

# Litter decomposition and soil microbial community composition in three Korean pine (*Pinus koraiensis*) forests along an altitudinal gradient

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## Abstract

**Background and aims** : The factors controlling litter decomposition and soil microbial community are important regulators of biogeochemical processes. Here we aim to explore controls on litter decomposition dynamics and soil microbial community composition in temperate forest by comparing three Korean pine forests along an altitudinal gradient.

**Methods** : Single- and mixed-species litter decomposition rates were determined by the litterbag method and soil microbial community composition was characterized by PLFAs.

**Results** : Litter decomposition rates decreased with altitude regardless of litter type, and were controlled by temperature. Generally, fine root litter decomposed more rapidly than leaf litter, and mixed-species litter decomposed faster than single-species. Soil microbial biomass C and N decreased with altitude and varied

differently among forest types in response to soil temperature and nutrient status. Fungal: bacterial PLFA ratios were significantly larger in forests receiving relatively poor litter quality inputs. Soil temperature, soil water content, total soil N and P were all directly related to the changes in total PLFAs among three forests.

**Conclusions** : In these Korean pines dominated temperate forests, environmental changes associated with altitude gradient drive decomposition dynamics and soil microbial community composition. This implies that climate change might be an important factor affecting these systems in the future.

**Keyword** Korean pine forest · Altitudinal gradient · Litter decomposition · Soil microbial biomass · PLFA

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## Introduction

Litter decomposition has profound effects on energy flow and the biogeochemistry of ecosystems. Decomposition of above- and belowground litter not only mineralizes nutrients in plant-available forms that support primary productivity, but it is also among the largest annual CO<sub>2</sub> fluxes to the atmosphere and exerts strong control over the global carbon cycle (Aerts 1997; Parton et al. 2007; van der Heijden et al. 2008). Therefore, a better understanding of the mechanisms and drivers that control the decomposition process is required to improve our ability to model and predict the responses of nutrient and carbon fluxes to future climate changes. This is especially crucial for northern forest ecosystems,

where decomposition rates are typically slower and more sensitive to warming (Shaver et al. 2000).

Generally, litter decomposition is controlled by the interaction between decomposers and substrate quality, which are both directly affected by environmental variables. At global or regional scales, climatic indices that incorporate both temperature and precipitation, like the actual evapotranspiration (AET), are typically the best predictors for litter decomposition rates (Meentemeyer 1978; Berg et al. 1993; Aerts 1997; Parton et al. 2007). Even at regional scales, environmental conditions, such as soil temperature and soil nitrogen availability, are considered important regulators in controlling litter decomposition (Salinas et al. 2011; Norris et al. 2013). For example, in a litter translocation experiment along a 2,800-m elevation gradient in Peruvian tropical forests, Salinas et al. (2011) found soil temperature explained 95 % of the variation in the decomposition rate. However, at the ecosystem scale, litter chemistry parameters are the best drivers for litter decomposition (Aerts 1997). Initial litter chemistry parameters often refer to N and lignin concentrations, or C: N and lignin: N, and litter with higher C: N or lignin: N decomposes at a relatively low rate (Taylor et al. 1989; Aerts 1997; Talbot and Treseder 2012).

Although many in situ decomposition experiments have focused on both leaf and fine root litter (Bloomfield et al. 1993; Majdi 2004; Cusack et al. 2009; Hobbie et al. 2010; Xiao et al. 2010; Wang et al. 2010a), the relationship between leaf litter and fine root litter decomposition is still not well understood. Compared to leaf litter, fine root typically has higher C: N or lignin content (e.g. Bloomfield et al. 1993; Abiven et al. 2005) and associated with its belowground site environment, fine root decomposition rates might not mirror those of leaf litter (Hobbie et al. 2010). Correspondingly, fine root decomposition rate might be higher (e.g. Ostertag and Hobbie 1999), or lower (e.g. Bloomfield et al. 1993; Majdi 2004) than leaf litter decomposition rate.

Soil microbes, functioning as decomposers, have an intense impact on litter decomposition processes, and even affect aboveground plant diversity and productivity (van der Heijden et al. 2008). The fluctuations of the soil microbial biomass pools are considered important drivers in affecting soil carbon and nutrient cycling. However, results regarding seasonal variations of soil microbial biomass C and N in recent decades are often contradictory (Bauhus and Barthel 1995; Diaz-Ravina

et al. 1995; Yang et al. 2010). For example, in temperate forests, maximal soil microbial biomass C or N may occur in spring (Diaz-Ravina et al. 1995), or summer (Gallardo and Schlesinger 1994; Zhong and Makeshin 2006; Yang et al. 2010), or show no significant seasonal variations (Von Lützwow et al. 1992; Bauhus and Barthel 1995), indicating that seasonal variations in soil microbial biomass might be determined by forest types, climatic factors, and soil conditions (Wardle 1992, 1998). Therefore, further research on seasonal variations and regulating factors in soil microbial biomass is needed to enhance our understanding of soil carbon cycling and nutrient transformation.

Plant communities can directly influence associated soil microbial communities through variable chemical products of litter decomposition and root exudation, and indirectly by modifying soil conditions and microclimate (Waldrop and Firestone 2004). In stands receiving mainly poor quality litter, soil microbial communities tend to have higher fungal: bacterial PLFA ratios than those receiving predominately high quality litters (Eiland et al. 2001; Bray et al. 2012), because fungi grow more slowly than bacteria and can decompose more recalcitrant materials (Rantalainen et al. 2004; Marschner et al. 2011). Previous studies indicate that soil microbial communities are influenced by soil conditions such as soil temperature (Rinnan et al. 2007; Frey et al. 2008; Feng and Simpson 2009), carbon and nutrient availability (Hogberg et al. 2007; Rinnan et al. 2007; Ushio et al. 2008), and soil pH (Fierer and Jackson 2006; Hogberg et al. 2007; Ushio et al. 2008). For example, Ushio et al. (2008) reported that the abundance of specific microbial biomarker lipids are correlated with soil pH, total C and N.

