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Different winter soil respiration between two mid-temperate plantation forests

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ABSTRACT

Larch and Chinese pine plantation forests are important carbon (C) sinks in the temperate regions, especially in China. However, their soil respiration in winter is still poorly studied. Here we explored the different microbial characteristics and winter soil respiration in larch and Chinese pine plantation forests in northeastern China, which has similar climate and basic soil characteristics. Results showed that both mean and cumulative winter soil CO_2 fluxes were significantly higher in Chinese pine forest (0.45 μmol $m^{-2} s^{-1}$ and 46.39 g C m^{-2}, respectively) than in larch forest (0.25 μmol $m^{-2} s^{-1}$ and 25.92 g C m^{-2}, respectively). Snow depth and inorganic nitrogen (N) could not explain the differences in winter soil respiration between the two sites. Instead, Chinese pine forest had higher soil microbial biomass, fungi abundance, F/B (ratio of fungi to bacteria), and extracellular enzymatic activities (EEAs) than larch forest, which could lead to higher winter soil respiration in Chinese pine forest than in larch forest. Our findings indicated that the thermal insulation effect of litter cover was important to winter soil respiration, especially when the snow cover depth was less than 30 cm. Soil microbes played a more important role in soil respiration than soil nutrient status and should be carefully considered for better estimation of the C budget in different forest ecosystems. Although soil respiration was higher in Chinese pine forest, soil organic C content was also higher, suggesting its better C sequestration capacity than larch forest.

1. Introduction

The balance between carbon (C) input and C output dominates the C budget of terrestrial ecosystems. Soil respiration is the main output of C from soil and winter soil respiration plays a vital role in annual C budget, accounting for 3–50% of annual C emissions in various ecosystems (Elberling, 2007; Liptzin et al., 2009; Monson et al., 2006; Schindlbacher et al., 2007; Schindlbacher et al., 2014; Shi et al., 2012; Shi et al., 2014; Wang et al., 2010). Temperate forest ecosystem is the biggest terrestrial C sink in the Northern Hemisphere, accounting for approximately 10% of global soil C stocks (Rasmussen et al., 2006). Especially in China, plantation forests cover 69.33 million hm^2, accounting for 36% of all forests areas (Chen et al., 2014). Winter soil respiration in these plantation forests could be significantly affected by global climate change and should not be neglected.

One of the characteristics of plantation forests is their simple species structure with low biodiversity. Therefore, plantations offer the opportunity to investigate singles species effects on forest C cycling. For example, previous studies showed that soil respiration was significantly higher in evergreen pine plantation forests than in deciduous larch plantation forest in northeastern China (Wang et al., 2006). However, no significant difference was observed in winter soil respiration among different forest types in a forest-steppe ecotone in north China (Wang et al., 2010). Different plant species affect C cycling differently mainly due to their distinct quantities and quality of litters (Kooch et al., 2017), root biomass (Bardgett et al., 1999; Kooch et al., 2017) and mycorrhizal fungi (Rillig et al., 2002; Vandenkoornhuyse et al., 2002), which could lead to differences in soil temperature, moisture and nutrients. These changes could further impact soil microbial activities (Rixen et al., 2008), microbial biomass (Bauhus et al., 1998; Durán et al., 2013; Tan et al., 2014), or microbial community structure (Puissant et al., 2015; Robroek et al., 2013). Snow depth under different plant species may also be different due to different interception effects by plant canopies. Because snow as an insulation layer could prevent soil freezing (Brooks

Abbreviations: C, carbon; N, nitrogen; F/B, ratio of fungi to bacteria; TDR, time domain reflectometry; PVC, polyvinyl chloride; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; TOC, total organic carbon; TN, total nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PLFA, phospholipid fatty acid; G^+, gram-positive bacteria; G^-, gram-negative bacteria; AM, arbuscular mycorrhizal; EAAs, extracellular enzymatic activities; PCA, principal component analysis; RDA, redundancy analysis

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et al., 2011), the difference in snow depth would result in distinct soil respiration. Until now, few studies have focused on the underlying mechanisms of species effects on winter soil respiration (Elberling, 2007).

The pine and larch are two major plantation species in the temperate region of China (Wang, 2006). Here we studied winter soil respiration in a mid-temperate pine plantation forest and a larch plantation forest. The objectives of our study were to assess species effects on winter soil respiration rate and to explore the underlying mechanisms. The hypothesis of our study was that winter soil respiration rate would be higher in larch forest than in pine forest because the less snow interception by larch canopy causes higher snow depth under larch, leading to higher soil temperature. We examined the differences of soil nutrients and characteristics of soil microbes in the two types of plantation forests to further explore the underlying mechanisms.

2. Materials and methods

2.1. Site description

The research site was located near Shenyang, Liaoning, northeast China (41°54′22″N, 123°35′48″E, elevation of 122 m). The research site has semi-humid temperate continental climate, with a long winter from November to the next March. The mean annual temperature is 8.3 °C with the minimum at −10.5 °C in January and the maximum at 24.8 °C in July, respectively. The mean annual precipitation is 726.2 mm, mainly falling between June and August, and the mean winter precipitation is 30 mm. The soils are sandy loam and are classified as aquic Brown soil by Chinese Soil Classification (equivalent to Typic Haplaqualf by USDA Soil Taxonomy). Both of the research forests are 20-year-old plantation forests, dominated by larch (*Larix gmelinii (Rupr.) Kuzen.*) and Chinese pine (*Pinus tabuliformis*), respectively. The distance between the two forests is about 200 m.

2.2. Experimental design

The experimental design was the completely randomized design with one-way treatment structure. There were two treatments: larch and Chinese pine species. Each treatment had five replicates (plot, 2.5 × 2.5 m each). Each plot contained 1 larch or Chinese pine tree. To mitigate the disturbance and the interactions among plots, a 50 cm buffer was used around the edge of each plot. This experiment was conducted from Dec. 18, 2014 to Apr. 1, 2015.

2.3. Microclimate monitoring

Continuous measurements of air temperature at 2 m height from the ground and soil temperature at 5 cm depth were recorded at 2-h intervals with Thermochron iButton (iButton DS1923-F5, Maxim Com.USA) in each plot. Soil volumetric water content at 0–5 cm depth was measured using time domain reflectometry 300 (TDR 300) soil moisture probe (Spectrum Technologies Inc., Plainfield; IL, USA). Snow depth was measured using a graduated stainless steel rod during soil respiration measurements.

