



Effects of experimental warming on soil N transformations of two coniferous species, Eastern Tibetan Plateau, China

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ABSTRACT

Previous research on the effects of tree species on soil processes has focused primarily on the role of leaf litter inputs and relatively few studies have considered the importance of plant roots and their associated ecological processes, especially under climate change. We therefore conducted an experiment to compare the impacts of two coniferous species via roots on soil N transformations and their responses to experimental warming using infrared heaters in the Eastern Tibetan Plateau. The infrared heater on average enhanced both air temperature and soil temperature by 2.0 °C and 3.7 °C, respectively. Warming did not affect soil organic C (SOC), total N (TN), microbial biomass C (MBC) and N (MBN), or their ratios (MBC/MBN) in both coniferous species plots. Effects of experimental warming on soil N availability varied with tree species and sampling dates. There were higher NO₃⁻ and lower NH₄⁺ concentrations in the *Picea asperata* than in the *Abies faxoniana* plots irrespective of warming treatment or sampling date, possibly caused by higher gross nitrification and denitrification rates in the *P. asperata*. Experimental warming significantly increased the net mineralization, net nitrification and denitrification rates on most sampling times in both species plots. Responses of gross nitrification to experimental warming significantly differed between the two species, and depended strongly on seasons. Gross nitrification and denitrification rates were markedly greater in the *P. asperata* than in the *A. faxoniana* plots, with *P. asperata* being more sensitive than *A. faxoniana* in response to experimental warming. Differences in the root morphology (i.e., root length, root type) and activity (i.e., root exudation, fine root vigor) between the two species could be largely responsible for the variation in the soil N cycling and its response to experimental warming. Taken together, our results indicate that tree species can differ in their effects on soil transformations and nutrient availability via roots and associated microbial processes. Further research is required regarding the exact mechanisms of tree species effects via roots on soil processes and function under climate change.

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1. Introduction

Global air temperatures are predicted to increase 1.8–4.0 °C over this century, with a greater warming occurring in the higher latitudinal and altitudinal ecosystems (IPCC, 2007). Warmer air temperatures would likely result in warmer soil temperatures which could, in turn, largely affect the biogeochemical processes of soils in these ecosystems, such as soil respiration and decomposition of soil organic matter (Rustad et al., 2001; Kirschbaum, 2004),

and hence the ecological consequences on soil processes arising from global warming have become the very important issues of global change research.

It has been widely recognized that tree species can exert a large influence on soil environment and its microflora and that these interactions in turn have considerable impact on plant growth and functioning of terrestrial ecosystems (Hobbie, 1992). Individual plant characteristics, such as life-span, biomass allocation, physiological traits and tissue chemical composition can play important roles in regulating soil processes such as soil organic matter decomposition and nutrient mineralization in forest ecosystems (Eviner, 2004). Previous research on the effects of tree species on soil processes has focused primarily on the role of leaf litter inputs (Binkley and Menyailo, 2005). Relatively few studies, however, have considered the importance of plant roots and their associated

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ecological processes (Ushio et al., 2008; Cheng, 2009), and thus those processes influenced by plant roots have been become one of the most important but least understood ways in which plants may affect nutrient cycling, especially under climate change (Jones et al., 2009).

Since tree species are very different with respect to root morphology and physiology (such as root biomass, mycorrhizal association, root type, quantity and quality of root exudation) as well as their nutrient requirements, plant effects on soil processes would be likely to vary with tree species (Richardson et al., 2009). Recent recognition of the importance of belowground C inputs has highlighted the need for more information on how tree species influence soil nutrient cycling via roots and associated ecological processes (Phillips and Fahey, 2006; Koranda et al., 2011), which is fundamental for characterizing nutrient acquisition capacity of different tree species. To date, relationships between tree species and soil N cycling through root–soil interactions are poorly understood (Kuzyakov, 2002; Drake et al., 2011). Therefore, there is still a strong need for more studies on N cycling under different climates and tree species for providing a more comprehensive view of belowground ecological processes.

The subalpine coniferous forest ecosystems in the Eastern Tibetan Plateau located at the transition zone from Qinghai–Tibet plateau to Sichuan basin could be very sensitive to global climate change, with important consequences for the global C and N balance (Wang et al., 2003). The magnitude of warming on the Tibetan Plateau is projected to be large relative to many other regions and the soils on the Tibetan Plateau contain large amounts of SOM (Jiang et al., 2009). Nutrient availability, mainly N, is the primary limiting factor for plant growth and productivity of subalpine coniferous forest ecosystems. Comparing response differences of soil N dynamics between tree species to warming could more accurately predict the impacts of global warming on subalpine coniferous forest ecosystems. Hence, in this study, we conducted an experiment to examine the effects of two coniferous species (i.e., *Picea asperata* and *Abies faxoniana*) via roots on soil nutrient availabilities and N transformations and their responses to experimental warming. We also investigated root morphological and physiological characteristics of the two coniferous species between treatments. These two tree species were chosen because: (1) both are widely distributed and important in subalpine coniferous ecosystems in western Sichuan; (2) the two species are both sympatric and co-occur naturally within subalpine ecosystems; (3) *A. faxoniana* is a dominant species in natural forests, whereas *P. asperata* is one of the most widely used species for reforestation in this region; and (4) microhabitats for the two species have slightly different ecological characteristics. *A. faxoniana* has a slightly higher tolerance to shade, and it is frequently found in relatively wetland areas. By contrast, *P. asperata* is less tolerant to shade and is normally distributed in more sunny exposed and drier habitats. We hypothesized that increasing temperature would stimulate the soil N transformations and nutrient availability, but that such effects would vary with tree species due to differences in intrinsic biological properties. This study is the first, to our knowledge, to investigate effects of species differences on N cycling and their responses to experimental warming.