Different altitudes which generate different forest types, soil and meteorological conditions have been recognized as powerful natural platforms to explore and quantify the influence of these factors on forest ecosystem processes (Wang et al. 2010b; Salinas et al. 2011). The north slope of the Changbai Mountain is an ideal transect for this kind of study because altitudinal gradients generate small-scale diversity of forest types and environmental conditions (Yang and Li 1985). Thus, in this study, we have compared litter decomposition dynamics and soil microbial properties among three natural forests dominated or co-dominated by Korean pine to better understand carbon and nutrient fluxes in temperate ecosystems along an altitudinal gradient on the Changbai Mountain. Our specific objectives

within these three Korean pine forest types are (1) to compare litter decomposition rates between fine root and leaf litter, and between single species and mixed-species litter, and (2) to elucidate the regulators driving the variations of soil microbial properties along an altitudinal gradient.

## Material and method

### Study site

The study sites are located on the northern slope of the Changbai Mountain Nature Reserve (extending from 41°43' to 42°26' N, 127°42' to 128°17' E), the largest protected temperate forest in the world, which was established in 1960 and joined the World Biosphere Reserve Network. This area has a temperate continental climate with long cold winters and warm summers. Mean annual temperature decreases from 4.9 °C at the foot of mountain to -7.3 °C on the mountaintop and annual mean precipitation varies from 750 to 1,350 mm.

There are four main vegetation types along the altitudinal gradient of the Changbai Mountain (from 740–2,691 m), namely broadleaved Korean pine mixed forest, spruce-fir forest, subalpine birch (*Betula ermanii*) forest, and alpine tundra (Yang and Li 1985). Three forest sites in this study were located between 750 to 1350 m with approximately 300 m as interval. The Broadleaved Korean pine Mixed Forest (hereafter abbreviated as BKF) is the primary old-growth forest present at low altitudes. With increasing altitude, the forest is co-dominated by Korean pine, larch (*Larix olgensis*) and fir (*Abies nephrolepis*), and is also accompanied with some broadleaved species in the mid- and understory (hereafter referred to as Transitional Forest and abbreviated as TF). At higher altitudes, coniferous forests are co-dominated by spruce (*Picea jezoensis*), fir and Korean pine (hereafter referred to as Korean pine Coniferous Forest and abbreviated as KCF). These three Korean pine forests are typical temperate forests in northeast China, and might be functioning as a potential carbon sink (Fang et al. 2001). In September 2011, we established three 20 m × 30 m plots at each Korean pine forest site for field measurements. All three forests were dominated or co-dominated by Korean pine, and the ratio of Korean pine basal area ranged from 36%–50% (more information about locations and community properties see Zhou et al. 2014). The soil in BKF is classified as

Eutric cambisols and in TF and KCF is classified as Humic cambisols (FAO-UNESCO 1988).

### Field measurement and laboratory

Air and soil temperature were measured continuously by temperature sensors with data loggers (DS1921G, Thermochron iButton, Maxim Integrated, CA, USA) during the growing season from late May to late September 2012. In each plot, one sensor was set up 1 m above the ground to measure air temperature and another was placed in the soil at a depth of 5 cm to measure soil temperature with an hourly recording frequency. Soil samples (0–10 cm) for measuring physical and chemical properties were collected from five randomly placed cores (3 cm diameter) in each plot in July 2012. The five replicates in each plot were pooled and mixed to get one composite sample. Gravimetric soil water content (SWC) was measured by oven-drying samples at 105 °C for 24 h. Soil bulk density was determined by weighing oven-dried samples of known volume by using a cutting ring (volume 100 cm<sup>3</sup>, inner diameter 5 cm). Compositing soil samples were air-dried, passed through a 2-mm sieve and manually cleaned of any visible plant tissues for laboratory analysis. Soil pH was measured in the 1:5 soil/water extract. The particle size distribution of the <2 mm particle fractions was determined using the laser detection technique. Soil organic carbon content was analyzed using the dichromate oxidation method, and soil total nitrogen content was determined using the Kjeldahl acid-digestion method with Kjeltac (2200 Auto Distillation, Foss Tecator, Höganäs, Sweden), and total P content was analyzed using the molybdenum blue colorimetric method with a UV/visible spectrophotometer after H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion (UV-2550, Shimadzu, Kyoto, Japan).

Decomposition rates of leaf and fine root litter were determined using the litterbag method (Berg et al. 1993). We collected fresh leaf litter in September 2011 from each plot within the three Korean pine forests using litter traps lined with 1 mm mesh openings. Half of the collected fresh leaf litter was used to determine the community (mixed-species) leaf litter decomposition rate, and the remainder was used to sort the Korean pine leaf litter to determine its decomposition rate. In BKF, mixed-species leaf litter includes Korean pine leaf litter and leaf litter from other broadleaved deciduous trees. Mixed-species leaf litter in the TF mainly includes leaf litter from Korean pine, fir and larch with a small