2.4. Measurement of CO_2 efflux and soil sampling

Soil CO_2 effluxes above the snow surface were measured using a Li-6400 soil CO_2 flux system (LI-COR INC., Lincon, NE, USA). Three polyvinyl chloride (PVC) collars (10.5 cm in diameter) were inserted into the snow surface and stabilized for 24 h before soil CO_2 efflux measurement, the height of PVC collar was determined by each snow depth, keeping 3 cm empty from snow surface to the upper edge of the collar (Elberling, 2007; Wang et al., 2010). The distance of each PVC collar to the nearest tree trunk was kept to be 50 cm to avoid tree trunk effect on soil respiration. The Li-6400 soil CO_2 flux system was kept in a heated container to keep its temperature above freezing point when air temperature was below −10 °C. Measurements were conducted from 9:00 am to 11:00 am on each sampling day (once a week). In the snow free period, soil CO_2 effluxes were also measured using the Li-6400 soil CO_2 flux system. Three PVC collars (10.5 cm in diameter) were inserted 3 cm into the soil and 3 cm height from the soil surface was left to above edge of the collar (Wang et al., 2010). The measurements were also made once a week between 9:00 am and 11:00 am.

The cumulative winter soil CO_2 fluxes were calculated as follows:

$$M = \Sigma(F_{i+1} + F_i)/2 \times (t_{i+1} - t_i) \quad (1)$$

where M is cumulative CO_2 fluxes; F is CO_2 flux; i is sampling number; t is sampling time.

2.5. Soil analysis

2.5.1. Soil inorganic N and dissolved organic C and N

Soil ammonium and nitrate were extracted with 2 M KCl solution (fresh soil: 2 M KCl = 1:5, shaken for 1 h) and then measured using the indophenol-blue and phenol disulphonic acid colorimetry, respectively (Lu, 1999). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted with 0.5 M K_2SO_4 solution (fresh soil: 0.5 M K_2SO_4 = 1:4, shaken for 1 h) and then analyzed using a TOC/TN analyzer (MultiN/C3100, analytikjena, Germany). The extractions were also analyzed for soil ammonium and nitrate using the indophenol-blue and phenol disulphonic acid colorimetry, respectively (Lu, 1999). Dissolved organic nitrogen (DON) was calculated as DON = TDN − (ammonium + nitrate), where the ammonium and nitrate contents were extracted using 0.5 M K_2SO_4 solution.

2.5.2. Soil microbial biomass

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Briefly, 20 g fresh soil was immediately extracted with 0.5 M K_2SO_4 (fresh soil: 0.5 M K_2SO_4 = 1:4, 200 rpm shaken for 1 h). Another 20 g fresh soil was fumigated with chloroform for 24 h in the dark and then was extracted with 0.5 M K_2SO_4 (fresh soil: 0.5 M K_2SO_4 = 1:4, 200 rpm shaking for 1 h). DOC and TDN content in both fumigated and unfumigated extracts were analyzed using a TOC/TN analyzer (MultiN/C3100, analytikjena, Germany).

MBC and MBN were calculated as follows:

$$MBC = E_C/0.45 \text{ and } MBN = E_N/0.54 \quad (2)$$

where E_C is the difference between fumigated and unfumigated DOC content; E_N is the difference between fumigated and unfumigated TDN content; 0.45 and 0.54 are two conversion factors for MBC and MBN, respectively (Brookes et al., 1985; Vance et al., 1987).

2.5.3. Soil microbial community structure

Soil microbial community structure was measured by analyzing soil microbial phospholipid fatty acid (PLFA) composition. PLFAs were analyzed using a modified Bligh-Dyer method (Wang et al., 2014). Briefly, total lipids were extracted from 4 g freeze-dried soil for 2 h with phosphate buffer: chloroform: methanol (0.8:1:2, v/v/v), and then the phospholipids were separated from other lipids on a silicic acid column. 19:0 methyl nonadecanoate (Sigma-Aldrich, USA) was added as an internal standard to quantify the PLFAs. Samples were analyzed by gas chromatograph (Agilent 7890, Agilent technologies, USA) with Ultra-2 column. We used the PLFAs i15:0, a15:0, i16:0, i17:0 and a17:0 as indicators of Gram-positive bacteria (G^+); 16:1ω7c, 17:1ω8c, 18:1ω7c, cy17:0 and cy19:0 were used as indicators for Gram-negative bacteria (G^-); 14:0, 15:0, 16:0 and 18:0 were used to indicate general bacteria; 10Me16:0, 10Me17:0 and 10Me18:0 were used as indicators for actinomycetes; both 18:1ω9c and 18:2ω6, 9c were used to indicate fungi; 16:1ω5c was used as the indicator for Arbuscular mycorrhizal (AM)

fungi (Kaiser et al., 2010a, 2010b; Frostegård et al., 2011).

2.5.4. Soil extracellular enzymatic activities

Four soil extracellular enzymatic activities (EAAs) were determined using fluorogenic substrates based on the method described by Saiya-Cork et al. (2002). The enzymes and substrates were: (1) α-glucosidase (EC 3.2.1.20) assayed with 4-methylumbelliferyl-α-D-glucosidase; (2) β-glucosidase (EC 3.2.1.21) assayed with 4-methylumbelliferyl-β-D-glucosidase; (3) cellobiohydrolase (EC 3.2.1.91) assayed with 4-methylumbelliferyl-β-D-cellobioside; (4) xylosidase (EC 3.2.1.37) assayed with 4-methylumbelliferyl-β-D-xylopyranoside. In order to ensure that the enzyme assays were under saturating substrate concentrations, the concentration of fluorogenic substrates was optimized before assaying soil enzyme activity (German et al., 2011). The optimized concentrations of fluorogenic substrates for α-glucosidase, β-glucosidase, cellobiohydrolase and xylosidase were 0.677, 0.677, 1.001, 0.617 mg L^{-1}, respectively. Briefly, soil suspensions were prepared with 2.00 g fresh soil mixed into 100 ml sodium acetate buffer (pH = 5.0) and stirred for 1 min using a magnetic stir plate. 200 μL soil suspensions and 50 μL substrates were added into assay 96-well microplates. 200 μL soil suspensions and 50 μL sodium acetate buffer (pH = 5.0) were added into blank 96-well microplates. 200 μL soil suspensions and 50 μL 4-methylumbelliferyl were added into quench 96-well microplates. 200 μL sodium acetate buffer (pH = 5.0) and 50 μL 4-methylumbelliferyl were added into standard 96-well microplates. Then, all 96-well microplates were incubated in the dark for 4 h at 25 °C. The reaction was terminated by adding 10 μL 0.5 mol L^{-1} NaOH. The fluorescence intensity was measured using a fluorimetric microplate reader (Synergy H1, BioTek, Winooski, VT), with excitation and emission wavelengths of 365 nm and 450 nm, respectively. A standard of time of 1 min was used between the addition of NaOH and the measurement of fluorescence. Soil EEAs were calculated according to the method described by German et al. (2011).

