Applied Soil Ecology 59 (2012) 60–67

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Nitrogen transformation in the rhizospheres of two subalpine coniferous species under experimental warming

Huajun Yin[a,b,c], Zhenfeng Xu[a,b], Zhi Chen[a,b], Yunyan Wei[a,b], Qing Liu[a,b,*]

[a] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China
[b] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chinese Academy of Sciences, Chengdu 610041, PR China
[c] Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu 610041, PR China

ARTICLE INFO

Article history:
Received 23 July 2011
Received in revised form 15 March 2012
Accepted 27 March 2012

Keywords:
Nitrogen transformation
Rhizosphere effect
Warming
Subalpine coniferous forest

ABSTRACT

Tree species can exert a strong influence on rhizosphere nutrient cycling through root and rhizosphere processes and create feedback in the patterns of nutrient cycling in forest ecosystems. In this study, we conducted an experiment to compare the rhizosphere effects of two coniferous species on N transformation as well as their responses to experimental warming using infrared heaters in the Eastern Tibetan Plateau. We examined the potential net N mineralization and nitrification rates, N availability, and microbial biomass C (MBC) and N (MBN) in rhizosphere soils of *Picea asperata* and *Abies faxoniana* plots and compared them to bulk soils. The infrared heater increased both the mean air and the soil temperatures by 1.5 °C and 2.1 °C respectively. Potential net N mineralization and net nitrification rates were generally greater in rhizosphere soils for the two conifers than in bulk soil, especially in the warmed plots. This led to higher NH_4^+ and NO_3^- concentrations in the rhizosphere soils. MBC and MBN were markedly higher in the rhizosphere soils relative to bulk soil in the study plots. In the control subplots of *P. asperata*, MBC, MBN, potential net N mineralization and net nitrification rates in the rhizosphere were 9.6%, 21.7%, 33.3% and 20.1% greater than in the bulk soil, respectively. MBC, MBN, potential net N mineralization and net nitrification rates in the control subplots of *A. faxoniana*, however, were 2.0%, 7.7%, 22.0% and 11.8% higher, respectively, in the rhizosphere than in the bulk soil; all of the variables were significantly lower than those of *P. asperata* subplots. Warming significantly promoted N transformation and nutrient availability by enhancing the rhizosphere priming effects for the two conifers, but the magnitudes of the rhizosphere effects on soil N transformation stimulated by warming were generally greater in *P. asperata* than in *A. faxoniana* subplots. Differences in the altered morphological and functional characteristics of the roots between the two species under experimental warming could be largely responsible for this variation. Taken together, the results indicated that the two species exhibited similar patterns but with considerably different magnitudes of rhizosphere effects on N transformations in response to experimental warming, implying different capacities of the two conifers to acquire nutrients and thereby altered the competitive and adaptive relationships between the tree species under climate change.

1. Introduction

The rhizosphere — the soil zone strongly influenced by plant roots — has been recognized as one of the key fine scale components in global carbon cycle research (Cheng, 2009). According to Kuzyakov et al. (2000), a priming effect (PE) is a short-term change in the turnover intensity (e.g., decomposition) of soil organic matter (SOM) caused by different factors, such as fertilization and planting. In the case of plant cultivation, the PE occurs in the direct vicinity of living roots. This effect is thus called the rhizosphere priming effect (RPE). Processes that are largely controlled or directly influenced by roots are often referred to as rhizosphere processes (Gobran et al., 1998). Consequently, an alteration of the physical, chemical or biological characteristics of the soil around the roots is known as a rhizosphere effect (Phillips and Fahey, 2008).

Many important aspects of plant–soil interactions such as plant nutrient acquisition, SOM decomposition, nutrient dynamics, and rhizosphere respiration are intimately coupled with plant roots and their associated rhizosphere processes (Cheng, 2009), which are crucial for plant growth and the functioning of terrestrial ecosystems. However, only limited effort has been made to link rhizosphere processes with soil processes, such as N cycling and

* Corresponding author at: Chengdu Institute of Biology, Chinese Academy of Sciences, No. 9 Section 4, South Renmin Road, P.O. Box 416, Chengdu 610041, PR China. Tel.: +86 28 8522 9115; fax: +86 28 8522 2753.
E-mail address: liuqing@cib.ac.cn (Q. Liu).

0929-1393/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsoil.2012.03.013

nutrient availability (Dinesh et al., 2010). Consequently, tree roots and their rhizosphere interactions are at the centre of many ecosystem processes yet remain highly uncertain (Bader and Cheng, 2007; von Lutzow and Kogel-Knabner, 2009). For example, available N and P have been found to be accumulated (Turpault et al., 2005), depleted (Chen et al., 2002) or unchanged (Ehrenfeld et al., 1997) in rhizosphere soil compared to bulk soil in different studies, which shows different results that are tightly linked to the soil conditions and tree species (Zhao et al., 2010).

It has been widely recognized that tree species can exert a large influence on the soil environment and its microflora (Hobbie, 1992). Previous research on the effects of tree species on soil functions has focused primarily on the role of leaf litter inputs, nutrient uptake or bulk soil processes (Binkley and Menyailo, 2005), and relatively few studies have considered the importance of spatially and temporally dynamic processes occurring in the roots and rhizosphere. As a result, rhizosphere processes have become one of the most important but least understood ways in which plants affect nutrient cycling (Cheng, 2009).

Because tree species are very different with respect to root morphology and physiology (e.g., root biomass; mycorrhizal association; root type; and the quantity and quality of C released from the root, i.e., root exudates) as well as their nutrient requirements (Jones et al., 2009), rhizosphere effects on nutrient cycling and soil microbial activity are likely to vary with tree species (Richardson et al., 2009). Knowledge of differences in nutrient cycling through rhizosphere processes among tree species is fundamental for characterizing the nutrient acquisition capacities of different tree species and for interpreting the influence of tree species on soil processes and functions (Ladygina and Hedlund, 2010). However, our ability to predict how tree species affect SOM decomposition and nutrient mineralization is constrained by our incomplete knowledge of the mechanisms influencing these processes and possible feedbacks between tree species and soil microbial processes, particularly under climate change (Hobbie, 1992; Gärdenäs et al., 2011). Therefore, there is still a strong need for more field studies on rhizosphere nutrient cycling for different climates and tree species to provide a more realistic view of rhizosphere processes (Zhao et al., 2010).