2. Materials and methods

2.1. Experiment design

The experiment was conducted in the Maoxian Ecological Station of the Chinese Academy of Sciences, Sichuan Province, China ($31^{\circ} 41' N$, $103^{\circ} 53' E$, 1820 m a.s.l.) where mean annual temperature, precipitation and evaporation are $8.9^{\circ}C$, 919.5 mm,

and 795.8 mm, respectively. Our experiment followed Wan et al. (2002) in using $165\text{ cm} \times 15\text{ cm}$ infrared heaters (Kalgo Electronics Inc, Bethlehem, PA, USA) to generate an artificially warmed environment. There were five pairs of $4\text{ m} \times 2\text{ m}$ plots (a warmed plot and a control plot). The indigenous soil of the all plots until the depth of 50 cm was replaced by the sieved topsoil (mesh size of 1 cm) from a coniferous forest, and any visible living plant material (i.e. roots) was manually removed from the sieved soil. The soil was classified as the mountain brown soil series (Chinese taxonomy), with the pH, total N, soil organic C and bulk density of 5.55, 4.54 g kg^{-1} , 78.04 g kg^{-1} and 0.887 g cm^{-3} , respectively. The warmed plot was heated by an infrared heater suspended 1.5 m above the middle of the plots. The infrared heater had a radiation output of approximately 100 W m^{-2} and its warming effect on soil temperature was spatially uniform in the warmed plots according to previous similar study (Wan et al., 2002). In the control plots, one “dummy” heater with the same shape and size as the infrared heater was suspended 1.5 m above the control plots to simulate the shading effects of the infrared heater. The control and the warmed plots were separated by 5 m to avoid heating the control plots.

Uniform four-year-old *P. asperata* and *A. faxoniana* seedlings from a local nursery were selected based on plant height and stem base diameter. In March 2007, twenty healthy seedlings per species were randomly planted on a common soil within each subplot. Artificial night warming was conducted from April 2007 to September 2011. During the experimental period, the warmed plots were heated from 7:00 pm to 7:00 am (12 h day^{-1}) on year-around, and all the plots were watered frequently as needed. Moreover, all litter within the plots was removed periodically to examine the “pure” effects of tree species via roots and associated microbial processes.

2.2. Measuring variables

Microclimate monitoring Air temperature (at the height of 20 cm above the ground) and soil temperatures (5 cm depth) were measured in four pairs of plots at 60 min intervals during the experiment period using DS1921 G Thermochron iButton data loggers (DS1921 G-F5, Maxim Integrated Products, Dallas Semiconductor Inc., Sunnyvale, California). Soil moisture content was measured in soil core samples (0–10 cm) collected twice monthly at all plots during the experiment period. The soil samples were dried for 12 h at $105^{\circ}C$ to determine the soil moisture.

2.3. Soil sampling

Soil samples were collected from the topsoil (0–15 cm) in early May, mid-July and late September of 2010. Five cores (3 cm in diameter, 15 cm deep) were randomly taken at each plot. The collected soil cores were mixed to get one composite fresh sample for each subplot, and delivered immediately to the laboratory for further analysis. Each composite sample was passed through a sieve (2 mm diameter), and any visible living plant material was manually removed from the sieved soil. The sieved soil was kept in the refrigerator at $4^{\circ}C$ and processed within one week for water content, organic carbon, total nitrogen, MBC, MBN, nitrate (NO_3^- -N), ammonium (NH_4^+ -N) and extractable inorganic N (N_{in} , NH_4^+ -N plus NO_3^- -N).

2.4. Soil analyses

Available N (ammonium and nitrate) was extracted with 2 M KCl extracting water solution. Ammonium and nitrate in extract were measured by colorimetry. Soil microbial biomass C (MBC) and N (MBN) were determined using the fumigation–extraction method (Vance et al., 1987). Soil extractable organic C and total N in the

K_2SO_4 extracts before and after the fumigation were quantified using a total C/N analyzer (Multi-N/C 2100, Analytik Jena AG, Germany). The released C and N were converted to MBC and MBN, respectively, using Kec: 0.45 and Ken: 0.45. Soil organic C (SOC) was measured using the potassium dichromatevitrion oxidation method, and soil total N (TN) was measured with the alkaline persulfate oxidation method (Sun et al., 2009). All results were expressed on an oven-dried soil basis.

Rates of net N mineralization and net nitrification in May, July, and August of 2010 were measured from *in situ* incubations using the buried bag technique (Adams et al., 1989). The incubations were performed using perforated PVC tubes (15 cm in height and 6 cm in diameter). Parafilm covered the top of each tube to avoid leaching of nitrate. The technique prevents plant uptake of mineralized nutrients but allows uptake by the microorganisms. The soil samples were kept in cool box to the laboratory and analyzed for ammonium and nitrate as the initial sample for measurement of net mineralization and net nitrification rates. The soil samples in the buried bags were retrieved after 30 days of incubation and analyzed as the final sample. The difference between the initial and final inorganic N concentrations ($\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$) was used to calculate net N mineralization rates. The difference between the initial and final $\text{NO}_3^- - \text{N}$ concentrations was used to calculate net nitrification rates.

Gross nitrification and denitrification rates were measured using the Barometric Process Separation (BaPS) instrument (UMS GmbH

Inc., Germany) through laboratory incubations, as described by Sun et al. (2009). Within each subplot, three intact soil cores were taken by soil containers with a diameter of 5.6 cm and a height of 4.1 cm. The soil containers were transported at coolers to the laboratory and processed immediately. The BaPS instrument was closed gas-tight and incubated for at least 24 h at a temperature of 25.0 °C.