2.5.5. Basic soil characteristics

Soil texture was measured by the pipette-sedimentation method (Gee and Bauder, 1986). Bulk density was determined by the core method (Burke et al., 1986). Soil gravimetric water content was determined by drying soil at 105 °C for 48 h. Soil pH was measured in deionized water (1:2.5 soil solution) using a pH meter (E-201-C, Leici, China). Soil total C and total N were analyzed using an elemental analyzer (Vario Macro Elementar, Germany).

2.6. Statistical analyses

A one-way ANOVA (Duncan's test at $p < .05$) and repeated measures ANOVA (LSD's test at $p < .05$) were used to analyze the effects of different vegetation species on environmental factors, soil nutrients, soil microbial characteristics and winter soil respiration. The relationships between winter soil respiration and microbial parameters and environmental variables were analyzed by linear regressions. Principal component analysis (PCA) and redundancy analysis (RDA) were performed in R Studio 3.2.0 (using the R packages vegan) to evaluate changes in microbial community composition and soil EEAs among plots with different plant species. A permutation test (999 permutations) was used to test the significance of soil EEAs, soil microbial community structure and environmental variables. All other statistical analyses were carried out in SPSS 19.0 (SPSS Inc., IL, USA).

3. Results

3.1. Basic soil characteristics

Soil total carbon (TC), C:N and litter layer thickness in larch plot were 28.67%, 11.26% and 48.78% lower than those in Chinese pine plot, respectively ($p < .05$, Table 1). No significant difference in soil texture, pH, bulk density and total nitrogen (TN) was found between these two types of plantation forests.

Table 1
Basic soil characteristics in studied larch and Chinese pine forests.

		Larch	Chinese pine
Soil texture	Sand (%)	44.97 ± 1.01a	43.36 ± 1.53a
	Silt (%)	24.90 ± 0.42a	26.77 ± 0.85a
	Clay (%)	30.13 ± 0.52a	29.88 ± 0.41a
pH		4.67 ± 0.02a	4.64 ± 0.03a
Bulk density (g cm^{-3})		1.19 ± 0.02a	1.14 ± 0.07a
Total carbon (%)		2.04 ± 0.08a	2.86 ± 0.27b
Total nitrogen (%)		0.18 ± 0.01a	0.22 ± 0.02a
C:N		11.98 ± 0.09a	13.50 ± 0.22b
Litter layer thickness (cm)		2.10 ± 0.74a	4.10 ± 0.95b

Values were reported as mean ± standard errors. Different lowercase letters denote significant differences at $p < .05$.

3.2. Air temperature and soil temperature

Soil temperature in both plots had the same temporal pattern as air temperature during the whole study period (Fig. 1). Soil temperature ranged from −4 °C to 0.5 °C during the snow cover period and from 0.5 °C to 6 °C during the snowmelt period, respectively (Fig. 1b). There was no significant difference in soil temperature between the two plots.

3.3. Snow depth, soil volumetric water content and soil respiration

Snow depth varied from 0 to 83.4 mm in larch plot and from 0 to 34.0 mm in Chinese pine plot, with the mean at 63.25 mm and 14.67 mm, respectively (Fig. 2a). Compared to Chinese pine forest, larch forest had 4.31 times deeper snow depth ($p < .05$). Soil volumetric water content ranged from 5.68% to 18.38% in larch forest and from 3.32% to 11.44% in Chinese pine forest, with the mean at 8.80% and 5.51%, respectively (Fig. 2b). The statistical analysis results showed that soil volumetric water content in larch plot was 59.70% higher compared with that in Chinese pine plot ($p < .05$). Temporal variations of winter soil CO_2 flux revealed that winter soil respiration was highest during the snowmelt period (Fig. 2c). Winter soil CO_2 flux was between 0.09 and 0.71 μmol $m^{-2} s^{-1}$ in larch plot and between 0.16 and 1.09 μmol $m^{-2} s^{-1}$ in Chinese pine plot, with the mean at 0.25 and 0.45 μmol $m^{-2} s^{-1}$, respectively. The statistical analysis results indicated that winter CO_2 flux was 43.50% lower in larch plot than that in Chinese pine plot ($p < .05$). Likewise, cumulative winter CO_2 fluxes and annual CO_2 fluxes in larch plot (25.92 g C m^{-2} and 479.68 g C m^{-2}, respectively) were 44.13% and 22.95% lower than those in Chinese pine plot (46.39 g C m^{-2} and 622.53 g C m^{-2}), respectively ($p < .05$) (Supplementary Fig. 2). The contribution of winter CO_2 fluxes to annual CO_2 fluxes were 5.40% and 7.5% in larch forest and Chinese pine forest, respectively.

3.4. Soil inorganic N and dissolved organic C and N

During the whole experimental period, soil nitrate and ammonium contents were all significantly higher in larch plot (25.66–31.89 mg kg^{-1} and 6.44–10.53 mg kg^{-1}, respectively) compared to those in Chinese pine plot (4.16–9.76 mg kg^{-1} and 2.99–8.71 mg kg^{-1}, respectively), except for soil nitrate during the snowmelt period, which had no significant difference between the two types of forests (Fig. 3a, b). For DON, larch plot had dramatically lower DON than Chinese pine plot by 28.16–33.16% (Table 2, $p < .05$). Soil DOC was significantly higher in larch plot than in Chinese pine plot during the snowmelt period ($p < .05$), but was not significantly different during the snow cover period (Table 2).