Our primary objective was to quantify and compare the rhizosphere effects of two subalpine coniferous species (*Picea asperata* and *Abies faxoniana*) on soil N transformations under experimental warming. We examined soil's chemical and biological properties associated with N transformation in rhizosphere and bulk soils of the two conifers and compared control and warmed plots. Root characteristics of the two coniferous species were also investigated 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 conifers are sympatric and co-occur naturally within subalpine ecosystems; and (3) *A. faxoniana* is a dominant species in natural forests, whereas *P. asperata* primarily functions as a keystone species in reforestation after logging. Previously, the stimulatory effects of warming on the growth of *P. asperata* and *A. faxoniana* seedlings have been observed, but the two tree species exhibit striking differences in belowground C allocation in response to experimental warming (Yin et al., 2008; Zhao and Liu, 2009). We hypothesize that warming would stimulate the rhizosphere effects of the two tree species on nutrient cycling by enhancing microbial activity but that the magnitude of these rhizosphere effects would vary with tree species due to differences in intrinsic biological characteristics. This study is the first, to our knowledge, to examine how experimental warming alters rhizosphere effects on N transformation and nutrient availability between different tree species.

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° 41′ N, 103° 53′ E, 1820 m a.s.l.) where mean annual temperature, precipitation and evaporation are 8.9 °C, 919.5 mm, and 795.8 mm, respectively. Our experiment followed Wan et al. (2002) in using 165 cm × 15 cm infrared heaters (Kalgo Electronics Inc., Bethlehem, PA, USA) to generate an artificially warmed environment. The experiment used a paired factorial design with a tree species treatment nested within five pairs of 2 m × 2 m warmed and control plots (10 plots total). 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 within the warmed plots according to a similar previous 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 warmed plots were separated by 5 m to avoid heating the control plots.

Each 2 m × 2 m plot was divided into two 2 m × 1 m subplots, and the indigenous soil of all subplots up to a depth of 50 cm was replaced by sieved topsoil from a coniferous forest. The soil was moderately moist and dark brown (Cambisol by the FAO classification). The basic soil properties as determined in March 2007 were as follows: pH 5.55, total N 4.54 g kg^{-1}, soil organic C 78.04 g kg^{-1} and bulk density 0.887 g cm^{-3}. Uniform 4-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 planted randomly in separate subplots within each plot. Artificial warming was conducted from April 2007 to December 2009, and the warmed plots were heated from 7:00 am to 7:00 pm (12 h day^{-1}) daily. The seedlings were watered frequently as needed. Moreover, all litters within the plots were removed to examine the pure effects of the two coniferous species on the soil via root and rhizosphere processes.

Soil and root sampling soils and fine roots were sampled for rhizosphere and bulk soil assays in late July 2008. Within each subplot, ten soil samples (taken around ten randomly selected seedlings) were taken from the topsoil (0–15 cm) with a 5 cm-diameter polyvinyl chloride core. To obtain a sufficient amount of rhizosphere soil, additional rhizosphere soil was also collected from harvested seedlings for growth analysis. The collected soils were placed into a sorting basin where large aggregates were gently broken apart. Fine roots (≤2 mm) and adhering rhizosphere soil were carefully picked out of the basin with fine forceps and shaken gently to remove loose soil. Soil adhering to fine roots after gentle shaking was defined as rhizosphere soil, whereas soil that did not adhere was defined as bulk soil (Phillips and Fahey, 2006). Currently, this method is the only and the most effective way in which a sufficient mass of rhizosphere soil could be collected for multiple process-based assays in the field (Badalucco and Kuikman, 2001). Within several hours of collection, rhizosphere soil was carefully separated from the fine roots by gently scraping away the adhering soil with fine forceps. Special care was taken to minimize the disturbance to fine root networks, and considerable effort was made to remove root hairs and fragments from soil samples.

The collected soils for each subplot were mixed thoroughly to obtain a composite fresh sample and delivered immediately to the laboratory for further analyses. Each composite soil sample was divided into two subsamples. One subsample was frozen for measuring NO_3^--N and NH_4^+-N concentrations, MBC and MBN, and potential net N mineralization and nitrification rates. All soil

samples were processed within one week (usually within 24 h) of collection from the field. The second subsample was air-dried to determine soil total N concentration and processed within 30 days of sampling but frequently sooner. The separated fine roots were carefully washed and analysed with the WinRHIZO image analysis system (Regent Instruments Inc., Sainte Foy, Québec, Canada), which was used to measure the length and diameter of each root. Coarse root mass, fine root biomass, specific root length (SRL), fine root length (RL) and root length density were calculated based on the measured data (Basile et al., 2007).

2.2. Environmental and plant measurements

2.2.1. Temperature and soil moisture

Air temperature (at the height of 20 cm above the ground) and relative humidity were measured using DS1923G temperature/humidity iButton data loggers, and soil temperatures (5 cm depth) were measured with DS1921G Thermochron iButton data loggers (DS1921G-F5, Maxim Integrated Products, Dallas Semiconductor Inc., Sunnyvale, CA) in four pairs of plots at 30 min intervals during the experiment period. 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 °C to determine soil moisture.

2.2.2. Growth and biomass analysis

In late August 2008, five randomly selected seedlings from each treatment were harvested 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. A fine root vigour (FRV) assay was conducted using the triphenyltetrazolium chloride (TTC) method as described by Liu et al. (2011). All plant parts were dried in an oven at 70 °C, to constant weight, and the R/S ratio (root/shoot mass ratio) was derived based on the measured data.