amount of broadleaves. Mixed-species leaf litter in the KCF was entirely from Korean pine, fir and spruce (more details see Zhou et al. 2014). The ratios of Korean pine leaf litter to total leaf litter in mixed-species leaf litter bags ranged from 0.45 to 0.52. Five g of oven-dried (60 °C) leaf litter was placed in a 1 mm mesh opening nylon bag with a dimension of 15 cm×15 cm. Leaf litterbags were placed on the forest floor in each plot (15 mixed-species leaf litter bags and 15 Korean pine leaf litter bags per plot) in late September. We also collected fresh fine root (<2 mm in diameter) litter in September 2011 from each plot of the three Korean pine forests using soil cores (8 cm diameter). Fine root litter was manually washed free of soil samples. Of the collected fine root samples, a portion of the sample was used to create mixed-species litter bags while the remaining fine root litter was sorted by species to create Korean pine single species litter bags. The ratios of Korean pine fine root litter to total fine root litter in mixed-species fine root litter bags ranged from 0.32 to 0.39. Fine root litterbags containing one g of oven-dried (60 °C) samples were placed in the soil at a depth of 5 cm in each plot (15 mixed-species fine root litterbags and 15 Korean pine litterbags per plot) in late September. In total, we placed 270 leaf litter bags and 270 fine root litter bags in the nine plots. Three litterbags for each litter category (mixed-species leaf litter, Korean pine leaf litter, mixed-species fine root litter, Korean pine fine root litter) in each plot were retrieved monthly to determine mass loss from May to September 2012. In the laboratory, adhering soil particles were carefully removed from the litter by rinsing with water. The litter was then oven-dried to a constant mass at 65 °C. Initial organic carbon, total nitrogen and phosphorous content of each litter category were measured using the same methods for soil. We used the following exponential function:  $Y_t = Y_0 \times e^{-kt}$  (Olson 1963) to determine the annual decay constant ( $k$ ).

Soil samples (0–10 cm) for measuring soil microbial properties were collected from five randomly located cores (3 cm diameter) in each plot in May, July and September 2012. The five replicates were passed through a 2-mm sieve and pooled together to get one composite sample for each plot. Fresh samples were immediately transported to the laboratory with a portable ice box and stored at 4 °C before analysis. Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) were measured by the fumigation-extraction method (Vance et al. 1987). Briefly, after adjusting to

approximately 60 % of water holding capacity, the fresh soil samples were incubated for 1 week in the dark at 25 °C. Then 20 g (dry weight equivalent) of soil samples were fumigated with  $\text{CH}_3\text{Cl}$  for 24 h and non-fumigated soil samples were extracted with 0.5 M  $\text{K}_2\text{SO}_4$ . The extracts were filtered through 0.45- $\mu\text{m}$  filters and analyzed with a Multi 3100 N/C TOC analyzer (Analytik Jena, Germany). SMBC and SMBN were calculated as the difference in extractable organic C and inorganic N contents between the fumigated and the non-fumigated samples using conversion factors ( $k_{ec}$  and  $k_{en}$ ) of 0.38 and 0.45 (Lovell et al. 1995), respectively.

The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by Bossio and Scow (1998). The separation and identification of extracted PLFAs were carried out according to the standard protocol of the Sherlock Microbial Identification System V<sub>4.5</sub> (MIDI) and a Gas Chromatograph (Agilent 6850, USA). The abundance of individual fatty acids was determined as relative n mol per g of dry soil and standard nomenclature was used. The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1 $\omega$ 7c, 16:1 $\omega$ 5c, i17:0, a17:0, 17:0cy, 19:0cy were chosen to represent the PLFAs of the bacterial group, and fungi were considered to be represented by the PLFAs 18:2 $\omega$ 6c (Frostegård and Bååth 1996; Bossio and Scow 1998). Two other abundant fatty acids, 16:0 and 18:1 $\omega$ 9c, were also used to analyze the microbial community composition. All of the PLFAs mentioned above were used to calculate the total PLFAs of soil microbial community. The ratio of fungal to bacterial PLFAs was also included in the data analysis. This ratio has often been used as an indicator of change in the soil microbial community structure (Bardgett et al. 1999).

#### Data analysis

Repeated measures ANOVA was used to test for the effects of forest type, sampling time and their interaction on soil microbial properties. One-way ANOVA was used to test the differences among the three Korean pine forests. Post-hoc analysis was performed by Duncan's test. Pearson's test was used to determine whether there were significant correlations between total PLFAs and soil physical and chemical variables. Sequential Bonferroni correction was used to adjust  $p$ -values during multiple comparisons (Holm 1979). All the statistical analyses were performed on SPSS 15.0 software

(SPSS, Chicago, IL, USA). Difference is significant at the 0.05 level.

Based on redundancy analysis and combined with the species weight, the principal response curves (PRC) can be used to interpret the extent and directions of composition changes under different experimental treatments with the control treatment as a reference (Lepš and Šmilauer 2003). Here, we used individual PLFA biomarkers as species and TF as a reference to compare the extent and directions of soil microbial composition changes of the three Korean pine forests. More specifically, the individual PLFA can be calculated by the following equation: the relative abundance of a species to the reference = EXP (value in curve  $\times$  value of species weight). If the relative abundance is greater than 1, it indicates the quantity of the individual PLFA is higher than the reference or vice versa. The PRC analyses were performed on CANOCO software for Windows 4.5 (Ithaca, NY, USA).

## Results

### Microclimate and soil variables

The average air temperature from late May to late September 2012 was 15.9, 13.2 and 11.6 °C ( $p < 0.001$ ), and soil temperature was 13.3, 8.7 and 7.3 °C for BKF, TF and KCF, respectively ( $p < 0.001$ ). Soil water content in BKF was significantly higher than in TF and KCF (Table 1). Soil organic carbon contents in the BKF and TF were significantly higher than in the KCF (Table 1). Soil total nitrogen decreased significantly with altitude. Total soil phosphorus in the BKF was significantly higher than in the TF and KCF. Both C : N ratio and C : P ratio differed significantly among forest types, with the largest value in the TF (Table 1). Soil clay and silt contents in the BKF were higher than in the TF and KCF, while soil sand content in the BKF was significantly lower than in the TF and KCF (Table 1).