2.5. Root characteristics analysis

Five randomly selected seedlings from each treatment were harvested in early August 2010, and then divided into leaf, stem and root components. Roots were rinsed free of soil, and 0.5 g samples of young white root were used to assay root activity immediately. All plant parts were dried in an oven at 70 °C to constant weight. R/S ratio (root/shoot mass ratio) was derived based on the measured data. Moreover, within each subplot, five soil samples were taken from the topsoil (0–15 cm) with a 5 cm-diameter polyvinyl chloride core. Fine roots (≤ 2 mm) were carefully separated with fine forceps, and the separated fine roots were carefully washed and analyzed with an image analysis system WinRHIZO (Regent Instruments Inc., Sainte Foy, Québec, Canada), which was used to measure the root length and the diameter of each root. Coarse root biomass, fine root biomass, coarse root/fine root mass ratio and fine root length (RL) were calculated based on the measured data. Fine root vigor (FRV) assay

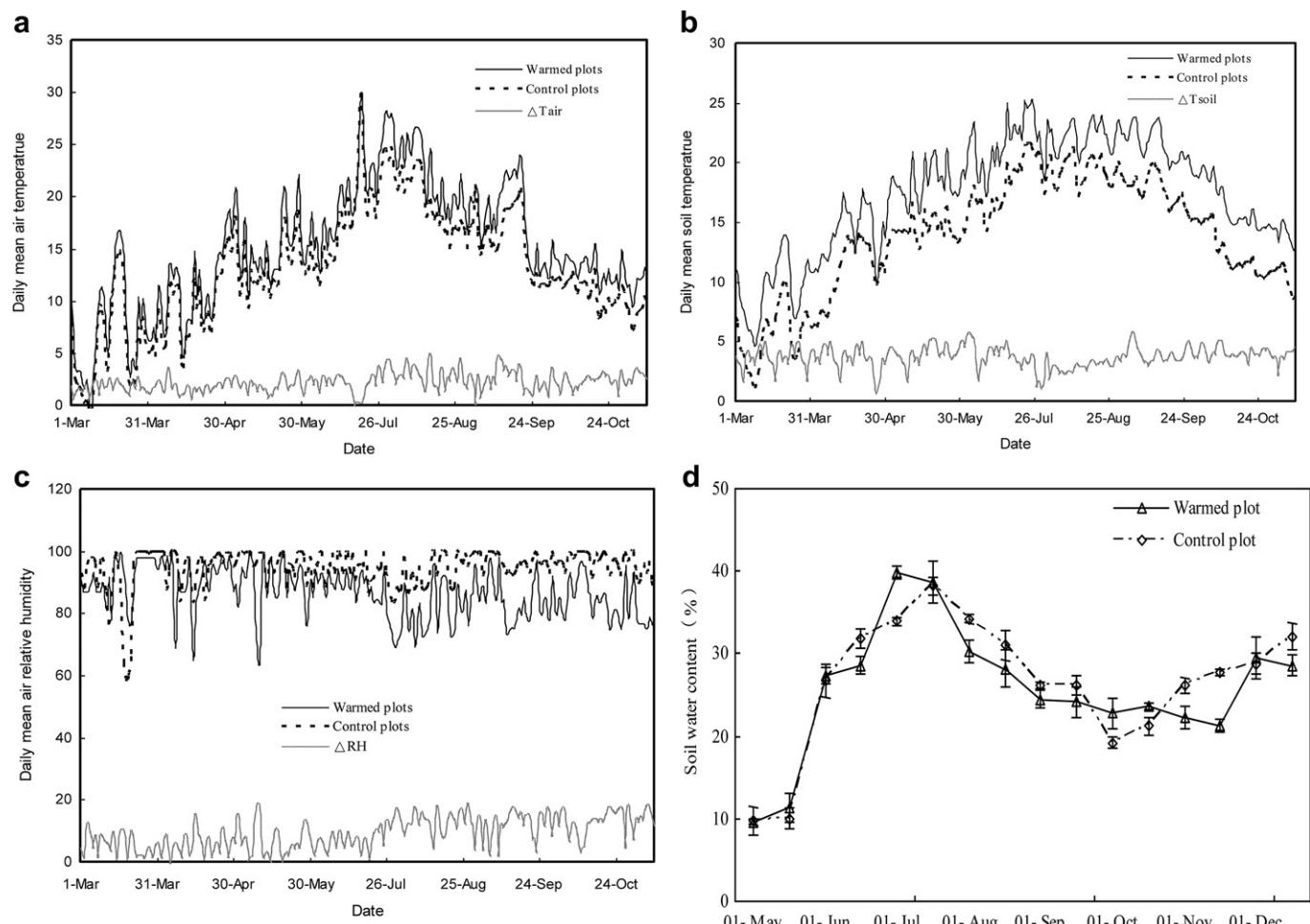


Fig. 1. Seasonal transitions and average differences in (a) daily mean air temperature at 20 cm above the ground, (b) daily mean soil temperature (5 cm depth), (c) mean air relative humidity and (d) mean soil water content (0–10 cm) between warmed plots (solid line) and control plots (dotted line). The lower gray lines (symbol for Δ) in a, b and c represent the daily mean differences in air temperature, soil temperature and air relative humidity between the warmed and control plots, respectively.

was measured by the triphenyltetrazolium chloride (TTC) method, as described by Basile et al. (2007).

2.6. Statistical analysis

Repeated measures ANOVA was used to examine the effects of warming, tree species, sampling date, and their interactions on all response variables. One-way analysis of variance was used to assess the effects of warming on root parameters. Before analysis, all data were tested for the assumptions of ANOVA. If data were heterogeneous, they were *In*-transformed before analysis. For specific sampling date, Student *t*-tests were used to compare the effects of the experimental warming. The statistical tests were considered significant at the $P < 0.05$ level. All statistical tests were performed using SPSS version 11.0.