2.2.3. Soil analyses

Total N content was measured using the alkaline persulphate oxidation method. Soil microbial biomass C (MBC) and N (MBN) concentrations were determined using the chloroform fumigation extraction method. MBC and MBN were calculated from the differences between total extractable C and N in the fumigated and unfumigated samples using efficiency factors (K_{ec} and K_{en}) of 0.45 and 0.54, respectively. All of the results were expressed on an oven-dried soil basis. Soil inorganic N (NH_4^+ and NO_3^-) and rates of potential net N mineralization and net nitrification were determined by extraction with 2 mol L^{-1} KCl. For soil inorganic N, recently collected soils (<24 h after sampling) were mixed with KCl (10:1), shaken for 1 h, and filtered with Whatman No. 42 Paper. For N transformations, control soils from the microbial biomass assay were extracted with KCl after a 15-d aerobic incubation at constant temperature (22 °C) in the dark, using the same extraction procedure as for inorganic N. Immediately following filtration, all KCl extracts were acidified with several drops of 6 mol L^{-1} HCl to prevent microbial growth and refrigerated at 4 °C until analysis. KCl extracts were analysed for NH_4^+-N and NO_3^--N concentrations on a flow-injected autoanalyser. Potential net nitrification, net ammonification and net N mineralization rates were calculated as the changes in NO_3^--N, NH_4^+-N and inorganic N before and after the 15-d incubation, respectively.

2.3. Calculations and statistics

Analyses were performed with the software Statistical Package for the Social Science (SPSS) version 11.0 (SPSS Inc., Chicago, IL). All response variables were averaged within each subplot, and the subplots were considered the experimental units. Non-normal data were log-transformed to meet conditions of normality and homogeneity of variance. A two-way analysis of variance was used to assess the main effects of warming, soil fraction and their interaction on soil parameters associated with N cycling. Individual treatment means were compared with Tukey's HSD test to determine whether they were significantly different at the 0.05 probability level. The magnitude of rhizosphere effect was calculated as the percentage difference of a given response variable between paired rhizosphere and bulk soil samples. One-way analysis of variance (ANOVA) was also performed to test the effects of tree species on the magnitude of rhizosphere effects for a given response variable.

3. Results

3.1. Warming effects of infrared heaters

Artificial warming significantly elevated both mean air (at 20 cm aboveground) and mean soil (at 5 cm depth) temperature by 1.5 °C, and 2.1 °C, above those of control plots, respectively (Fig. 1). The average air relative humidity was slightly lower in the warmed plots compared to the control plots, with humidities of 79.0% in the warmed plots and 82.3% in the control plots (Fig. 1c). Soil water content was not significantly affected by experimental warming (Fig. 1d).

3.2. Soil properties

Warming significantly increased soil NO_3^--N concentration in the plots with the exception that NO_3^--N concentration did not differ significantly between treatments in the rhizosphere soil of *A. faxoniana* subplots. In contrast, the NH_4^+-N and total N concentrations were not significantly affected by warming in the plots (Table 1). There were no significant differences in total N, NH_4^+-N or NO_3^--N concentrations between the bulk and rhizosphere soils of either species. No significant interactions were found between warming and soil fraction for total N, NH_4^+-N or NO_3^--N concentrations (Table 1).

3.3. Soil microbial biomass and N transformations

MBC, MBN, potential net N mineralization and net nitrification rates were markedly increased by warming for the two coniferous species with the exception that MBN in *P. asperata* subplots and MBC in the bulk soil of *A. faxoniana* subplots did not differ significantly between treatments (Table 1). MBC, MBN, potential net N mineralization and net nitrification rates were significantly higher in the rhizosphere soil of *P. asperata* subplots compared to the bulk soil except that the net N mineralization and net nitrification rates did not differ significantly between the soil fractions. Similarly, MBC, MBN, potential net N mineralization and net nitrification rates were generally higher in the rhizosphere soil of *A. faxoniana* subplots compared to the bulk soil, but the differences between the rhizosphere and bulk soils were not significant for any of the response variables in *A. faxoniana* subplots (Table 1). The correlated changes in MBC and MBN resulted in no significant change in the MBC/MBN ratio under warming or between the soil fractions in the plots, but MBC/MBN ratios were significantly higher in *P. asperata* subplots than in *A. faxoniana* subplots, irrespective of the soil fraction. Significant interactive effects of warming and soil fraction were detected for MBC and potential net N mineralization rate in the *P. asperata* subplots and for potential net N mineralization and net nitrification rates in the *A. faxoniana* subplots (Table 1).

