processes should be simulated (DeLong et al., 2011). As such, this study has three important implications. First, microbial datasets that have information about enzyme activities and soil microbial properties contribute to improved parameterization of ecosystem models (Xu et al., 2017). Information about the spatial patterns of, and factors that control, microbial properties and enzymatic activities can enrich the datasets that are used to parameterize models of microbial processes (Wang et al., 2013). Secondly, knowledge about microbial community structure and its environmental controls can give a better understanding of how microbes adapt to changing environments, which is the main direction of model development (Schimel and Schaeffer, 2012). Information about edaphic controls on microbial processes is critical for developing new modeling frameworks with improved links with field experimental data (Abramoff et al., 2017). Finally, the information generated in this study about the divergence of the dominant factors that control soil microbial properties across forests is extremely valuable for improving our understanding of soil microbial ecology and forest management.

5 Conclusions

In this study, we characterized the soil microbial communities and enzyme activities and factors that controlled them in various forest types across three different climatic zones. We found that

forest types with specific soil conditions supported the development of distinct soil microbial communities with variable functions. The soil total PLFAs, bacteria, G^+, G^-, and actinomycete were much higher in the conifer broad-leaved mixed forests than in the coniferous forests and the broad-leaved forests. The soil BG and NAG activities were much higher in the coniferous forest than in the conifer broad-leaved mixed forests and the broad-leaved forests. Except AP, soil enzyme activities were highest in warm temperate zone. Soil tPLFAs, bacteria, G^- increased from temperate zone to subtropical zone, but fungi was in reverse. The litter TN, soil temperature, and soil clay contents were important predictors of the variance in soil enzyme activities in temperate, warm temperate, and subtropical zones, respectively, while litter and soil nutrient ratios were significant predictors of the variance in soil microbial communities. We also found that SMC, soil temperature, soil pH, and the soil N/P ratio were common drivers of variations in the soil microbial community structure and enzyme activities across the different forest types in the three climatic zones. Forests within the same climatic zones had similar soil microbial communities and enzyme activities, and these patterns were mainly determined by the litter input, soil micro-environment, and soil nutrient ratios. The data in this study is extremely valuable for improving our understanding of soil microbial ecology and forest management.

Data accessibility. Requests for data and materials should be addressed to N.H. (henp@igsnrr.ac.cn) and G.Y. (yugr@igsnrr.ac.cn).

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

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Figure captions

Figure 1. Soil enzyme activities under different forest types in different climatic zones. BG, b-1, 4-glucosidase; NAG, b-1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase. The capital letters A, B, C, and D represent the variations in the enzyme activities of BG, NAG, LAP and AP, respectively. 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 2. 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 3. 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 4. 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.

Figure S1. Redundancy analysis (RDA) ordination biplot of soil enzyme activities and environmental properties for the 12 forests.

Figure S2. Redundancy analysis (RDA) ordination biplot of soil microbial community structure and environmental properties for the 12 forests.