3. Results

3.1. Microclimate

The infrared heaters caused expected warming effects within the experimental plots. Daily air temperature (at 20 cm above-ground) and soil temperature (at 5 cm depth) within warmed plots were on average increased by 2.0 °C and 3.7 °C, respectively, as compared to control plots (Fig. 1 a and b). The mean air relative humidity was slightly lower in the warmed plots compared with the control plots, with humidities of 86.52% in the warmed plots and 92.58% in the control plots (Fig. 1c). Moreover, there was no obvious difference in soil water content between control (30.41%) and warmed plots (28.55%) (Fig. 1d).

3.2. Root traits

Experimental warming did not significantly affect either root/shoot mass ratio (R/S) or coarse root/fine root mass ratio (C/F) in

both coniferous species, except that C/F ratio was significantly decreased by warming in the *P. asperata* plots, which may have resulted from relatively more biomass partitioning to the fine roots in response to experimental warming (Fig. 2A and B). Warming markedly increased the fine root length in both tree species but enhanced the FRV in the *P. asperata* plots (Fig. 2C and D). Both fine root length and FRV were generally higher in the *P. asperata* than in the *A. faxoniana* plots, irrespective of warming treatment.

3.3. Soil carbon pools and microbial properties

Experimental warming did not significantly affect SOC, TN, or C:N ratio in either tree species among three sampling dates (Table 1; Table 2). Likewise, there were no significant warming effects on the microbial biomass C (MBC), microbial biomass N (MBN) and MBC/MBN ratio in both species among sampling dates (Table 1), except that MBC/MBN ratio in the *P. asperata* plots was significantly increased by experimental warming only in September (Table 1). Irrespective of warming treatment, MBC/MBN ratio was generally higher in the *P. asperata* than in the *A. faxoniana* plots, with an average of 8.01 in the *A. faxoniana* and 9.58 in the *P. asperata* plots, respectively (Table 1). In addition, the MBC, MBN and MBC/MBN ratio all significantly varied among three sampling dates (Table 2).

3.4. Soil nutrient pools

Concentrations of $\text{NH}_4^+ - \text{N}$, $\text{NO}_3^- - \text{N}$ and N_{in} were significantly different between the two species in either warmed or control plots (Table 2). $\text{NH}_4^+ - \text{N}$ concentrations in the *A. faxoniana* plots were greater than those of *P. asperata*, whereas the $\text{NO}_3^- - \text{N}$ concentrations were significantly lower in the *A. faxoniana* compared to the *P. asperata* (Fig. 3A–C). The $\text{NO}_3^- - \text{N}$ was the dominant form of N_{in} , and the $\text{NO}_3^- - \text{N}$ concentrations accounted for more than 80% of N_{in} in both species plots (Fig. 3A–C). Consequently, there was a similar

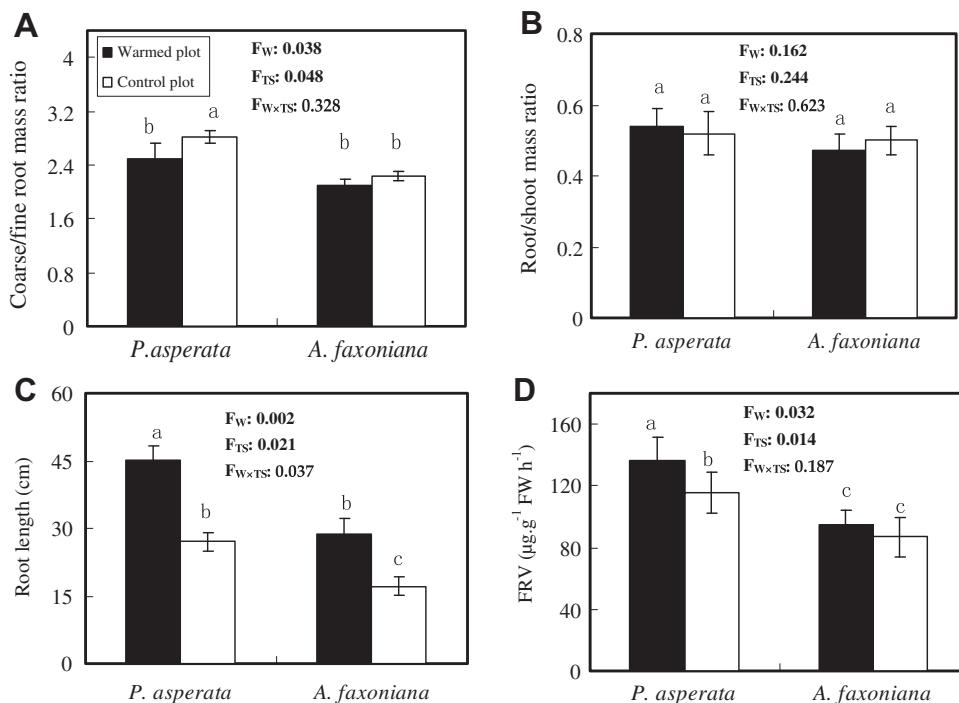


Fig. 2. Effects of experimental warming on coarse root/fine root mass ratio (C/F, A), root/shoot mass ratio (R/S, B), fine root length (C) and fine root activity (FRV, D) of *P. asperata* and *A. faxoniana* seedlings. Values are means \pm SD with the sample size $n = 5$. The bars with different letters are significantly different from each other. F_W : warming effects; F_{TS} : tree species effects; $F_{W \times TS}$: interactions effects of warming \times tree species.

Table 1

Effects of experimental warming on soil organic C (SOC), total N (TN), C:N ratio, microbial biomass C (MBC), microbial biomass N (MBN) and MBC: MBN ratio (C) in *P. asperata* and *A. faxoniana* plots.