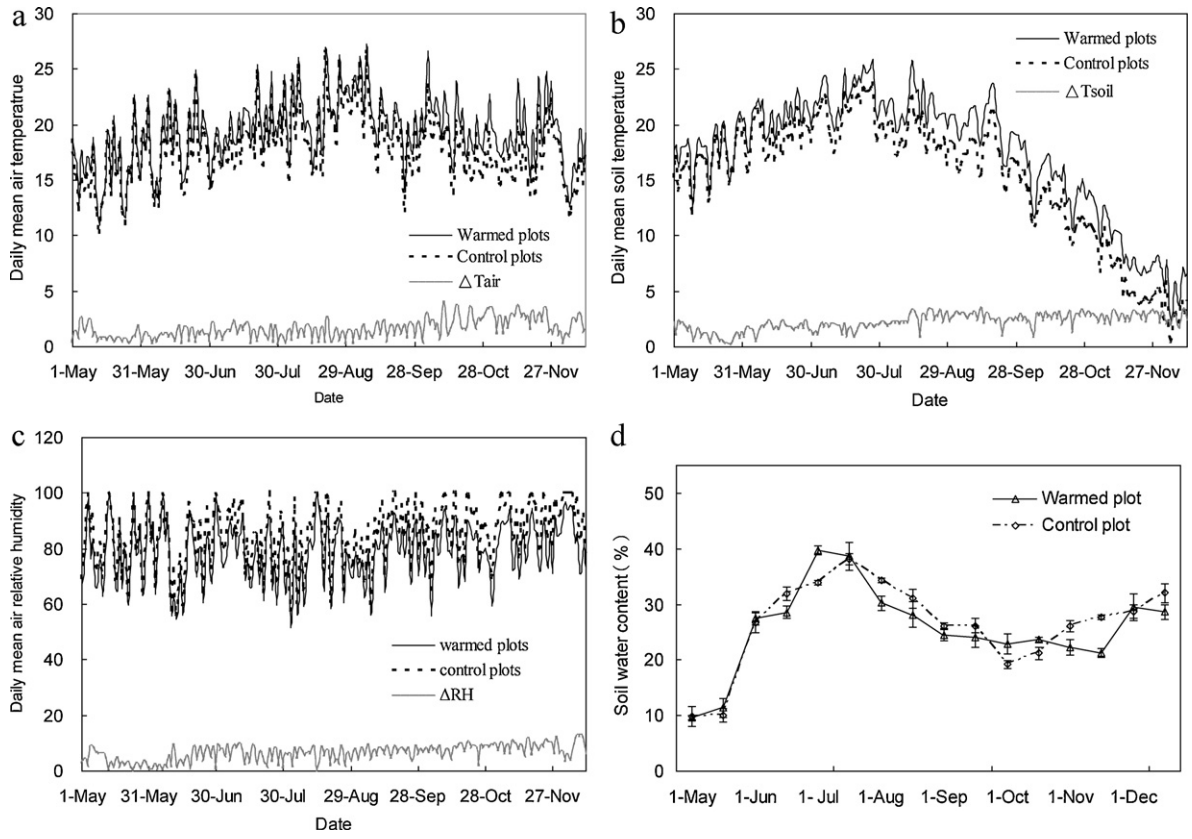

Fig. 1. Seasonal transitions and average difference in (a) daily mean air temperature at 20 cm above the ground, (b) daily mean soil temperature (5 cm depth), (c) mean air relative humidity and (d) mean water content (0–10 cm) between warmed plots (solid line) and control plots (dotted line). The lower gray lines (symbol for △) in (a), (b) and (c) represent the daily mean differences in the air temperature, soil temperature and air relative humidity between the warmed and control plots, respectively. All the data were measured and recorded in 2 h intervals for whole days and smoothed by moving-averages. The scales of the *x*-axis are 30 day intervals staring from 1 May to 15 December 2008.

3.4. Species differences in the rhizosphere effects under warming

Positive rhizosphere effects on MBC, MBN, potential net N mineralization and net nitrification rates were generally observed in both the control and warmed plots, but the magnitude of the rhizosphere effects on soil microbial biomass and N transformations varied significantly between the two tree species (Fig. 2). In the control subplots of *P. asperata*, MBC, MBN, potential

Table 1
Effects of warming and soil fraction on soil properties associated with N transformations in the plots. Values are the mean ± SE of five replicates. Values followed by the same letter in the same row are not significantly different between treatments at a $P < 0.05$ significance level. Significant effects of the two factors as well as of the interactions are indicated in the last three columns. BSCP, bulk soils in control plots; BSWP, bulk soils in warmed plots; RSCP, rhizosphere soils in control plots; RSWP, rhizosphere soils in warmed plots; W, warming effect; SF, soil fraction effect; W × SF, warming × soil fraction interaction effect; NS, not significant.

Soil properties	Treatment						
	BSCP	BSWP	RSCP	RSWP	W	SF	W × SF
P. asperata							
Total N	4.22 ± 0.08a	4.39 ± 0.02a	4.56 ± 0.13a	4.46 ± 0.16a	NS	NS	NS
NO_3^--N (mg kg^{-1})	5.87 ± 0. 16b	6.22 ± 0.14a	5.89 ± 0.35b	6.60 ± 0.33a	*	NS	NS
NH_4^+-N (mg kg^{-1})	4.44 ± 0.26b	4.49 ± 0.29ab	4.53 ± 0.41ab	4.75 ± 0.19a	NS	NS	NS
MBC (mg kg^{-1})	473.05 ± 38.36d	619.79 ± 65.73b	518.37 ± 72.45c	737.53 ± 40.79a	***	*	*
MBN (mg kg^{-1})	91.02 ± 3.95b	99.67 ± 5.90b	110.81 ± 5.56a	127.54 ± 12.66a	NS	*	NS
Microbial C/N	5.19 ± 0.36a	6.22 ± 0.41a	4.67 ± 0.24a	5.78 ± 0.34a	NS	NS	NS
Net N mineralization (mg N kg^{-1} day^{-1})	0.09 ± 0.02c	0.39 ± 0.09b	0.12 ± 0.03c	0.60 ± 0.11a	***	*	*
Net nitrification (mg N kg^{-1} day^{-1})	0.10 ± 0.01c	0.34 ± 0.05b	0.12 ± 0.01c	0.51 ± 0.13a	***	*	NS
A. faxoniana							
Total N	4.19 ± 0.13a	4.30 ± 0.15a	4.47 ± 0.14a	4.51 ± 0.17a	NS	NS	NS
NO_3^--N (mg kg^{-1})	6.05 ± 0.33b	6.44 ± 0.28a	6.19 ± 0.33ab	6.54 ± 0.17a	*	NS	NS
NH_4^+-N (mg kg^{-1})	4.49 ± 0.18a	4.50 ± 0.14a	4.56 ± 0.13a	4.69 ± 0.11a	NS	NS	NS
MBC (mg kg^{-1})	561.94 ± 99.91b	635.59 ± 70.64ab	573.28 ± 37.85b	684.94 ± 22.21a	**	NS	NS
MBN (mg kg^{-1})	143.59 ± 15.05b	160.57 ± 8.73a	154.59 ± 10.77b	188.05 ± 15.48a	*	NS	NS
Microbial C/N	3.91 ± 0.18a	3.95 ± 0.27a	3.71 ± 0.21a	3.72 ± 0.13a	NS	NS	NS
Net N mineralization (mg N kg^{-1} day^{-1})	0.12 ± 0.02b	0.71 ± 0.12a	0.15 ± 0.04b	0.86 ± 0.16a	***	NS	*
Net nitrification (mg N kg^{-1} day^{-1})	0.17 ± 0.02b	0.67 ± 0.14a	0.19 ± 0.02b	0.81 ± 0.16a	***	NS	*

* Significant differences between treatments at $P < 0.05$.
** Significant differences between treatments at $P < 0.01$.
*** Significant differences between treatments at $P < 0.001$.