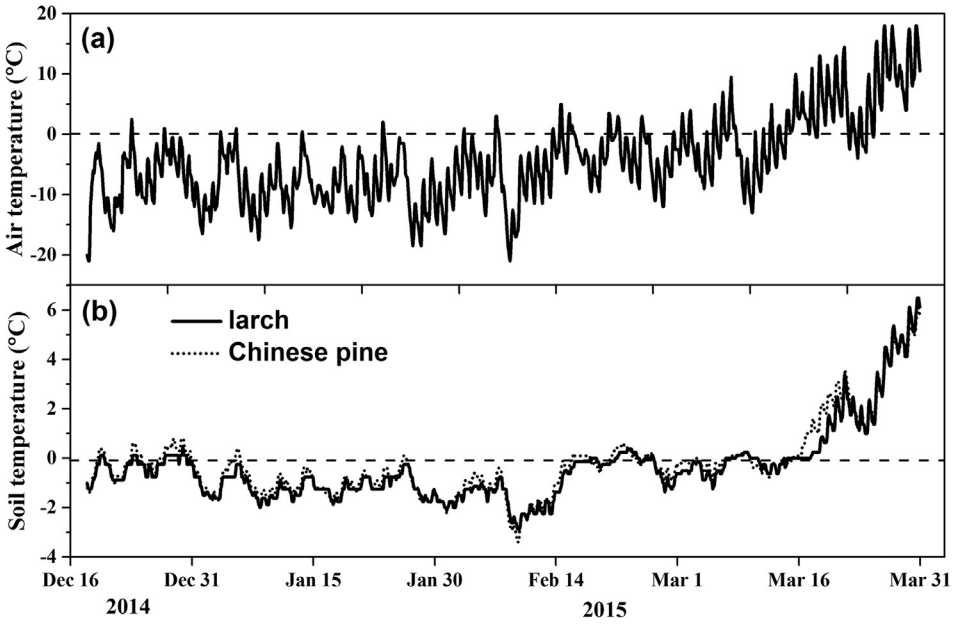

Fig. 1. Air temperature at 2 m aboveground (a) and soil temperature at 5 cm depth (b) in Chinese pine plot (short dot line) and larch (blank line) plot.

3.5. Soil microbial biomass C and N

Soil microbes were more abundant during the snow melt period than during the snow cover period in both types of forests as indicated by MBC and MBN (Table 2). The statistical analysis results indicated that soil MBC and MBN were 34.40% and 29.56% smaller in larch forest than those in Chinese pine forest during the snow cover period, respectively ($p < .05$), but no observable difference was found during the snowmelt period (Table 2). Soil MBC:MBN was not statistically significantly different between the two types of forests during the whole experimental period (Table 2).

3.6. Soil microbial community structure

Soil microbial communities were dramatically different in different types of forests (Table 2). During the snow cover period, the abundance of G^+ bacteria in larch plot was significantly larger compared to that in Chinese pine plot ($p < .05$), but the abundances of G^- bacteria and total fungi in larch plot were significantly smaller compared to those in Chinese pine plot ($p < .05$). During the snowmelt period, the

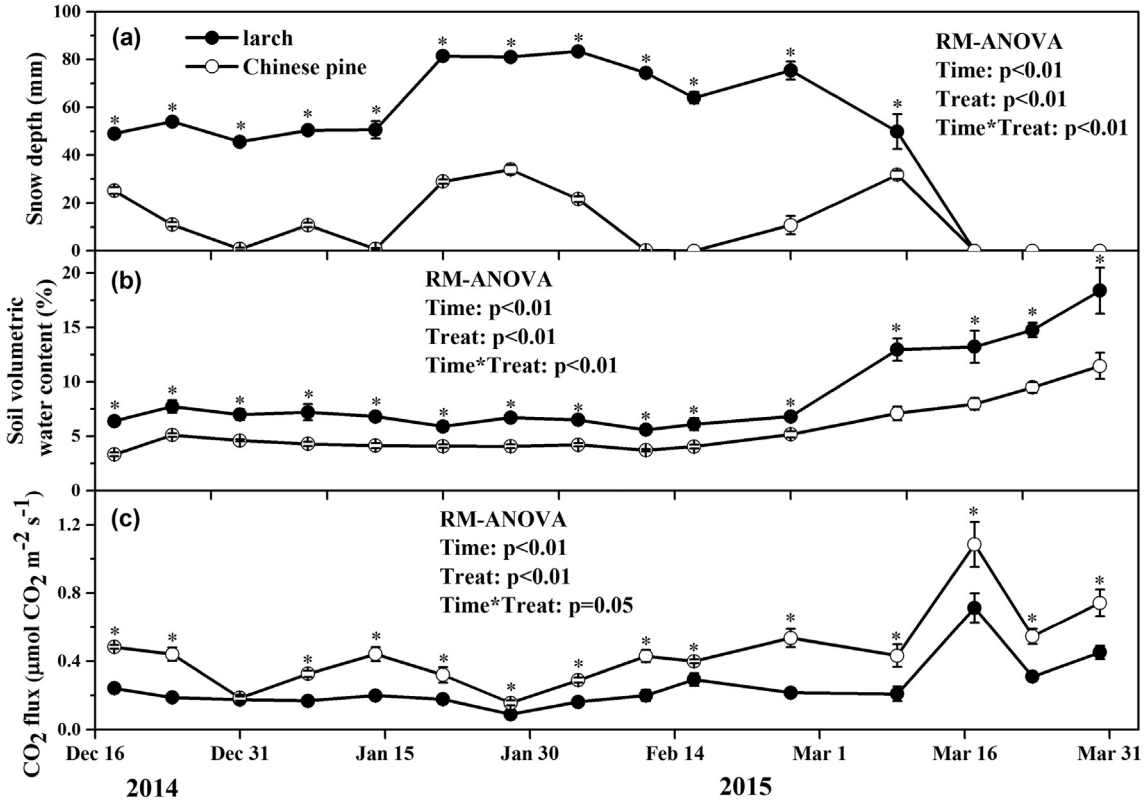

Fig. 2. Temporal variations of snow depth (a), soil volumetric water content (b) and CO_2 flux (c) in larch and Chinese pine forests. The vertical bars represent standard errors (n = 5). Asterisk stands for significant differences ($p < .05$).