### Litter decomposition dynamics

C : N ratios of all litter categories (except Korean pine leaf litter) in the BKF were significantly lower than in the TF and KCF (Table 2). Meanwhile, mixed species leaf and fine root litter generally had lower C : N ratios than Korean pine leaf and fine root litter (Table 2). The mass loss (in comparison with its initial mass) of Korean pine leaf litter in the BKF was significantly higher than

**Table 1** soil characteristics (0–10 cm) of three Korean pine forests along an altitudinal gradient in Changbai Mountain, China

Forest type	Soil water content (%)	pH	m	SOC (g/kg)	m	TP (g/kg)	C : N	C : P	Soil texture		
									Clay, <2 $\mu$ m (%)	Silt, 2–50 $\mu$ m (%)	Sand, 50–2,000 $\mu$ m (%)
BKF	77.3(10.3)a	4.8(0.1)a	0.74(0.01)a	73.3(6.8)a	6.1(0.2)a	1.13(0.01)a	12.1(0.8)c	66.5(11.6)c	3.3(0.2)a	2.4(0.1)b	2.8(0.2)ab
TF	37.8(4.0)b	4.5(0.1)a	0.73(0.02)a	62.6(5.9)a	2.3(0.2)b	0.25(0.03)b	27.0(2.1)a	260.1(28.4)a	51.0(1.4)a	37.8(0.1)b	40.9(1.3)b
KCF	31.4(5.2)b	4.9(0.2)a	0.82(0.06)a	32.4(7.1)b	1.6(0.2)c	0.22(0.01)b	19.5(2.4)b	146.7(33.3)b	45.7(1.5)b	59.9(0.2)a	56.4(1.6)a

BKF broadleaved Korean pine mixed forest; TF transitional forest; KCF Korean pine coniferous forest; SOC soil organic carbon; TP total soil nitrogen; TN total soil phosphorus; C : N the ratio of soil organic carbon to total soil nitrogen; C : P the ratio of soil organic carbon to total soil phosphorus. Values are mean  $\pm$  SE ( $n = 3$ ). In each column, different letters indicate statistically significant differences ( $p < 0.05$ )

**Table 2** Initial leaf and fine root litter chemical content in the three Korean pine forests along an altitudinal gradient in Changbai Mountain, China

	C (g/kg)	N (g/kg)	P (g/kg)	C : N	C : P
Korean pine leaf litter					
BKF	513.8(3.4)c	10.2(0.9)a	1.09(0.05)b	43.4(3.1)a	473.6(20.0)b
TF	555.5(2.7)a	11.9(0.2)a	0.96(0.03)b	46.6(0.8)a	579.5(15.7)a
KCF	535.6(8.2)b	10.8(0.5)a	1.24(0.03)a	49.5(1.3)a	433.1(13.9)b
Korean pine fine root litter					
BKF	512.8(6.6)a	10.5(0.3)a	1.41(0.05)a	48.7(1.4)b	365.8(14.3)c
TF	529.5(14.0)a	8.1(0.2)b	0.97(0.04)c	65.3(3.4)a	547.9(32.7)a
KCF	528.8(4.1)a	9.3(0.6)ab	1.15(0.01)b	57.3(4.0)ab	459.9(1.4)b
Mixed-species leaf litter					
BKF	508.2(5.7)b	14.4(0.0)a	1.19(0.02)a	35.2(0.3)c	428.8(11.4)b
TF	558.3(9.6)a	9.18(0.6)c	1.02(0.05)b	57.4(4.3)a	551.6(34.4)a
KCF	544.5(10.6)a	11.7(0.2)b	1.09(0.04)ab	46.7(1.0)b	499.0(16.1)ab
Mixed-species fine root litter					
BKF	487.0(2.1)b	14.0(0.6)a	1.32(0.05)a	34.9(1.6)b	371.1(14.2)b
TF	494.1(3.8)ab	9.0(0.2)b	1.00(0.05)b	55.1(1.6)a	497.2(27.9)a
KCF	508.3(7.1)a	9.1(0.5)b	1.14(0.04)b	56.5(3.9)a	449.0(21.3)a

*BKF* broadleaved Korean pine mixed forest; *TF* transitional forest; *KCF* Korean pine coniferous forest. Values are mean±SE ( $n=3$ ). Different letters indicate statistically significant differences ( $p<0.05$ )

in the KCF, while the decay constant ( $k$  value) of Korean pine leaf litter in the BKF was significantly higher than in the TF and KCF (Fig. 1a; Table 3). The mass loss and decay constant of Korean pine fine root litter in the BKF were significantly higher than in the TF and KCF (Fig. 2b; Table 3). Both mass loss and the decay constant of mixed-species leaf litter decreased significantly with altitude (BKF>TF>KCF) (Fig. 1c; Table 3). The mass loss of mixed species fine root litter in the BKF was significantly higher than in the TF and KCF, while the decay constant of mixed-species fine root litter decreased significantly with altitude (Fig. 1d; Table 3). The mass loss of fine root litter (about 20 %) during the winter (the first 8 months, i.e. from September 2011 to May 2012) was higher than leaf litter (about 10 %) (Fig. 1a, b, c, d). The most striking difference of mass loss for litter in the BKF compared to the other two forest types were detected from the 9 months incubation period (Fig. 1a, b, c, d).