Soil properties	Months					
	May		July		September	
	Control plots	Warmed plots	Control plots	Warmed plots	Control plots	Warmed plots
<i>P. asperata</i>						
SOC (g kg^{-1})	69.3 ± 1.4 ^a	71.5 ± 2.4 ^a	75.4 ± 4.5 ^a	70.9 ± 5.9 ^a	74.5 ± 4.3 ^a	78.60 ± 6.36 ^a
Total N (g kg^{-1})	4.1 ± 0.1 ^a	4.2 ± 0.2 ^a	4.0 ± 0.4 ^a	3.9 ± 0.3 ^a	4.0 ± 0.5 ^a	3.9 ± 0.4 ^a
C/N ratio	17.1 ± 1.3 ^a	17.2 ± 1.2 ^a	19.0 ± 1.3 ^a	18.0 ± 1.0 ^a	19.2 ± 2.2 ^a	20.1 ± 2.2 ^a
MBC (mg kg^{-1})	1014.5 ± 113.9 ^a	931.8 ± 92.4 ^a	883.2 ± 83.7 ^a	888.1 ± 39.9 ^a	941.5 ± 89.4 ^a	1024.9 ± 88.6 ^a
MBN (mg kg^{-1})	114.7 ± 12.7 ^a	107.7 ± 9.8 ^a	104.7 ± 12.7 ^a	95.0 ± 6.8 ^a	87.5 ± 10.8 ^a	81.7 ± 6.6 ^a
Microbial C/N	8.8 ± 1.3 ^a	8.0 ± 1.1 ^a	8.4 ± 1.2 ^a	8.9 ± 1.0 ^a	10.8 ± 1.6 ^a	12.5 ± 1.0 ^a
<i>A. faxoniana</i>						
SOC (g kg^{-1})	68.47 ± 1.42 ^a	72.3 ± 1.7 ^a	74.9 ± 0.8 ^a	74.5 ± 1.0 ^a	73.6 ± 1.0 ^a	71.4 ± 1.4 ^a
Total N (g kg^{-1})	4.0 ± 0.2 ^a	3.8 ± 0.3 ^a	4.0 ± 0.2 ^a	4.1 ± 0.3 ^a	3.8 ± 0.2 ^a	4.0 ± 0.2 ^a
C/N ratio	17.3 ± 1.4 ^a	18.9 ± 1.7 ^a	18.7 ± 0.8 ^a	18.1 ± 1.0 ^a	19.3 ± 1.0 ^a	17.8 ± 1.4 ^a
MBC (mg kg^{-1})	916.5 ± 25.9 ^a	890.9 ± 75.3 ^a	890.5 ± 76.4 ^a	874.4 ± 57.9 ^a	1037.6 ± 41.5 ^a	1174.1 ± 109.9 ^a
MBN (mg kg^{-1})	133.0 ± 10.3 ^a	112.9 ± 8.0 ^a	116.3 ± 9.0 ^a	115.8 ± 15.4 ^a	118.7 ± 10.6 ^a	115.9 ± 7.9 ^a
Microbial C/N	6.9 ± 0.8 ^a	7.1 ± 0.8 ^a	7.7 ± 1.0 ^a	7.6 ± 1.2 ^a	8.7 ± 1.4 ^b	10.1 ± 1.0 ^a

Values are means ± SD with the sample size $n = 4$. Values in rows with different letters denote significant differences between control and warmed plots at $P < 0.05$ level for a given variable and tree species. Data printed in bold indicates a significant ($P < 0.05$) difference between control and warmed plots.

seasonality in the concentrations of N_{in} and NO_3^- –N in both species plots. In *P. asperata* plots, experimental warming did not significantly affect concentrations of the NH_4^+ –N, NO_3^- –N and N_{in} throughout the study period. In *A. faxoniana* plots, however, experimental warming significantly increased the NH_4^+ –N concentrations, whereas a significant decrease in the concentrations of NO_3^- –N and N_{in} in May and July and a significant increase in concentrations of the NO_3^- –N and N_{in} in September were observed (Fig. 3A–C). There were three-way interactions of warming, tree species and sampling date on the concentrations of NH_4^+ –N, NO_3^- –N and soil extractable inorganic N (N_{in}) (Table 2).

3.5. Soil N transformations

In the incubation experiments in the absence of plants (buried bag technique), experimental warming induced a remarkably higher net N mineralization and net nitrification rates on all sampling dates in both species plots (Fig. 4A and B). Although the net nitrification rates differed significantly among sampling dates, the statistical analyses showed that the effects of warming, tree species and their interactions were independent of sampling date. In addition, the interactive effects of warming and sampling date were significant on the net nitrification but not on the net mineralization rates (Table 2).

Warming, tree species, sampling date and their interactions all showed significant effects on the soil gross nitrification and denitrification rates (Table 2). In the *P. asperata* plots, warming significantly increased the gross nitrification rates in July and September. In contrast to *P. asperata*, warming significantly increased the gross

nitrification rate of the *A. faxoniana* plots only in September, but remarkably lowered the gross nitrification rates in May and July (Fig. 4C). Experimental warming significantly increased the denitrification rates on all sampling dates in both species plots (Fig. 4D). Gross nitrification and denitrification rates were significantly different over three months, and were markedly greater in the *P. asperata* than in the *A. faxoniana* plots regardless of warming treatment and sampling date (Table 2; Fig. 4D).

4. Discussion

In order to study the projected effects of future climate warming on terrestrial ecosystem, various warming facilities have been employed in field experiments, such as open-top chambers, greenhouse, soil-heating cables, and infrared heaters. Among these warming methods, infrared heaters most closely simulate the processes of climate warming by enhancing downward infrared radiation. The efficacy of employing infrared heaters in global warming studies had also been evaluated and validated in the last ten years (Wan et al., 2002; Liu et al., 2011). Considerable evidence is now available showing that water content within warmed plots is generally lower compared to control plots (Marion et al., 1997), which is similar to the general results found in the present study, with a 6.02% reduction of air relative humidity and 1.86% of soil water content inside the warmed plots. However, this warming-induced decline in water content may be less important, because moisture content in the warmed plot still remained at a relatively high level due to frequent rainfall. It should be noted, however, that the present results on the influences of experimental warming on

Table 2

Results of repeated measures ANOVA showing the P values for responses of soil organic C, total N, C:N ratio, nitrate, ammonium, extractable inorganic N (N_{in}), MBC, MBN, MBC/MBN, net mineralization, net nitrification, gross nitrification and denitrification to warming (W), tree species (TS) and sampling date (D). P values smaller than 0.05 are bold.