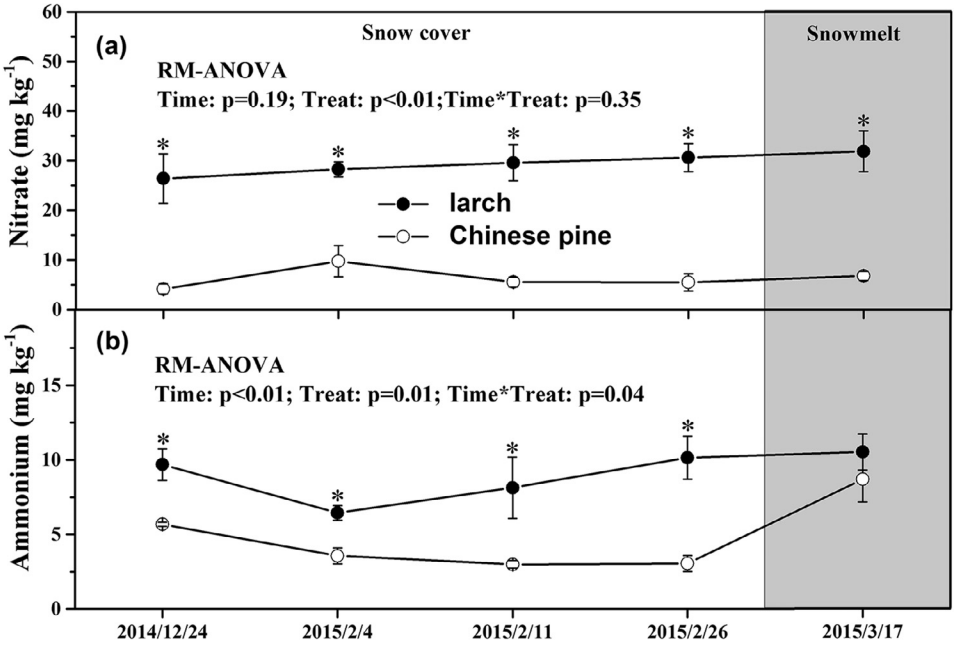

Fig. 3. Effects of different plant species on the concentrations of soil nitrate (a), ammonium (b). The shaded areas denote the experimental snowmelt period. The vertical bars represent standard errors (n = 5). Asterisk stands for significant differences ($p < .05$).

abundance of actinomycete was significantly higher in larch plot than in Chinese pine plot ($p < .05$), but the abundances of total fungi and AM fungi were significantly lower in larch forest than in Chinese pine forest ($p < .05$). The abundance of total bacteria was not statistically significantly different between two types of forests during the whole study period. The F/B ratio in Chinese pine forest was significantly higher than that in larch forest during the whole study period (Table 2, $p < .05$). G^+/G^- was significantly higher in larch plot than in Chinese pine plot during the snow cover period ($p < .05$), but had no apparent difference during the snowmelt period between the two types of forest (Table 2).

The PCA based on PLFA data explained 87.53% of total soil microbial community structure variance on the first two axes (52.66% and 34.87% for PC1 and PC2, respectively, Fig. 4a) during the snow cover period and explained 73.80% of total soil microbial community structure variance on the first two axes (53.24% and 20.56%, for PC1 and PC2, respectively Fig. 4b) during the snow melt period. Examination of the PCA loading indicated that PLFAs 18:1ω7c, 16:0, 18:1ω9c, c19:0 and PLFAs 17:1ω8c, 18:1ω7c, 18:2ω6,9c, 18:1ω9c mainly contributed to PC1 during the snow cover and snow melt period, respectively (Fig. 4). Soil microbial community structure composition was significantly different between larch and Chinese pine plots on PC1 ($p < .05$) during the whole study period, but no marked difference was observed on PC2.

3.7. Soil extracellular enzymatic activities

Soil β-glucosidase and cellobiohydrolase activities in larch plot were 16.78–41.10% and 30.94–53.76% smaller than those in Chinese pine plot, respectively (Fig. 5b, c, $p < .05$). Soil α-glucosidase activity in larch plot was also lower than that in Chinese pine plot, although the effect was not statistically significant (Fig. 5a). However, no marked difference in soil xylosidase activity was found between the two types of forests (Fig. 5d).

Table 2
Dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MBC:MBN, and phospholipid fatty acid (including gram-positive bacteria (G^+), gram-negative bacteria (G^-), total bacterial, total fungi, actinomycete, arbuscular mycorrhizal (AM) fungi, ratio of fungi to bacteria (F/B), and ratio of G^+ to G^- (G^+/G^-)) in larch and Chinese pine forests during the snow cover period (Feb. 4, 2015) and snow melt period (Mar. 17, 2015). p values of repeated measures ANOVA on the effects of different plant species and time on these variables were shown in the last three columns.

		Snow cover period		Snow melt period		Time	Treat	Time*Treat
		Larch	Chinese pine	Larch	Chinese pine			
DOC (mg kg^{-1})		280.5 ± 8.0	285.0 ± 9.6	329.5 ± 11.5	279.7 ± 9.6	0.09	0.02*	0.05*
DON (mg kg^{-1})		14.0 ± 0.4	21.0 ± 1.0	19.8 ± 0.6	27.5 ± 1.6	< 0.01**	< 0.01**	0.72
MBC (mg kg^{-1})		167.2 ± 5.7	254.8 ± 8.6	356.3 ± 26.1	310.3 ± 16.0	< 0.01**	0.25	< 0.01**
MBN (mg kg^{-1})		14.4 ± 1.2	20.4 ± 0.9	28.1 ± 1.4	30.5 ± 1.5	< 0.01**	0.01*	0.17
MBC:MBN		11.7 ± 1.0	12.1 ± 0.5	16.9 ± 1.1	15.2 ± 2.1	0.01*	0.65	0.41
PLFA nmol g^{-1}	G^+	21.9 ± 0.9	17.2 ± 1.1	21.9 ± 0.3	20.3 ± 0.8	0.08	0.01*	0.08
	G^-	13.9 ± 1.3	20.3 ± 0.5	21.8 ± 0.4	20.5 ± 0.6	< 0.01**	0.03*	< 0.01**
	Total bacterial	48.6 ± 3.0	54.2 ± 1.6	62.1 ± 0.9	59.6 ± 2.6	< 0.01**	0.54	0.07
	Total fungi	7.1 ± 0.6	10.5 ± 1.2	7.9 ± 0.3	9.8 ± 0.7	0.95	0.02*	0.25
	Actinomycete	8.1 ± 0.5	7.7 ± 0.5	9.8 ± 0.3	8.4 ± 0.4	0.01*	0.12	0.16
	AM fungi	1.2 ± 0.1	1.4 ± 0.1	1.1 ± 0	1.3 ± 0.1	0.18	0.04*	0.54
F/B		0.14 ± 0.01	0.19 ± 0.02	0.13 ± 0	0.17 ± 0.01	< 0.01**	0.02*	0.45
G^+/G^-		1.61 ± 0.11	0.85 ± 0.07	1.01 ± 0.03	0.99 ± 0.03	< 0.01**	< 0.01**	< 0.01**