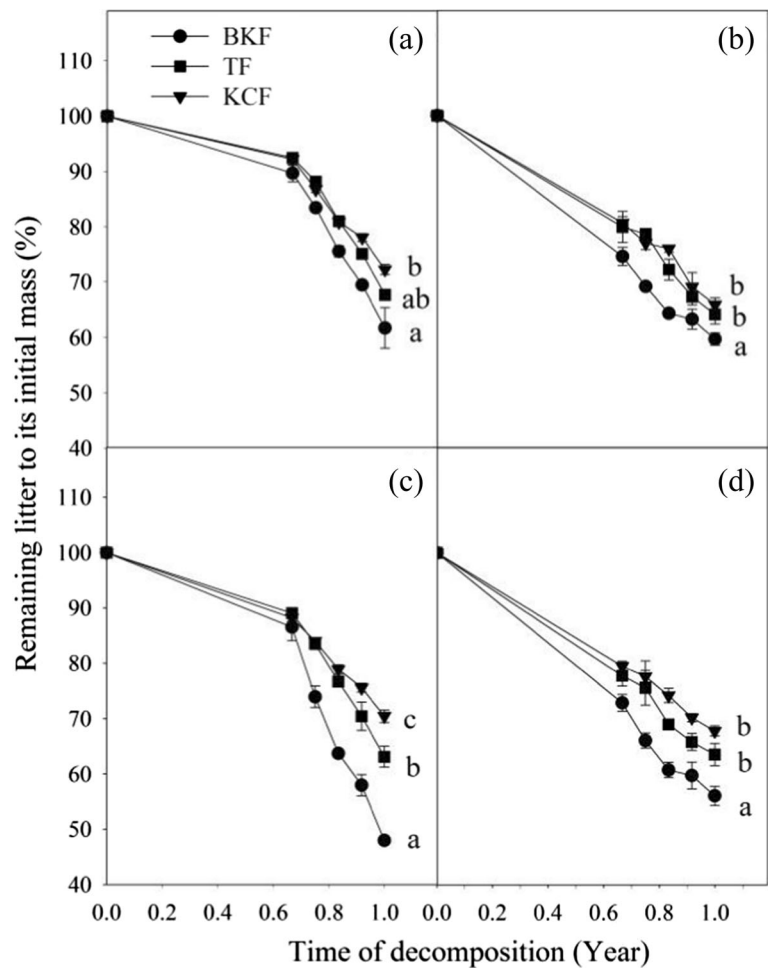
#### Soil microbial properties

SMBC, SMBN and SMBC: SMBN ratio differed significantly among forest types (Fig. 2 a, b, c). SMBC and

SMBN in the BKF were significantly higher than in the TF and KCF, while SMBC: SMBN ratio in the TF forest was significantly higher than in the BKF and KCF (Fig. 2 a, b, c). SMNC, SMBN and SMBC: SMBN ratio also significantly differed among sampling times (Fig. 2 a, b, c). In the BKF, SMBC and SMBN were much higher in May and July than in September; in the TF, SMBC and SMBN were much higher in May than at other sampling times; in the KCF, SMBC and SMBN showed negligible variations during the growing season 2012 (Fig. 2a, b).

Total PLFAs, bacterial PLFAs, and F: B ratio differed significantly among forest types (Fig. 3a, b, d). Total PLFAs and bacterial PLFAs in the BKF were significantly higher than in the TF and KCF, while F: B ratio in the BKF was significantly lower than in the TF and KCF (Fig. 3a, b, d). Total PLFAs, bacterial PLFAs, and Fungal PLFAs also significantly differed among sampling times (Fig. 3 a, b, c). Total PLFAs were significantly correlated with soil microbial biomass C and N ( $R^2=0.846$  and  $0.849$ , respectively, Fig. 4). Meanwhile, a series of bivariate comparisons using correlation analyses showed that total PLFAs were strongly related to soil temperature, soil water content, total soil N and P (Table 4).

**Fig. 1** Remaining Korean pine leaf litter **a**, Korean pine fine root litter **b**, mixed-species leaf litter **c** and mixed-species fine root litter **d** to their initial mass in the three Korean pine forests from September 2011 to September 2012. Leaf litter was placed on the forest floor while fine root litter was placed at a depth of 5 cm in the soil. BKF, TF and KCF represents broadleaved Korean pine mixed forest, transitional forest and Korean pine coniferous forest, respectively. Values are mean±SE ( $n=3$ ). Values with a different letter are significantly different for mass loss (%) ( $p<0.05$ )



According to the PRC analysis, in reference to the TF, soil microbial community in the BKF and KCF changed along sampling times. However, the relative abundance of most PLFA biomarkers was less variable, only the PLFA biomarker a15:0 was more abundant and variable in the BKF and less abundant in the KCF when referring to the TF (Fig. 5). The quantity variances of the PLFA biomarkers 15:0 and 18:2 $\omega$ 6 among the three Korean pine forests were smaller than other PLFA biomarkers (Fig. 5).

## Discussions

### Litter decomposition dynamics

Overall, the annual decay constant ( $k$ ) and mass loss in this study declined with increasing altitude regardless of