Factor	Organic C	Total N	C:N ratio	Nitrate	Ammonium	N_{in}	MBC	MBN	MBC/MBN	Net mineralization	Net nitrification	Gross nitrification	Denitrification
Warming	0.810	0.302	0.767	0.043	0.023	0.975	0.059	0.366	0.276	<0.001	<0.001	<0.001	<0.001
Tree species	0.170	0.082	0.793	<0.001	<0.001		0.046	0.016	<0.001	0.015	0.063	0.415	0.003
Date	<0.001	0.122	<0.001	<0.001	0.056	<0.001	<0.001	0.004	<0.001	0.130	<0.001	0.025	<0.001
W × TS	0.780	0.160	0.804	0.062	0.208	0.001	0.062	0.216	0.052	0.920	0.481	<0.001	0.005
W × D	0.037	0.359	0.023	0.002	0.003	0.002	0.022	0.065	0.003	0.117	<0.001	0.003	0.002
TS × D	0.009	0.060	0.007	<0.001	<0.001	<0.001	<0.001	0.070	0.001	0.264	0.011	<0.001	<0.001
W × D × TS	0.123	0.0054	0.006	0.013	0.017	0.001	0.362	0.492	0.108	0.603	0.220	<0.001	0.038

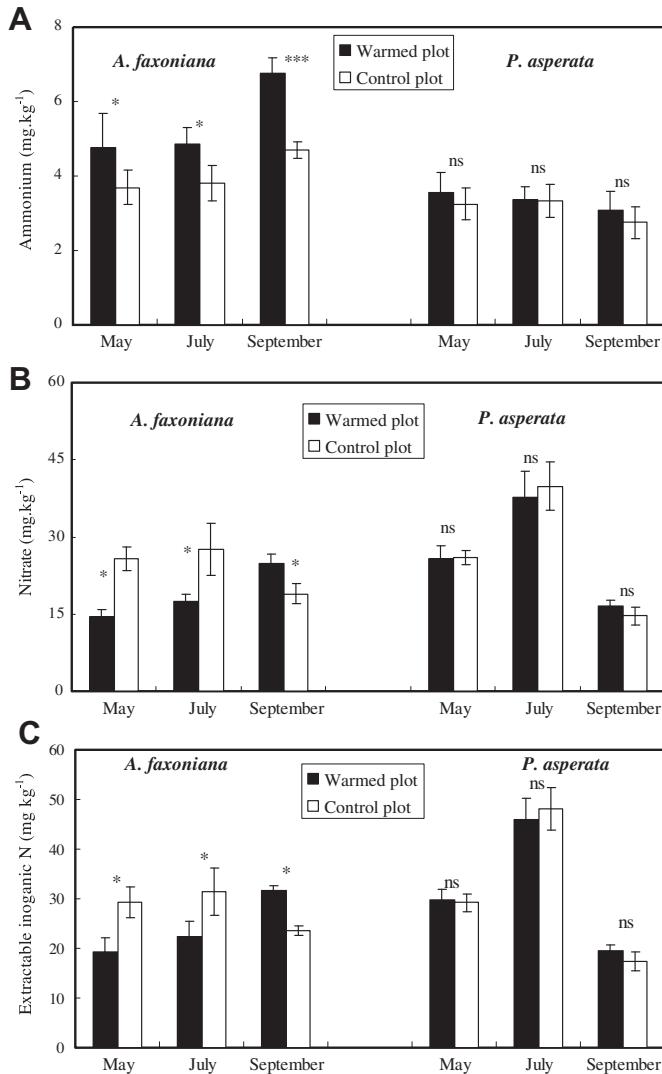


Fig. 3. Effects of experimental warming on soil ammonium (A), nitrate (TN, B) and extractable inorganic N (N_{in} , C) in *P. asperata* and *A. faxoniana* plots. *** $P < 0.001$, * $P < 0.05$, ns: not significant. Values are means \pm SD with the sample size $n = 4$.

rhizosphere processes should be considered with caution since the warming treatment was only conducted at night in our study, whereas night warming and continuous warming might have differential impacts on terrestrial ecosystem processes. It is essential to incorporate the differential effects of asymmetric warming on N transformation into simulating and predicting the responses and feedbacks of forest ecosystems N cycling to global warming.

It is difficult to study the pure effects of tree species via roots on soil processes and its responses to climate change, since associated ecological processes mediated by roots are believed to be temporally dynamic due to biotic (e.g., plant growth, plant phenological stages, tree age and belowground C flux) and abiotic factors (e.g., soil moisture, fertility, pH and nutrient availability) (Bader and Cheng, 2007; Zhu and Cheng, 2011), and hence the influences of different tree species may be masked by the pedology of the site, variability between sites and former occupation. In the present study, both tree species were grown under a common soil with similar field management, and the litter within the plots was removed periodically in order to disrupt the effects of litter inputs on soils. Therefore, the differences in soil N cycling between the

two coniferous species were assumed to reflect the potential effects of experimental warming and the intrinsic biological traits of the different tree species.