** Highly significant ($p < .01$).
* Significant ($p < .05$).

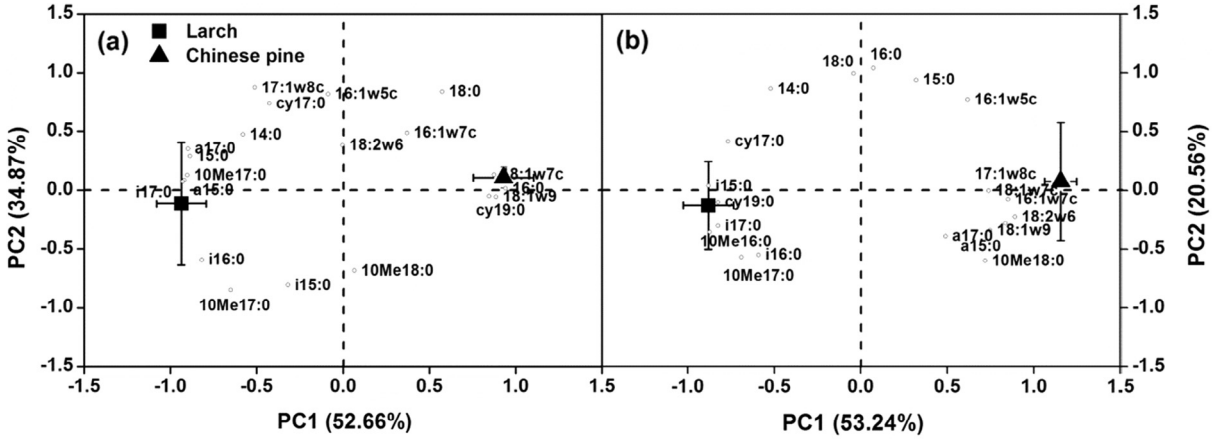

Fig. 4. Principal component analysis (PCA) of soil microbial community structure in larch and Chinese pine forests during the snow cover period (Feb. 4, 2015, a) and snow melt period (Mar. 17, 2015, b). The horizontal and vertical bars represent standard errors (n = 5).

3.8. Redundancy analysis of soil microbial community structure, soil extracellular enzymatic activities and environmental variables

RDA indicated that soil microbial community structure was significantly explained by MBC ($r^2 = 0.693$, $p < .001$), MBN ($r^2 = 0.490$, $p = .004$), DOC ($r^2 = 0.568$, $p = .005$), DON ($r^2 = 0.386$, $p = .014$), TC ($r^2 = 0.541$, $p = .002$), C:N ($r^2 = 0.771$, $p < .001$), and soil moisture ($r^2 = 0.770$, $p < 0.001$). Soil EEAs was significantly explained by TC ($r^2 = 0.428$, $p = .013$), C:N ($r^2 = 0.648$, $p < .001$), PC1 ($r^2 = 0.632$, $p < .001$) and total fungi abundance ($r^2 = 0.352$, $p = .017$) (Fig. 6). Other factors had no obvious correlations with soil microbial community structure or soil EEAs.

3.9. Relationships between CO_2 flux and microbial parameters

Correlation analysis on all data of the two sites showed that soil CO_2 flux was significantly positively correlated with MBC and MBN during the snow cover period, but not during the snow melt period. Soil CO_2 flux had significantly positive correlations with fungi abundance, F/B, β-glucosidase, and cellobiohydrolase during both the snow cover and the snow melt periods (Fig. 7). Conversely, although a significant relationship between CO_2 flux and soil temperature existed in each forest, when data of the two sites were lumped, soil CO_2 flux did not correlate with soil temperature any more during either the snow cover or the snow melt period (Supplementary Fig. 1).

4. Discussion

We found that mean winter soil respiration rate and cumulative winter soil respiration in Chinese pine forest (0.45 μmol $m^{-2} s^{-1}$; 46.39 g C m^{-2}) was higher than in larch forest (0.25 μmol $m^{-2} s^{-1}$; 25.92 g C m^{-2}) in the studied mid-latitude forest plantation ecosystems. Previous studies reported that average winter CO_2 efflux ranged from 0.32 μmol $m^{-2} s^{-1}$ to 0.71 μmol $m^{-2} s^{-1}$ in evergreen forests (Hubbard

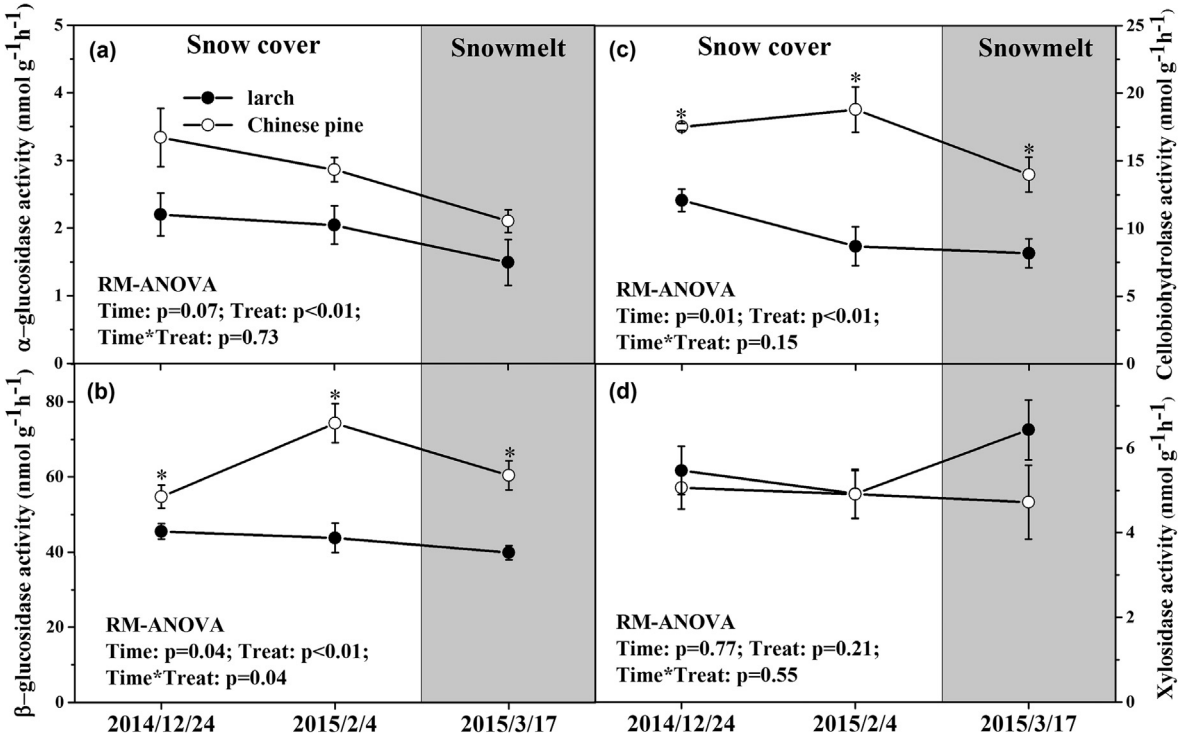

Fig. 5. Effects of different plant species on α-glucosidase (a), β-glucosidase (b), cellobiohydrolase (c) and xylosidase (d) activities. The vertical bars represent standard errors (n = 5). Asterisk stands for significant differences ($p < .05$).