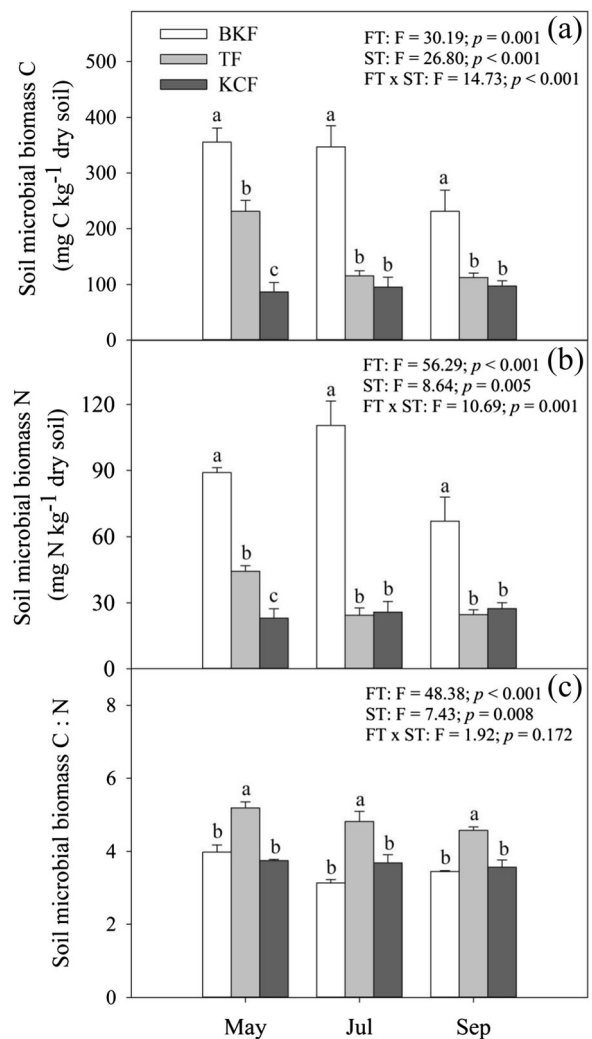
litter type and litter quality. While recognizing many factors could contribute to this pattern, we suggest that temperature was the dominant regulator controlling the litter decomposition rates along this altitude. Similarly, many litter decomposition experiments, which take advantage of altitudinal gradients, have also considered temperature as the best predictor of explaining the variation across elevations (Scowcroft et al. 2000; Wang et al. 2010b; Salinas et al. 2011). Suitable temperature could accelerate litter decomposition by enhancing litter microbial metabolic activity (Schindlbacher et al. 2011). Implications of temperature on soil microbial community along this altitudinal gradient (see below) further proved the role of temperature in influencing litter decay by modifying microbes. Also, temperature could affect litter decomposition by influencing the rates of mineralization and nutrient supply (Aerts 1997; Salinas et al. 2011), since fresh litter typically contains insufficient

**Table 3** Mean annual decay constant ( $k$ ) of leaf and fine root litter in the three Korean pine forests along an altitudinal gradient in Changbai Mountain, China

	$k$	$R^2$	$p$
Korean pine leaf litter	0.38(0.02)a	0.77	0.022
BKF	0.30(0.00)b	0.72	0.032
TF	0.27(0.01)b	0.80	0.015
KCF			
Korean pine fine root litter	0.51(0.00)a	0.99	< 0.001
BKF	0.41(0.02)b	0.95	< 0.001
TF	0.38(0.02)b	0.96	< 0.001
KCF			
Mixed-species leaf litter			
BKF	0.56(0.02)a	0.77	0.021
TF	0.37(0.01)b	0.79	0.018
KCF	0.30(0.01)c	0.89	0.005
Mixed-species fine root litter			
BKF	0.57(0.03)a	0.98	< 0.001
TF	0.44(0.02)b	0.98	< 0.001
KCF	0.37(0.01)c	0.99	< 0.001

BKF broadleaved Korean pine mixed forest; TF transitional forest; KCF Korean pine coniferous forest. Values are mean±SE ( $n=3$ ). Different letters indicate statistically significant differences ( $p<0.05$ )

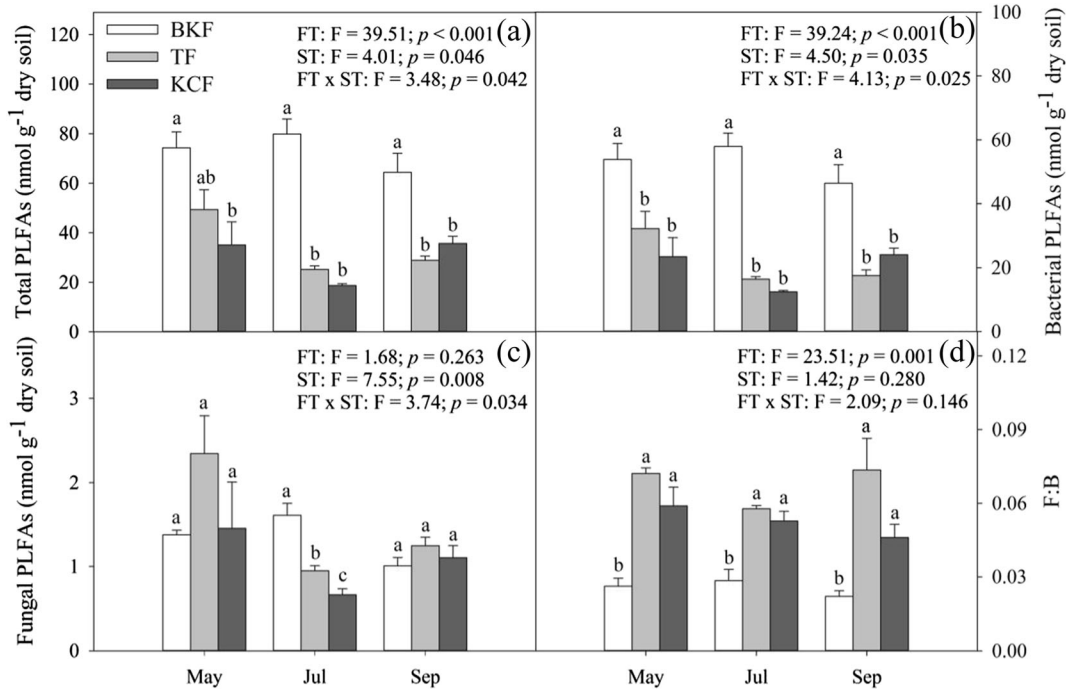
nutrients to meet the growth and maintenance demand of decomposers, thereby requiring decomposers to access exogenous nutrients (Hobbie and Vitousek 2000; Norris et al. 2013). This is particularly important for higher altitude and relatively infertile forests, like the Transitional Forest and Korean pine Coniferous Forest in this study. Although, temperature is considered the overriding environmental factor affecting litter decomposition along this altitude, the nature of litter chemistry likely has a major influence on the decay rates. In this study, initial litter C: N ratio, which is widely used as an index characterizing litter quality (Taylor et al. 1989; Aerts 1997; Talbot and Treseder 2012), was lower in the BKF than the TF and KCF (Table 2). Additionally, our results indicate that the annual decay constant and mass loss of mixed-species litter were generally higher than single-species litter. This may be due to differences in litter quality between mixed and single species litter. Single-species litter has higher C: N ratio and therefore lower quality litter compared to mixed-species (Table 2). This is in agreement with previous studies (e.g. Salamanca et al. 1998; Vos et al. 2013).