Tables

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

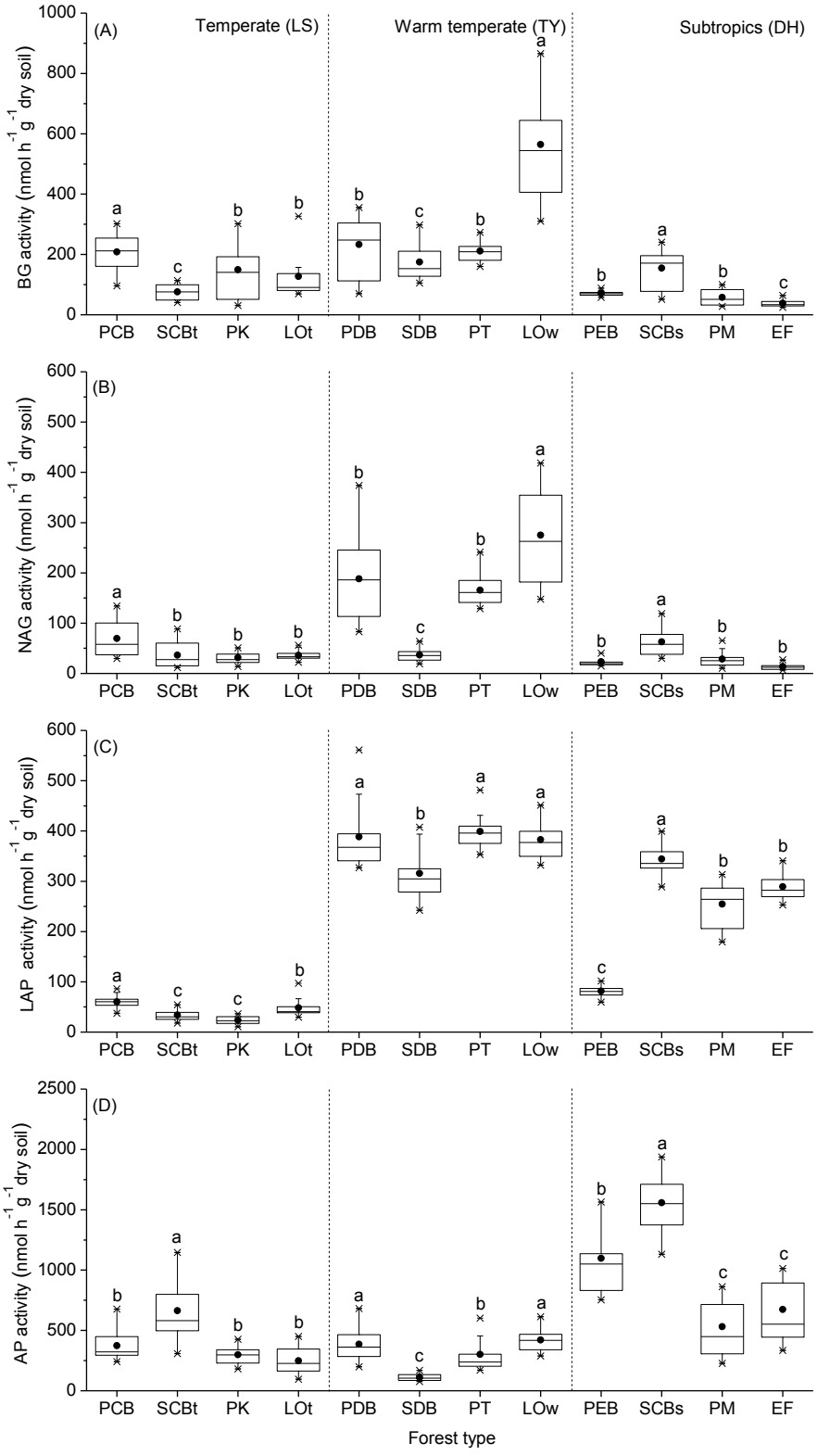
Areas[a]	XiaoXing'an Mountain (LS)				Taiyue Mountain (TY)				Dinghu Mountain (DH)			
Sampling date	Jul.5 2013				Jul.28 2013				Aug.15 2013			
Latitude(°)	47.19				36.70				23.17			
Longitude(°)	128.90				112.08				112.54			
Climatic zone	Temperate				Warm temperate				Subtropical			
MAT (℃)	0.3				6.2				20.9			
MAP (mm)	676				662				1927			
Altitude (m)	401				1668				240			
Soil type	Cryumbrept				Eutrochrepts				oxisol			
Vegetation type[b]	PCB(M)	SCBt(M)	PK(C)	LOt(C)	PDB(B)	SDB(B)	PT(C)	LOw(C)	PEB(B)	SCBs(M)	PM(C)	EF(B)
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 (℃)	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	28.47c	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 TC	460.50b	489.66a	476.48b	414.26c	507.47a	456.64b	509.65a	435.00c	422.65c	451.69b	521.11a	520.51a
Litter TN	10.87c	20.23a	14.86b	16.10b	10.38b	12.23a	9.59b	13.97a	14.1c	16.38b	17.25a	17.38a
Litter C/N	43.11a	24.03c	31.96b	25.54c	48.56a	37.82b	53.16a	30.82c	28.67a	27.06a	30.31a	29.85a

[a] PCB, SCBt, PK, and LOt 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 LOw represent primary deciduous broad-leaved forest, secondary deciduous broad-leaved forest, *Pinus tabulaeformis* forest and *Larix olgensis* forest, respectively. PEB, SCBs, PM, and EF represent primary evergreen broadleaved forest, secondary conifer and broadleaf mixed forest, *Pinus massoniana* forest and *Erythrophleum fordii* forest, respectively. The letters in the bracket after the vegetation type represent M, conifer broad-leaved mixed forest; C, coniferous forest; B, broad-leaved forest. 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.

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.