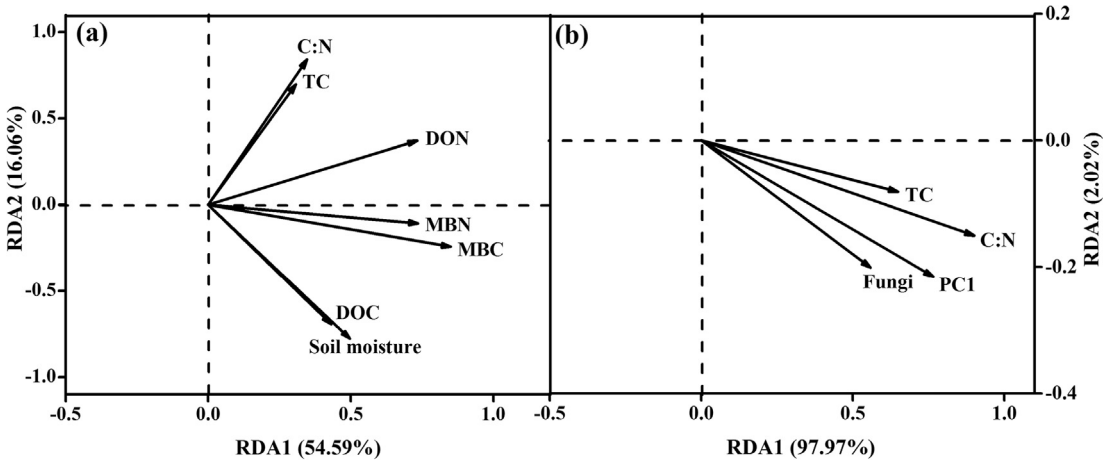

Fig. 6. Redundancy analysis (RDA) of soil microbial community structure (a), soil extracellular enzymatic activities (EEAs) (b) and environmental variables. PC1: principal component analysis of soil microbial community structure on the first axis; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; C:N: ratio of total carbon to total nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; TC: total carbon.

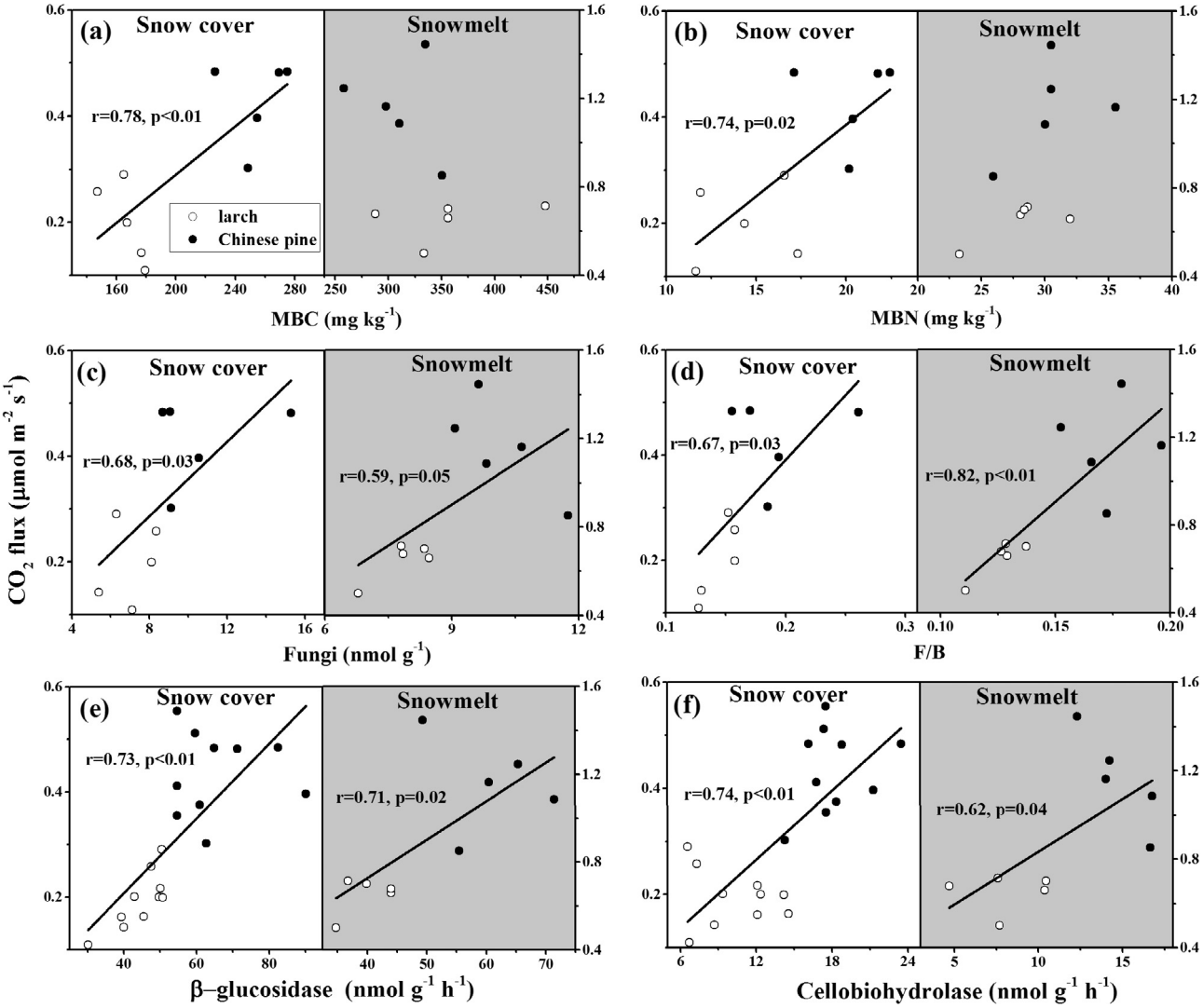

Fig. 7. Relationships between CO_2 flux and microbial biomass carbon (MBC) (a), microbial biomass nitrogen (MBN) (b), fungi abundance (c), ratio of fungi to bacteria (F/B) (d), β-glucosidase (e), and cellobiohydrolase (f) in both larch (white) and Chinese pine (black) forests during the snow cover and snow melt periods. The relationships were established using data of both forests in each period.