**Fig. 2** Soil microbial biomass C **a**, N **b** and C: N ratio **c** for the three Korean pine forests in May, July and September 2012. BKF, TF and KCF represents broadleaved Korean pine mixed forest, transitional forest and Korean pine coniferous forest, respectively. Values are mean±SE ( $n=3$ ). Within each month, values with a different letter are significantly different ( $p<0.05$ ). Repeated measures ANOVA results (F and  $p$  values) of forest type (FT), sampling time (ST) and their interaction (FT×ST) are shown in the insets

Previous reviews have suggested that leaf litter decomposition depends more heavily on climatic variables (Berg et al. 1993; Meentemeyer 1978; Aerts 1997; Parton et al. 2007), whereas root litter decomposition may be more sensitive to root chemistry (Silver and Miya 2001). However, despite the higher C: N ratio of fine root compared to leaf litter in this study, we still found that fine root litter generally decomposed more rapidly than leaf litter for both Transitional Forest and Korean pine Coniferous Forest. Similarly, many previous works have

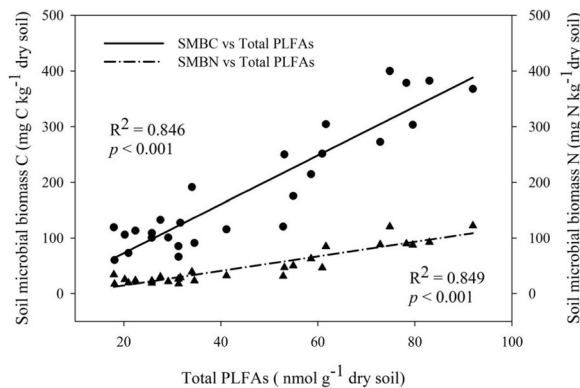




**Fig. 3** Total PLFAs **a**, bacterial PLFAs **b**, fungal PLFAs **c**, and F: B ratio **d** for the three Korean pine forests in May, July and September 2012. F: B indicates the ratio of fungal to bacterial PLFAs. BKF, TF and KCF represents broadleaved Korean pine mixed forest, transitional forest and Korean pine coniferous forest,

respectively. Values are mean±SE ( $n=3$ ). Within each month, values with a different letter are significantly different ( $p<0.05$ ). Repeated measures ANOVA results (F and  $p$  values) of forest type (FT), sampling time (ST) and their interaction (FT×ST) are shown in the insets

also noted the higher decomposability of fine root than leaf litter (Ostertag and Hobbie 1999). Apart from the effects of fine root litter quality, we propose that preferable belowground environmental conditions, like consistent temperature and humidity, available exogenous nutrients for microbial colonization, and the composition and activity of the decomposer communities, might be attributed to the faster decomposition rate of fine roots.

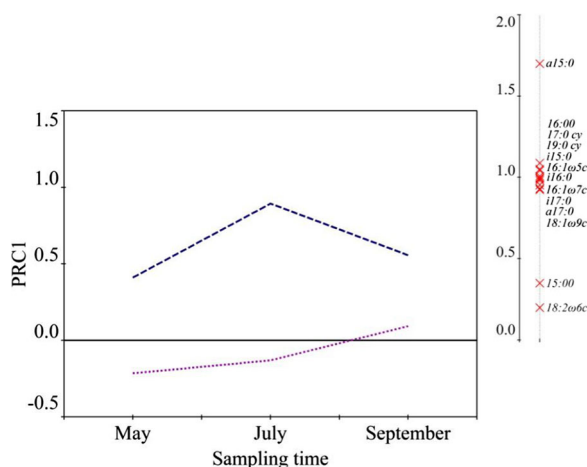


**Fig. 4** Correlated relationships between soil microbial biomass (C and N) and total PLFAs

**Table 4** Summer of Pearson correlation coefficients between total PLFAs and covariates,  $p$ -values for individual pair-wise comparisons and adjusted  $p$ -values for multiple comparisons.  $p$ -values were adjusted using Holm’s sequential Bonferroni correction (Holm 1979)

	Total PLFAs		
	r	$p$ -value	Corrected $p$ -value
TP	0.99	< 0.0001	< 0.001
TN	0.97	< 0.0001	< 0.001
ST	0.96	< 0.0001	< 0.001
SWC	0.94	< 0.0001	< 0.001
Silt	0.86	0.003	0.016
Sand	−0.85	0.003	0.015
SOC	0.67	0.048	0.192
Clay	0.67	0.049	0.147
SBD	−0.36	0.348	0.696
pH	0.09	0.817	0.817

TP total soil phosphorus; TN total soil nitrogen; ST soil temperature; SWC soil water content; SOC soil organic carbon; SBD soil bulk density