Microbes play critical roles in C and nutrient transformation in forest soils, and slight changes in the microbial biomass or community structure may affect soil C and N cycling (Xu et al., 2010). Therefore, soil microbial properties have been proposed to be the potential indicators of impacts of global warming on soils. In our study, MBC, MBN and MBC/MBN ratio were not sensitive to warming irrespective of tree species and sampling date. The lack of a marked warming effect on soil microbial biomass was consistent with the studies conducted in arctic tundra, tallgrass prairie and subalpine forest (Zhang et al., 2005; Biasi et al., 2008; Xu et al., 2010). The effects of warming on soil microbial biomass, however, varied among studies. For example, Liu et al. (2011) reported that warming significantly increased MBC and MBN in rhizosphere soils of spruce seedlings. The variability of these results may reflect the diversity of soils and plant species.

Temperature is a key factor that regulates soil processes and N turnover, and a lot of studies have demonstrated that soil N availability increases with soil warming (Rustad et al., 2001; Sardans et al., 2008). Nevertheless, our results clearly demonstrated that effects of warming on soil N availability varied with tree species and sampling date (Fig. 3A–C). Moreover, individual N-forms (ammonium or nitrate) also exhibited different responses to experimental warming in *A. faxoniana* plots, and depended strongly on sampling date (Fig. 3A–C). In addition, increasing temperature is expected to increase soil microbial activity and soil N transformation (Rustad et al., 2001). In this study, experimental warming also caused significant increases in the net N mineralization and nitrification rates in the two species plots, and the results are consistent with some previous studies which have demonstrated significant positive relationships between temperature and soil N mineralization rates (Melillo et al., 2002; Xu et al., 2010). Moreover, our results showed that the denitrification was very sensitive to increasing temperature, and experimental warming caused a significant increase in the soil denitrification rates and consequently N_2O fluxes from soils, similar results have also been reported previously (Abdalla et al., 2009).

4.1. Species differences in N transformation in response to experimental warming

In this study, soil processes and functions are mainly regulated by plant roots through rhizosphere processes since aboveground litter inputs are periodically removed from experimental plots (Zhu and Cheng, 2011). There were higher $NH_4^+ - N$ and lower $NO_3^- - N$ concentrations in the *A. faxoniana* plots compared to the *P. asperata*. Soil nutrient availability was reflected in the pattern of microbial processes: the gross nitrification and denitrification rates within the *P. asperata* plots were on average 1.61 times and 2.61 times higher than those in the *A. faxoniana* plots, respectively. There are several possible underlying mechanisms resulting in the difference of tree species effects on the soil N transformations and nutrient availability.

Firstly, the differences in the root morphological characteristics and root activity between the two conifers could be largely responsible for the variation of tree species effects on soil nutrient pools and N turnover. *P. asperata* had higher fine root length than that of *A. faxoniana*, with an average of 35.5 cm in the *P. asperata* and 21.3 cm in the *A. faxoniana* plots, respectively (Fig. 2C). A host of studies have shown that plant root length is positively correlated with root-released C (Xu and Juma, 1994; Darwent, 2003). Also, FRV was remarkably higher in the *P. asperata* than in the *A. faxoniana* plots in either warmed or control plots (Fig. 2D). As a result, the nutrient uptake mechanisms and respiration rate of roots can differ between