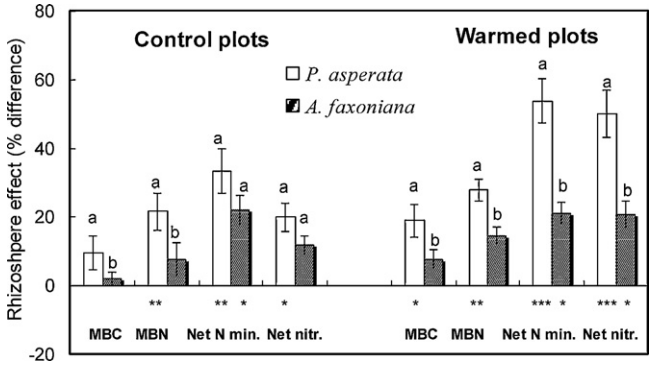

Fig. 2. Rhizosphere effects on soil microbial biomass C (MBC), microbial biomass N (MBN), net N mineralization (net N min.) and net nitrification (net nit.) in control and warmed plots of *P. asperata* and *A. faxoniana* tree species. Rhizosphere effects are presented as means (and standard errors) for a given response variable within control and warmed plots. Effects differences significantly greater than zero are noted by the symbols ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Differences letters indicate significant differences between *P. asperata* and *A. faxoniana* tree species for a given response variable ($\alpha = 0.05$).

net N mineralization and net nitrification rates in the rhizosphere were 9.6%, 21.7%, 33.3% and 20.0% greater, respectively, than in the bulk soil. The rhizosphere effects for MBC, MBN, potential net N mineralization and net nitrification rates in the control subplots of *A. faxoniana*, however, were 2.0%, 7.7%, 22.0% and 11.8%, respectively, over the bulk soil, and the values were significantly lower compared to *P. asperata* (Fig. 2). A similar result was found in the warmed plots of the two coniferous species: the rhizosphere effects on MBC, MBN, potential net N mineralization and net nitrification rates were 144.8%, 91.0%, 154.9% and 139.2% greater, respectively, in the *P. asperata* subplots than in the *A. faxoniana* subplots (Fig. 2). Experimental warming also markedly increased the magnitude of rhizosphere effects on soil microbial biomass and N transformation compared to the control plots for the two conifers.

3.5. The effects of warming on the response variables related to root traits

Experimental warming generally increased the coarse root mass and fine root mass and decreased the specific root length (SRL) for the two coniferous species, but significant differences between the treatments were observed only in *P. asperata* (Table 2). There were significant increases in the root length (RL), root length density and fine root vigour (FRV) in the warmed plots compared with the control plots for the two coniferous species, but RL and FRV in *P. asperata* were generally higher than in *A. faxoniana* in either warmed or control plots. Warming did not significantly affect either the root/shoot mass ratio (R/S) or the coarse root/fine root mass ratio (C/F) except that the C/F ratio was significantly decreased by warming in *P. asperata*, which may indicate relatively more biomass partitioning to the fine roots in response to experimental warming (Table 2). The results suggested that the root morphology of *P. asperata* seedlings tended to be more sensitive to experimental warming compared to *A. faxoniana* seedlings.

4. Discussion

4.1. Rhizosphere soil sampling in the field

Despite the relatively small percentage of total soil volume that the rhizosphere occupies, rhizosphere processes may be more important than bulk soil processes for soil functions and regulate virtually all aspects of nutrient cycling (Badalucco and Kuikman, 2001). Quantifying the magnitude and direction of rhizosphere effects in the field is challenging owing to the inherent structural and functional complexity of rhizosphere systems and the lack of suitable methods for studying rhizosphere processes without physically altering the plant–soil system (Phillips and Fahey, 2008). Most previous studies of rhizosphere effects in trees have been conducted either on tree seedlings growing in pots, where the rhizosphere soil is defined merely as the soil from a potted plant relative to the soil from unplanted control pots (Priha et al., 1998), or on roots trained to grow along root windows (Norton and Firestone, 1996). Although such methods may elucidate the mechanisms by which tree roots influence rhizosphere processes, they provide limited insight into the ecological relevance of rhizosphere effects in the field. This is especially true for forest soils (Jones, 2003). In this study, we employed the adhering soil method to quantify the rhizosphere effects on soil N transformation and microbial activity. Although not without significant drawbacks, this approach is currently the only and the most effective method for studying rhizosphere effects in the field because it enabled us to obtain a sufficient amount of soil for process-based assays (Badalucco and Kuikman, 2001). The efficacy of employing the adhering soil method for quantifying rhizosphere effects on soil N transformation has also been evaluated and validated by multiple studies (Phillips and Fahey, 2006; Phillips et al., 2011; Zhao et al., 2010).

It is difficult to study the physicochemical and microbial properties of rhizosphere soil in situ in response to climate change. This is because the rhizosphere effects are temporally dynamic due to interactions between biotic (e.g., plant growth, plant phenological stages, tree age, C allocation patterns) and abiotic factors (e.g., soil moisture, fertility, pH and nutrient availability) (Bader and Cheng, 2007; Berg and Smalla, 2009; Zhu and Cheng, 2011). Therefore, the effects of temperature on specific tree species relative to rhizosphere soil microbial responses may be masked by the pedology of the site, edaphic and environmental variability, and management effects (Phillips and Fahey, 2008). In this study, both tree species were grown in a common soil with similar field management. The differences in the rhizosphere processes between the two conifers were therefore assumed to reflect the potential effects of experimental warming and the intrinsic biological traits of the different tree species.

4.2. Rhizosphere effects on N transformation and nutrient availability

The present results on the influences of tree species on rhizosphere processes should be considered with caution because all soil microbiological assays were performed in the absence of roots, and thus, the differences between the rhizosphere and bulk soil processes may result in part from root disturbance rather than from actual rhizosphere effects. Consequently, these results only represent a potential rate of reaction that cannot be extrapolated to field processes. However, the potential influences of root removal on rhizosphere effects may be less important because all microbiological assays were conducted on fresh soil, usually within 24 h of collection from the field.