et al., 2005; McDowell et al., 2000; Schindlbacher et al., 2007; Sommerfeld et al., 1993; Sommerfeld et al., 1996), which were generally higher than values in deciduous forests with a range from 0.16 μmol $m^{-2} s^{-1}$ to 0.37 μmol $m^{-2} s^{-1}$ (Mariko et al., 2000; Suzuki et al., 2006; Winston et al., 1997; Wang et al., 2010; Zimov et al., 1993; Zimov et al., 1996). Therefore, our results are within previously reported ranges and suggested significant plant species effects on soil respiration (Yohannes et al., 2011). We then investigated the underlying mechanisms of the higher winter soil respiration in Chinese pine forest than in larch forest.

First, the differences in snow depth could not explain the differences in winter soil respiration between the two sites. Although our hypothesis of higher snow depth in larch forest (average, 6.3 cm) than in Chinese pine forest (average, 1.5 cm) was supported, our hypothesis of higher soil respiration in larch forest was not supported. Due to the insulation effects of snow cover (Brooks et al., 2011), it is expected that larch forest would have higher soil temperature, and thereby higher winter soil respiration (Aanderud et al., 2013; Li et al., 2008; Wang et al., 2010). Two reasons may have contributed to the observed opposite pattern. First, litter layer played a role in regulating soil temperature. Larch forest had thinner litter layer (averaged 2.1 cm) than Chinese pine forest did (averaged 4.1 cm). If we added up the depths of both litter layer and snowpack, the difference between the two types of forests got much smaller. Second, the snowpack did not effectively decouple soil temperatures from colder air temperatures in our study site due to the thin snow layer (0–8.3 cm, Fig. 2a). Previous studies also reported limited insulation effects when snow depth was less than 30 cm (Cline, 1997; Steinweg, et al., 2008; Wang et al., 2010). Our research site is located in mid-latitude region where snow pack does not usually reach more than 20 cm (Peng et al., 2010), and hence, although the snow depth was different between the two types of forests, soil temperature was not different (Fig. 1b). Moreover, we did find significantly higher soil moisture in larch forest than in Chinese pine forest (Fig. 2b), probably due to the thicker snow depth in larch forest. Although previous studies found higher soil moisture would stimulate soil respiration when soil water content was below optimum (Yohannes, et al., 2011) and our study forests are relatively dry, it is not the case in our study. Therefore, the effects of soil moisture on soil respiration were either minor or counterbalanced by other factors in our studied plantation forests.

Second, differences in soil N content could not explain the differences in winter soil respiration between the two sites either. We found both soil ammonium and nitrate concentrations were higher in larch forest than in Chinese pine forest (Fig. 3). Previous studies found higher soil N availability would lead to higher soil respiration (Yan et al., 2011), while we found the opposite. Although this might be due to more uptake of soil inorganic N in Chinese pine forest than in larch forest, we believe other factors might have overridden the effects of soil nutrients on soil respiration in different species types of forests in our study.

Finally, differences in soil microbes were able to explain the differences in winter soil respiration between the two sites. We found Chinese pine forest had higher MBC, MBN, fungi abundance (Table 2) and β-glucosidase and cellobiohydrolase activities (Fig. 5) than larch forest. What's more, the significantly positive relationships between soil CO_2 flux and MBC, MBN, fungi abundance, F/B, β-glucosidase, and cellobiohydrolase (Fig. 7) provided further evidence. Although the abundance of actinomycetes was higher in larch forest during the snowmelt period (Table 2), its relative proportion in total microbial biomass was low (10%). Fungi are believed to be important for the decomposition of recalcitrant C (De Boer et al., 2005; Meidute et al., 2008; Schneider et al., 2012), and therefore the higher fungi abundance was beneficial to soil C emission.

We believe two major reasons could explain the differences in winter soil respiration between the two types of forests. First, soil microbial respiration is an important component of total soil respiration, especially in winter, and the contribution of microbial respiration to total respiration was reported to be 50–87% (Puissant et al., 2015; Ruehr and Buchmann, 2010; Schindlbacher et al., 2007). Microbial respiration process is a common process which is performed by most microorganisms (Sun et al., 2016). Therefore, higher abundance of soil microbes usually induces higher microbial respiration rate. Both β-glucosidase and cellobiohydrolase enzymes are necessary in the decomposition of labile and recalcitrant C resources (Bandick and Dick, 1999). Higher abundances of these enzymes (Fig. 5) were consistent with higher MBC and fungi in Chinese pine forest than in larch forest. The positive correlations between soil respiration and MBC and MBN during both the snow cover and the snow melt periods in both forests (Supplementary Fig. 4) pointed to the importance of soil microbes. In addition, the higher DOC and DON may also be a potential reason of the higher microbial biomass and soil respiration in Chinese pine forest than in larch forest. Second, autotrophic respiration in Chinese pine forest may also be higher than in larch forest. The limitation of this study is that we did not differentiate root respiration from microbial respiration. A previous study indicated that evergreen forests had a longer photosynthesis period and more root biomass and root exudates than deciduous forests, which may result in higher root respiration (Wang et al., 2006). Future studies should differentiate root respiration and microbial respiration to better understand the differences in soil respiration between the two sites.

5. Conclusions

In our study, winter soil respiration rate was higher in Chinese pine forest than that in larch forest although the climate and basic soil characteristics were the same between the two forests. This phenomenon was not explained by the differences in snow depth or soil inorganic N, but was explained by the higher soil microbial biomass, fungi abundance, F/B ratio, and EEAs in Chinese pine forest than in larch forest. Our study suggested that soil microbes may be more important than soil nutrients to soil respiration, especially in winter. Additionally, different plant respiration and rhizosphere activities between the two types of forests could also contribute to the different soil respiration and should be further studied. The similar soil temperature between the two sites suggested that the insulation effect of the litter layer should not be ignored while studying the effect of snow cover on winter soil respiration, especially when the snow depth is less than 30 cm. The variations in winter soil respiration due to forest management could have important consequences on soil C cycling and should be taken into account for accurately assessing and modeling the global C budgets in the future.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.11.029.

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