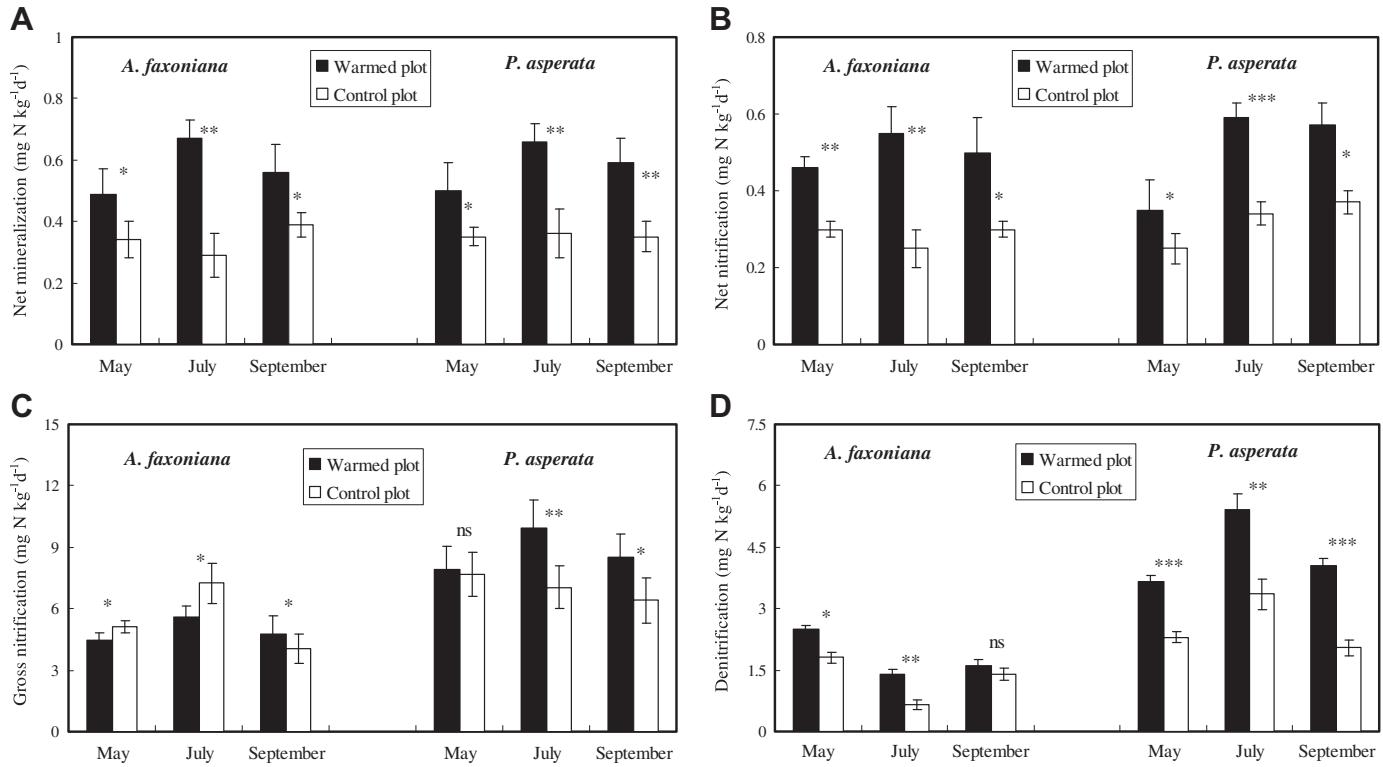


Fig. 4. Effects of experimental warming on rates of net mineralization (A), net nitrification (B), gross nitrification (C) and denitrification (D) in *P. asperata* and *A. faxoniana* plots. ***P < 0.001, **P < 0.01, *P < 0.05, ns: not significant. Values are means \pm SD with the sample size $n = 4$.

tree species, and these processes may have profound impacts on the root exudation and microbial feedbacks to N cycling (Hinsinger et al., 2009; Phillips et al., 2011). Although the available data on root exudation remain limited in the present study, a preliminary experiment has indicated that *P. asperata* species has higher concentrations of total organic carbon (TOC) and total organic nitrogen (TON) than that of *A. faxoniana*, with $4.07 \mu\text{g C cm}^{-1}$ root h^{-1} and $0.35 \mu\text{g N cm}^{-1}$ root h^{-1} in *P. asperata*, and $3.55 \mu\text{g C cm}^{-1}$ root h^{-1} and $0.24 \mu\text{g N cm}^{-1}$ root h^{-1} , respectively. In addition to amount of root exudates, the composition of root exudates may also differ between the tree species (Grayston et al., 1996).

Secondly, the differences in the amount and composition of root exudates between the tree species may result in the establishment of different microbial communities and thus translated into differences in N transformation processes and nutrient availability, as suggested previously (Jones et al., 2009; Phillips et al., 2011). The proposition for altered microbial community composition between the two species was also supported in part by our results (Table 1). Microbial biomass C/N ratio, which has been used to indicate the relative abundance of bacteria versus fungi at a coarse level (Zhao et al., 2010), was generally higher in the *P. asperata* (9.85) than in the *A. faxoniana* plots (8.01), indicating that the microbial community in the *P. asperata* was more fungi-abundant compared to the *A. faxoniana* (Paul and Clark, 1989). Other possibilities related to the difference of tree species effects on the soil N cycling may be due to root type, plant nutrient uptake (i.e. preferred for N-forms), and different capacity for enzyme synthesis and for acquiring nutrients between species (Gessler et al., 1998; Koranda et al., 2011). However, the exact mechanisms underlying tree species differences in the regulating soil N cycling are unknown and warrants further study, and more attention should be paid to ascertain which plant traits actually determine their tree species effects via roots on the soil microbial activity and microbially mediated processes.

In line with our initial hypothesis, although increasing temperature generally stimulated the soil N turnover and nutrient availability, the stimulated effects of warming on N cycling varied with tree species. The effect size of warming on soil N transformations was relatively greater in the *P. asperata* plots (ranging from 32.8% to 41.0%) compared to the *A. faxoniana* (ranging from 18.1% to 22.8%). Rhizosphere effect on soil processes and functions is largely determined by the amount and type of C released from the roots, while belowground C allocation and root characteristics have been thought to be the two main aspects controlling root exudates (Badri and Vivanco, 2009). In the present study, the C/F ratio of *P. asperata* seedlings was significantly increased by experimental warming, but was not affected for the *A. faxoniana* species (Fig. 2A). Thus, the differences in C/F ratio between the two species could be partially responsible for the variation in soil N transformation in response to experimental warming, as indicated by the results of our current study and previous studies (Liu et al., 2011). In addition to altered belowground C allocation, it is possible that the greater root exudation in the *P. asperata* resulted from warming-induced changes in the root morphological characteristics. In our study, the differences on the root length between the two coniferous species were further enhanced by experimental warming (Fig. 2C). Darwent (2003) reported that decreased exudation from *Hordeum vulgare* L. roots resulted from reductions in root length and in the numbers of root tips due to altered nutrient conditions. Furthermore, it is also possible that the greater exudation in the *P. asperata* seedlings might be associated with warming-induced changes in the FRV. The FRV of *P. asperata* seedlings was significantly increased by experimental warming, but was not affected for the *A. faxoniana* species (Fig. 2D). As discussed above, these alterations will have profound impacts on the nutrient uptake and labile root exudates, thereby intensifying their influences on rhizosphere priming effect and associated soil microbial processes (i.e., soil organic matter decomposition) (Cheng, 2009; Phillips et al., 2011). Unfortunately,

there is almost no quantitative information about the belowground C flux of the two species between the treatments due to methodological difficulties and the limitations of our experimental design. Further examination of root exudation and fine root turnover in response to experimental warming and with more detailed characterization of root traits and belowground C inputs would be a worthwhile focus of future studies.

In conclusion, this present study demonstrated that the responses of soil N transformation and nutrient availability to experimental warming depended, to some degree, on tree species and season with *P. asperata* being more sensitive than *A. faxoniana* in gross nitrification and denitrification rates, implying different capacities of the two coniferous species to acquiring nutrients and thereby altered the competitive abilities and adaptive relationships between the tree species under climate change. As a result, the response differences of soil N cycling to experimental warming between the two coniferous species could have important implications for subalpine coniferous forest dynamics. It should be kept in mind, however, that the present results on the influences of tree species on the N cycling should be considered with caution since the data came from seedlings, and the experiment period was only lasted three years. Future longer-term studies that establish mechanistic links between belowground C inputs and soil N cycling will greatly advance our understanding of how tree root–soil-microbial interactions influence belowground ecological processes of forest ecosystems under global climate change.

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