**Fig. 5** Principal response curves with species weights based on the PLFAs biomarker data. The proportion of variability explained by the first response curves was 77.2 % ( $F=87.7$ ,  $p=0.004$ ). The *dashed line* represents the broadleaved Korean pine mixed forest, the *dotted line* represents the Korean pine coniferous forest, and the horizontal axis represents the transition forest which was used as reference. The vertical axis on the right shows the PLFAs biomarker weights

### Soil microbial properties

Our results indicate that the amounts and seasonal patterns of soil microbial biomass differed significantly among the three Korean pine forests. Many factors are considered to elucidate the effects of vegetation type on soil microbial biomass (Wardle 1992, 1998). For example, soil carbon and nutrient availability, determined by the quantity and quality of substrates via leaf litter inputs and root exudations, are crucial drivers that affect soil microbial biomass (Wardle 1992, 1998; Wang and Wang 2007; Yang et al. 2010), since SMBC and SMBN were proven to have strong positive correlations with total nitrogen and phosphorus (Wang and Wang 2007; Yang et al. 2010). Moreover, soil temperature is responsible for the size of soil microbial biomass by influencing microbial metabolic activity (Schindlbacher et al. 2011), this is particularly important for higher altitudes where soil temperature typically lags behind air temperature in spring. Correspondingly, significantly higher SMBC and SMBN in the BKF than in the TF and KCF are mainly attributed to higher soil organic carbon, total N and P (Table 1), and more preferable soil temperature. Three Korean pine forests in this study exhibited different seasonal patterns in soil microbial biomass. Higher SMBC and SMBN in spring (May) and summer (July) for the BKF might be ascribed to the

combination of the burst of N mineralization, which increases the nutrient availability from freeze-thaw events, (Schimel and Clein 1996), the increase in labile substrate by tree root growth associated exudations (Kuzakov 2002), and the suitable soil moisture and temperature during these periods. Additionally, we found remarkably higher SMBC: SMBN ratio in the TF (which has higher soil C: N ratio) than in the BKF and KCF. The intense uptake and immobilization of N into soil microbial biomass to maintain the necessary metabolism in N limited stands might be responsible for this phenomenon.

The quantity of PLFAs extracted from soil microbes has also been widely used as an indicator of characterizing microbial biomass (Frostegård and Bååth 1996; Fierer et al. 2003), and it has been reported that there is a positive linear relationship between  $\text{CHCl}_3$ -extractable biomass and PLFA-analysis estimated biomass (Fierer et al. 2003). In this study, the relative sizes and seasonal patterns of total PLFAs are generally similar to that of soil microbial biomass. This is also evident from the positive correlations between soil microbial biomass and total PLFAs (Fig. 4). Thus, factors driving soil microbial community changes might resemble that of soil microbial biomass for the three Korean pine forests. In general, fungi grow more slowly than bacteria, and can decompose more recalcitrant and complex organic materials (Rantalainen et al. 2004; Marschner et al. 2011), all this directly results in a tendency for soil microbial communities to maintain high fungal: bacterial PLFA ratios in sites receiving predominantly poor quality litter (Eiland et al. 2001; Bray et al. 2012). Our results also indicated higher fungal: bacterial PLFA ratios in the TF and KCF which received relatively poor quality leaf and fine root litter (higher C: N ratios, Table 2) than the BKF.

Using the spatiotemporal variations of PLFAs in soils to interpret the changes of specific microbial taxonomic groups should be more prudent since the same PLFA biomarker may exist in the membranes of microbes belonging to different taxonomic groups. However, certain trends can be generalized in accordance with specific PLFAs belonging to the same taxonomic group. According to PRC analysis, branched fatty acid a15:0 (a gram-positive indicator) was more abundant and variable in BKF when referred to TF, while 18:2 $\omega$ 6c (a widely used fungal indicator) was relative less abundant and stable. This indicates soil in BKF was dominated by bacteria, reflecting the previous result of lower fungal:

bacterial PLFA ratio in BKF, and provided evidence for a shift in the function and composition of microbial communities among the three Korean pine forests along this altitudinal gradient.

Additionally, many soil variables, such as soil temperature, pH, carbon and nutrients, have been identified to influence soil microbial community (Fierer and Jackson 2006; Hogberg et al. 2007; Rinnan et al. 2007; Ushio et al. 2008; Feng and Simpson 2009). In this study, we found total PLFAs were strongly correlated with soil temperature, soil water content, soil total N and total P, which further explained that the growth of soil microbes for the three Korean pine forests along this altitudinal gradient was predominantly affected by climate (in particular soil temperature and moisture) and soil nutrient status. Unlike other studies which considered soil pH as an important factor explaining variations in soil microbial community between sites (Wardle 1998; Fierer and Jackson 2006; Ushio et al. 2008), we did not detect a correlation between soil pH and total PFLAs. This might be ascribed to the narrow range of pH values among the three Korean pine forests (Table 1).

## Conclusions

Taking advantage of the altitudinal gradient offered by the Changbai Mountain, we have compared differences of litter decomposition dynamics and soil microbial community among three Korean pine forests, and explored the drivers controlling these variations. Having found that temperature is the predominant factor controlling litter decay along this altitude gradient, we have also noted that litter quality still plays an important role. From CHCl<sub>3</sub>-extractable biomass and PLFA-analysis, we have identified that soil temperature and nutrient availability are the main regulators influencing the growth of soil microbes in the three Korean pine forests along this altitude. The response of litter decomposition to temperature will affect rates of mineralization, nutrient supply, and therefore the carbon cycle, especially in cool temperate forest ecosystems. Although we have explored temperature as a main factor controlling litter decay rate and soil microbial growth along this altitude, further studies, like litter translocation experiments, are needed to quantify the sensitivity of litter decay rate to temperature in order to predict its response to future global climate change.

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