It is suggested that microbial growth in the rhizosphere is limited by N rather than C at the present site (Xu et al., 2010). In N-limited soils, rhizosphere effects may not increase rhizosphere N availability because nutrient-limited rhizosphere microbes immobilize N (Phillips and Fahey, 2006). This is confirmed in the present study. There were no significant differences in the concentrations of NH_4^+ and NO_3^- in rhizosphere soil compared to bulk soil for the two conifers. The rhizosphere effects of tree species on concentrations of inorganic N vary widely. For example, significantly higher NH_4^+ and lower NO_3^- concentrations in rhizosphere soil relative to bulk soil were observed by Zhao et al. (2010) in three plantation stands. However, Wang et al. (2001) observed significant depletion

Table 2
The effects of warming on coarse root mass, fine root mass, root/shoot mass ratio, coarse root/fine root mass ratio, fine root length, specific root length and fine root activity of *P. asperata* and *A. faxoniana* seedlings.

Root characteristics	*P. asperata*		*A. faxoniana*	
	Control plots	Warmed plots	Control plots	Warmed plots
Coarse root mass (g)	1.52 ± 0.40b	1.71 ± 0.24a	1.39 ± 0.26a	1.41 ± 0.46a
Fine root mass (g)	0.54 ± 0.14b	0.69 ± 0.17a	0.62 ± 0.11a	0.74 ± 0.30a
Coarse/fine root mass ratio	2.81 ± 0.43a	2.48 ± 0.29b	2.24 ± 0.26a	2.10 ± 0.28a
Root/shoot mass ratio	0.54 ± 0.05a	0.54 ± 0.07a	0.47 ± 0.05a	0.50 ± 0.04a
Specific root length ($m\,g^{-1}$)	1.11 ± 0.12a	0.79 ± 0.19b	1.05 ± 0.27a	1.01 ± 0.07a
Root length (cm)	27.13 ± 2.03b	45.04 ± 2.43a	17.13 ± 2.07b	28.77 ± 3.45a
Root length density ($cm\,cm^{-3}$)	0.08 ± 0.03b	0.15 ± 0.05a	0.08 ± 0.04b	0.17 ± 0.07a
Fine root activity (TPF $\mu g\,g^{-1}$ FW h^{-1})	111.59 ± 13.88b	136.79 ± 11.82a	86.79 ± 12.7b	94.65 ± 9.37a

of both NH_4^+ and NO_3^- in the rhizosphere soil of Norway spruce (*P. abies*) and European beech (*Fagus sylvatica*) seedlings. Ehrenfeld et al. (1997) found that NH_4^+ and NO_3^- were not influenced by live roots in mineral soil. The variability of these results may reflect the diversity of soil fractions and morphological and physiological differences between tree species.

In our plots, potential net N mineralization and net nitrification rates were generally greater in the rhizosphere soil than in the bulk soil, especially in the warmed plots (Table 1). Root-induced stimulation of N mineralization has been reported in most previous studies of forest tree species (Phillips and Fahey, 2006), which may result primarily from positive rhizosphere priming effects on SOM decomposition (Cheng, 2009) or increased faunal grazing (Ehrenfeld et al., 1997). The greater net nitrification rate in the rhizosphere soil relative to the bulk soil did not result in the accumulation of NO_3^--N (Table 1), indicating that the uptake of NO_3^--N by the plant roots and rhizosphere microbes counterbalanced the nitrification. This proposition is further supported in part by the data on soil microbial biomass. MBN was markedly higher in the rhizosphere soil compared to the bulk soil in the plots, which may be the result of increased microbial immobilization of N (Zhao et al., 2010).

Temperature is a key factor that regulates soil processes and N turnover, and many studies have demonstrated that the soil microbial activity and N transformation increase with experimental warming (Rustad et al., 2001; Melillo et al., 2002). Similar results were also found in our study; MBC, MBN, potential net N mineralization and net nitrification rates were significantly increased by experimental warming in both rhizosphere and bulk soils for the two conifers. The simultaneous increases in potential net N mineralization rate and MBN in response to warming suggest that tree roots stimulate gross N mineralization in this study, because the gross N mineralization rate is the sum of net N mineralization and N immobilization. Our results also indicated that the magnitudes of rhizosphere effects on N transformation were markedly increased by experimental warming compared to the control plots for the two conifers. N transformation is tightly coupled with SOM decomposition, which can be strongly accelerated by root exudation (i.e., rhizosphere priming effect) (Jackson et al., 2008; Gärdenäs et al., 2011). One possible interpretation for the stimulated rhizosphere effects was that experimental warming substantially intensified the rhizosphere priming effects on SOM decomposition by enhancing the flux of C belowground (i.e., root exudation), thus resulting in a higher temperature sensitivity for microbial activity and microbially mediated processes (i.e., SOM decomposition and N mineralization) in response to experimental warming (Phillips et al., 2011; Zhu and Cheng, 2011).

4.3. Species differences in rhizosphere effects and their response to warming

In this study, soil processes and functions are primarily regulated by plant roots through rhizosphere processes because aboveground litter inputs are periodically removed from experimental plots. The two coniferous species had a similar pattern of rhizosphere effects on soil mineralization and nutrient availability, but *P. asperata* had greater rhizosphere effects on N transformations compared to *A. faxoniana* species (Fig. 2). The similar pattern and greatly varied magnitude of rhizosphere effects on nutrient cycling for the different tree species have also been observed in previous limited studies (Wang et al., 2001; Phillips and Fahey, 2006). Although the exact mechanisms behind this phenomenon are unknown, we propose several possible underlying mechanisms to explain this observation based on ancillary data. These mechanisms are primarily related to the differences between the two conifers in terms of the root traits, quantity and chemical quality of C flux to the rhizosphere soil and associated microbial processes.