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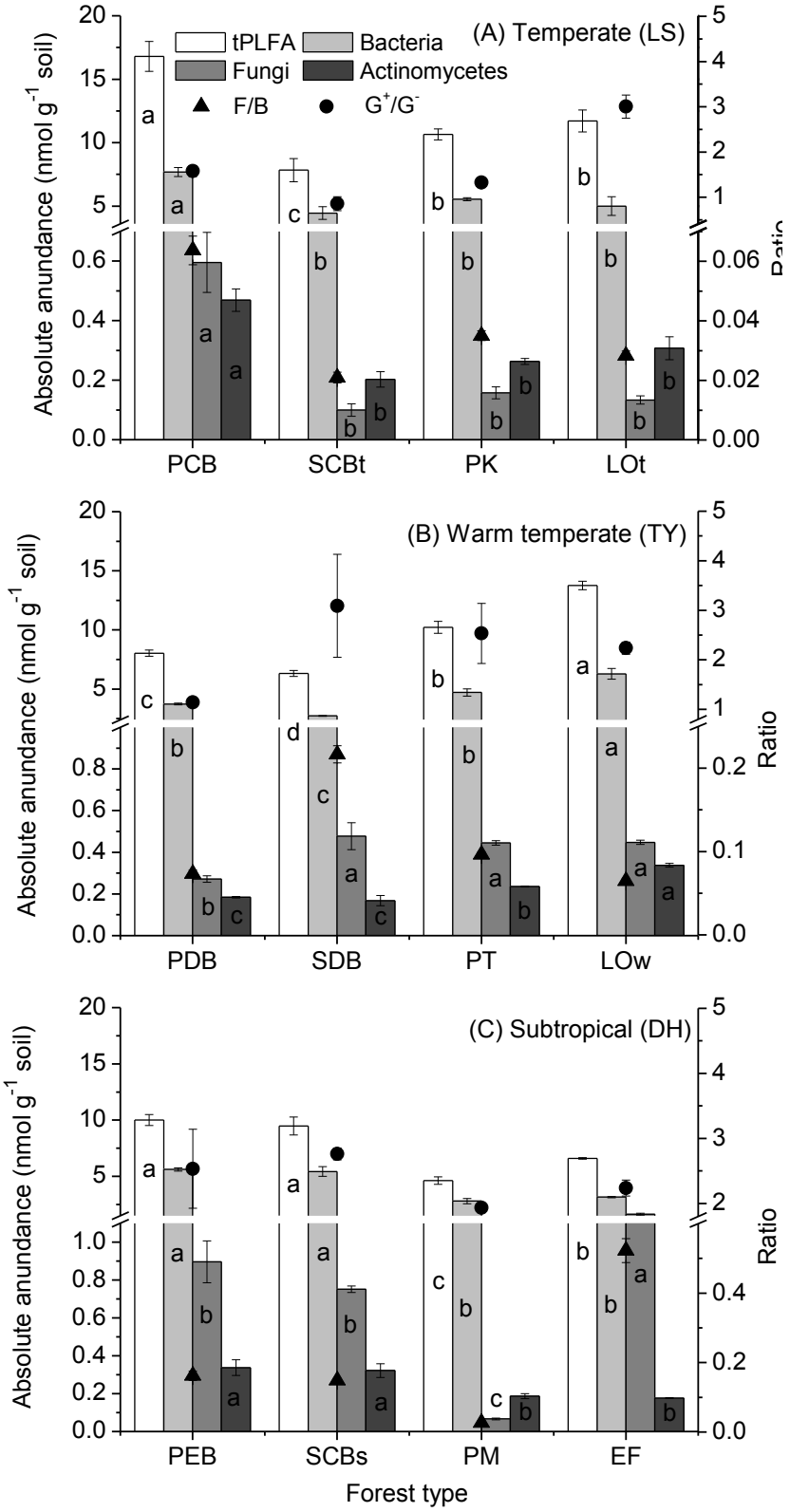