First, the differences in root morphological and functional characteristics between the two conifers could be largely responsible for the variation in tree species effects on N transformation and microbial activity. For example, the fine root length of *P. asperata* was markedly higher than that of *A. faxoniana* in both the warmed and the control plots (Table 2). A host of studies have demonstrated that plant root length is positively correlated with root-released C (Xu and Juma, 1994; Darwent et al., 2003). Additionally, FRV was remarkably higher in *P. asperata* compared to *A. faxoniana* subplots in both the warmed and control plots (Table 2). As a result, the nutrient uptake mechanisms and respiration rate of roots can differ between tree species, and these processes may have profound impacts on the quantity and chemical quality of root exudates released to the rhizosphere and associated microbial feedbacks to N cycling (Hinsinger et al., 2009; Phillips et al., 2011). Although the available data on root exudation or other rhizodeposits remain limited in this study, the preliminary experiments have indicated that *P. asperata* had higher concentrations of total organic carbon (TOC), total organic nitrogen (TON) and total sugar (TS) compared to *A. faxoniana* (data not shown). Consequently, the differences in the amount and/or composition of root exudates between the two 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 (Kuzyakov, 2002; Gärdenäs et al., 2011; Phillips et al., 2011). The proposition for altered microbial community composition between tree species was also supported in part by our results (Table 1). The microbial biomass C/N ratio, which has been used to indicate the relative abundance of bacteria versus fungi at a coarse level, was generally higher in *P. asperata* (5.46 on average) than in *A. faxoniana* subplots (3.87 on average), indicating that the microbial community of *P. asperata* was more fungi-abundant compared to *A. faxoniana* (Paul and Clark, 1989).

Alternately, the variation in the magnitude of rhizosphere effects between the two coniferous species was most likely due to the effects of root type differences. The two tree species had contrasting biological traits and root systems; *P. asperata* was less shade-tolerant, and its root system was typically fibrous with well-developed lateral roots. In contrast, *A. faxoniana* had a slightly

higher tolerance to shade with a taproot system and relatively poorly developed later roots (Li, 1990). Other possibilities related to the differences in rhizosphere effects between the tree species include the different capacities of the two species for enzyme synthesis and for acquiring nutrients through the roots and hyphae (Koranda et al., 2011). However, the exact mechanisms underlying the regulation of rhizosphere nutrient cycling in different tree species are unknown and require further investigation. More attention should be paid to ascertain which plant traits actually determine the rhizosphere effects via roots on microbial activity and microbially mediated soil processes for a species.

Consistent with our hypothesis, although warming generally promoted the rhizosphere effects of the two coniferous species on N transformation and soil microbial activity, the magnitude of stimulation of the rhizosphere effects on N transformation in response to experimental warming varied with tree species. The size of the stimulated rhizosphere effect on soil N transformation by experimental warming was generally greater in the *P. asperata* subplots (ranging from 19.0% to 50%) than in the *A. faxoniana* subplots (ranging from 7.8% to 20.9%). Rhizosphere effects on soil processes and functions are determined by the amount and type of C released from the roots, whereas belowground C allocation and root morphological characteristics have been thought to be the two primary aspects controlling root exudates (Badri and Vivanco, 2009). In the present study, although warming did not significantly affect the R/S ratios of the two tree species, the C/F ratio of *P. asperata* seedlings was significantly increased by experimental warming, resulting in relatively more biomass partitioning to fine roots in response to warming, whereas the C/F ratio of *A. faxoniana* seedlings was not affected by experimental warming. Consequently, the differences in the C/F ratio between the two species could be partially responsible for the variation in the rhizosphere effects on N transformation in response to experimental warming (Liu et al., 2011). In addition, it is possible that greater root exudation in *P. asperata* resulted from warming-induced changes in root morphological characteristics. In our study, the differences in fine root length between the two coniferous species were further enhanced by experimental warming (Table 2). Darwent et al. (2003) reported that decreased exudation from *Hordeum vulgrare* 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 root exudation in *P. asperata* seedlings resulted from warming-induced changes in FRV. The FRV of *P. asperata* seedlings was significantly increased by experimental warming but was not affected for *A. faxoniana* species (Fig. 2). As discussed above, these alterations will have profound impacts on the root nutrient uptake and the quantity and quality of root exudates (Aerts and Chapin, 2000), thereby intensifying their influences on rhizosphere effects and associated microbial processes (Cheng, 2009; Phillips et al., 2011). Unfortunately, there is almost no quantitative information about the root exudates of the two conifers between the treatments due to methodological difficulties and the limitations of our experimental design. Further examination of root exudation in response to experimental warming and with more detailed characterizations of root morphology and belowground C allocation would be a worthwhile focus of future studies.

In conclusion, the current results suggested that the two coniferous species had similar patterns of rhizosphere effects on N transformations and microbial activity in response to experimental warming but with considerably different magnitudes, implying different capacities of the two tree species to acquire the necessary nutrients. Differences in the magnitude (and possibly mechanisms) of rhizosphere effects on nutrient cycling under experimental warming between tree species could have important implications for forest dynamics. Variation in the ability to stimulate nutrient availability through rhizosphere processes between the tree species is likely to influence the outcome of competition for nutrient resources between *A. faxoniana* and *P. asperata* under climate warming. However, the present results on rhizosphere processes should be considered with caution because the data came from seedlings and the experiment period was only two years. Future longer term studies that establish mechanistic links between belowground C input and rhizosphere nutrient cycling will greatly advance our understanding of how plant–soil–microbial interactions influence belowground ecological processes of forest ecosystems under global climate change.

Acknowledgements

We are grateful to two anonymous reviewers and the section editor for their helpful comments and suggestions. We thank the staff in the Maoxian Mountain Ecosystem of CERN Research Station for their kind help with field investigations. This study was supported jointly by the National Natural Science Foundation of China (nos. 30800165, 31100446 and 31070533), and Knowledge Innovation Engineering of the Chinese Academy of Sciences (KSCX2-YW-Z1023).

References

Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67.
Badalucco, L., Kuikman, P.J., 2001. Mineralization and immobilization in the rhizosphere. In: Pinto, R. (Ed.), The Rhizosphere: Biochemistry and Organic Substances at the Soil–Plant Interface. Marcel Dekker, New York, pp. 159–196.
Bader, N.E., Cheng, W.X., 2007. Rhizosphere priming effect of *Populus fremontii* obscures the temperature sensitivity of soil organic carbon respiration. Soil. Biol. Biochem. 39, 600–606.
Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant Cell. Environ. 32, 666–681.
Basile, B., Bryla, D.R., Salsman, M.L., Marsal, J., Cirillo, C., Johnson, R.S., Dejong, T.M., 2007. Growth patterns and morphology of fine roots of size-controlling and invigourating peach rootstocks. Tree Physiol. 27, 231–241.
Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.
Binkley, D., Menyailo, O., 2005. Gaining insights on the effects of tree species on soils. In: Binkley, D., Menyailo, O. (Eds.), Tree Species Effects on Soils: Implications for Global Change. Springer, New York, NY, pp. 1–16.
Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., 2002. Phosphorus dynamics in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D Don.). Soil Biol. Biochem. 34, 487–499.
Cheng, W., 2009. Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. Soil Biol. Biochem. 41, 1795–1801.
Darwent, M.J., Paterson, E., McDonald, A.J., Tomos, A.D., 2003. Biosensor reporting of root exudation from *Hordeum vulgare* in relation to shoot nitrate concentration. J. Exp. Bot. 54, 325–334.
Dinesh, R., Srinivasan, V., Hamza, S., Parthasarathy, V.A., Aipe, K.C., 2010. Physicochemical, biochemical and microbial properties of the rhizosphe soils of tree species used as supports for black pepper cultivation in the humid tropics. Geoderma 158, 252–258.
Ehrenfeld, J.G., Parsons, W.F.J., Han, X.G., Parmelee, R.W., Zhu, W.X., 1997. Live and dead roots in forest soil horizons: contrasting effects on nitrogen dynamics. Ecology 78, 348–362.
Gärdenäs, A.I., Ågren, G.I., Bird, J.A., Clarholm, M., Hallin, S., Ineson, P., Kätterer, T., Knicker, H., Nilsson, S.I., Näsholm, T., Ogle, S., Paustian, K., Persson, T., Stendahl, J., 2011. Knowledge gaps in soil carbon and nitrogen interactions e from-molecular to global scale. Soil Biol. Biochem. 43, 702–717.
Gobran, G.R., Clegg, S., Courchesne, F., 1998. Rhizospheric processes influencing the biogeochemistry of forest ecosystems. Biogeochemistry 42, 107–120.
Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant soil 321, 117–152.
Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. Trends Ecol. Evol. 7, 336–339.
Jackson, L.E., Burger, M., Cavagnaro, T.R., 2008. Roots, nitrogen transformations, and ecosystem services. Annu. Rev. Plant Physiol. 59, 341–363.
Jones, D.L., 2003. Rhizosphere methods in agroforestry research. In: Schroth, G., Sinclair, F.L. (Eds.), Trees, Crops and Soil Fertility: Concepts and Research Methods. CAB International, Wallingford, UK, pp. 289–301.
Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 321, 5–33.
Koranda, M., Schnecker, J., Kaiser, C., Fuchslueger, L., Kitzler, B., Stange, C.F., Sessitsch, A., Zechmeister-Boltenstern, S., Richter, A., 2011. Microbial processes and

community composition in the rhizosphere of European beech – The influence of plant C exudates. Soil Biol. Biochem. 43, 551–558.
Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sc. 165, 382–396.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32, 1485–1498.
Ladygina, N., Hedlund, K., 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biol. Biochem. 42, 162–168.
Li, C.B., 1990. Ecological Study of Sichuan Forest. Sichuan Published House of Science & Technology, Chengdu.
Liu, Q., Yin, H.J., Chen, J.S., Zhao, C.Z., Cheng, X.Y., Wei, Y.Y., Lin, B., 2011. Belowground responses of *P. asperata* seedlings to warming and nitrogen fertilization in the Eastern Tibetan plateau. Ecol. Res 26, 637–648.
Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 13, 2173–2176.
Norton, J.M., Firestone, M.K., 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biol. Biochem. 28, 351–362.
Paul, E.A., Clark, F.E., 1989. Soil Microbiology and Biochemistry. Academic Press, San Diego, CA.
Phillips, R.P., Fahey, T.J., 2006. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. Ecology 87, 1302–1313.
Phillips, R.P., Fahey, T.J., 2008. The influence of soil fertility on rhizosphere effects in northern hardwood forest soils. Soil Sci. Soc. Am. J. 72, 453–461.
Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol. Lett. 14, 187–194.
Priha, O., Lehto, T., Smolander, A., 1998. Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant Soil 206, 191–204.
Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321, 305–339.
Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126, 543–562.
Turpault, M.P., Utérano, C., Boudot, J.P., Ranger, J., 2005. Influence of mature Douglas fir roots on the solid soil phase of the rhizosphere and its solution chemistry. Plant Soil 275, 327–336.
von Lutzow, M., Kogel-Knabner, I., 2009. Temperature sensitivity of soil organic matter decomposition – what do we know? Biol. Fert. Soils 46, 1–15.
Wan, S., Luo, Y., Wallace, L., 2002. Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. Global Change Biol. 8, 754–768.
Wang, Z.Y., Göttlein, A., Bartonek, G., 2001. Effects of growing roots of Norway spruce (*P. abies* [L.] Karst.) and European beech (*F. sylvatica* L.) on rhizosphere soil solution chemistry. J. Plant Nutr. Soil Sc. 164, 35–41.
Xu, J.G., Juma, N.G., 1994. Relations of shoot C, root C and root length with root-released C of two barley cultivars and the decomposition of root-released C in soil. Can. J. Soil Sci. 74, 17–22.
Xu, Z.F.H., Xiong, R., Wan, P., Cao, C., Liu, G.Q., 2010. Initial soil responses to experimental warming in two contrasting forest ecosystems, Eastern Tibetan plateau, China: nutrient availabilities, microbial properties and enzyme activities. Appl. Soil Ecol. 46, 291–299.
Yin, H.J., Liu, Q., Lai, T., 2008. Warming effects on growth and physiology in the seedlings of the two conifers *P. asperata* and *A. faxoniana* under two contrasting light conditions. Ecol. Res. 23, 459–469.
Zhao, C., Liu, Q., 2009. Growth and photosynthetic responses to two coniferous species experimental warming and nitrogen fertilization. Can. J. For. Res. 39, 1–11.
Zhao, Q., Zeng, D.H., Fan, Z.P., 2010. Nitrogen and phosphorus transformations in the rhizospheres of three tree species in a nutrient-poor sandy soil. Appl. Soil Ecol. 46, 341–346.
Zhu, B., Cheng, W.X., 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Global Change Biol. 17, 2172–2183.