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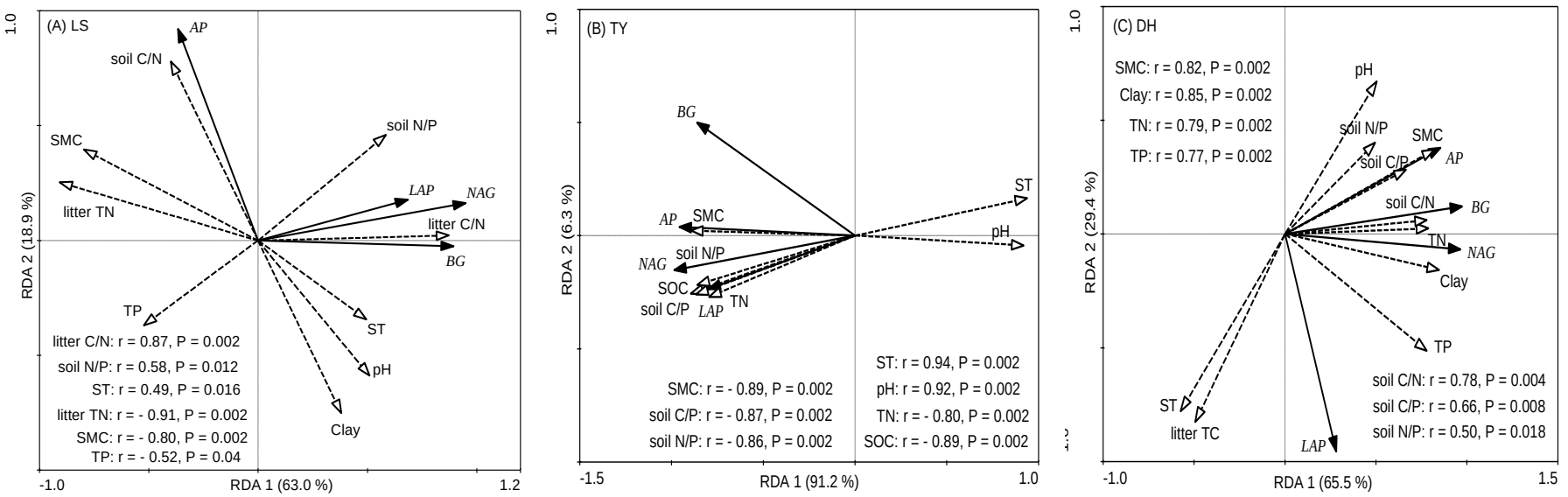

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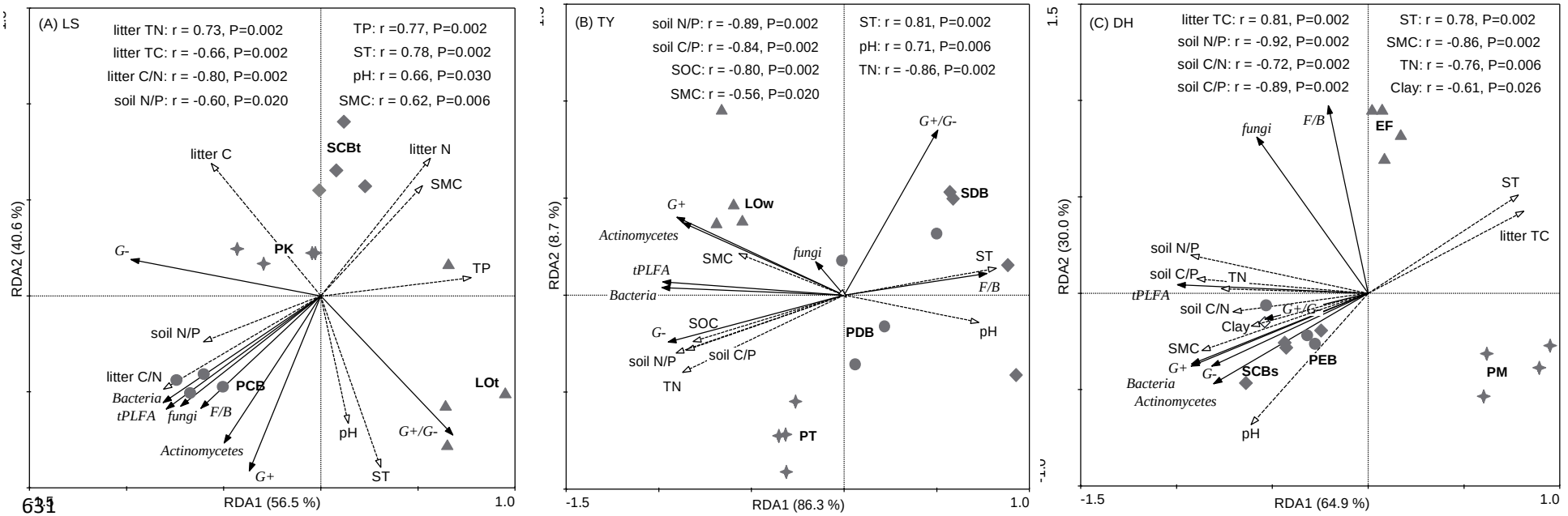

631

632 **Figure 4.** Redundancy analysis (RDA) ordination biplot of soil microbial community structure and environmental properties for different forest types in different climatic zones (A. Liangshui;
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635