



Original article

Positive effects of night warming on physiology of coniferous trees in late growing season: Leaf and root

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ARTICLE INFO

Article history:

Received 21 October 2015

Received in revised form

3 February 2016

Accepted 3 February 2016

Available online 22 February 2016

Keywords:

Gas exchange

Net influx of NH_4^+ and NO_3^- in root

Root nitrate reductase activity

Root physiology

ABSTRACT

Previous studies about the effects of experimental warming on tree species have focused primarily on response of morphology and physiology in leaf and biomass allocation in the growing season, and a few studies considered the importance of roots. Based on the available evidence, it is unclear whether photosynthesis rate is enhanced by night warming in late autumn an issue that deserves further investigation. Thus, we exposed two coniferous species, *Picea asperata* and *Abies faxoniana*, to night warming continued throughout the year to investigate morphological and physiological responses of roots and leaves in the autumn. The results showed that night warming caused significant increases in net influxes of NH_4^+ and NO_3^- in *P. asperata* seedlings corresponding well with net H^+ efflux and net influx of O_2 . Meanwhile, night warming had a positive effect on foliar gas exchange such as net photosynthesis rate, apparent quantum efficiency, dark respiration rate and maximum quantum efficiency of PS II, and nitrate reductase activity of roots. Additionally, root morphology such as total roots length, surface area, specific root area and specific root length was also stimulated by night warming. In contrast, night warming decreased concentrations of non-structural carbohydrate in leaves and roots of both species in autumn. The present study demonstrates that night warming would enhance late autumn leaf photosynthetic rate, and increase N uptake capacity of roots.

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1. Introduction

There is an overall trend of climate warming at the global scale, with more pronounced warming in the higher latitudinal and altitudinal regions (IPCC, 2013). Consequently, a cascade of studies have investigated plants responses to global warming (Lemmens et al., 2006; Vicca et al., 2007; Danby and Hik, 2007; Leppalammi et al., 2013; Zheng et al., 2013), such as structure, physiology and function of leaves (Jin et al., 2011; Zheng et al., 2013), root lifespan

and physiology (Bai et al., 2012; Yin et al., 2014), plant phenology (Körner and Basler, 2010) and carbon gain (Gimeno et al., 2012) as well as net primary productivity (Hudson and Henry, 2009). It is reported that globally averaged maximum and minimum temperatures have both increased since 1950 (IPCC, 2013), which tends to affect carbon assimilation and consumptions in plants, because photosynthesis in most plants occurs during the daytime and is more sensitive to daily maximum temperature, whereas plants respiration occurs throughout the whole day (Atkin et al., 2013), and is therefore influenced by both daily maximum and minimum temperature (Peng et al., 2013). To our knowledge, a great deal of attention has been focused primarily on continuous warming or day warming (Zhao and Liu, 2009; Zheng et al., 2013), while little is known about the effect of night warming on trees, especially on their root N uptake rates.

It has been widely recognized that effects of global warming on plants varied by season, meteorologic conditions, phenology and plant organs (Gimeno et al., 2012; Yin et al., 2013, 2014). Alteration in plant phenology is one of the most sensitive and observable

Abbreviations: AQE, apparent quantum efficiency; Fv/Fm, maximum quantum efficiency of PS II; NR, nitrate reductase; NSC, non-structural carbohydrate; PAR, photosynthetically active radiation; P_{max} , light-saturated rate of photosynthesis; P_n , net photosynthesis rate; R_d , dark respiration rate; SLA, specific leaf area; SRA, root specific surface area; SRL, specific root length; TF, triphenylformazan; TTC, triphenyltetrazolium chloride.

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biological response to global warming (Bronson et al., 2009; Morin et al., 2010; Polgar and Primack, 2011). Some field (Rollinson and Kaye, 2012) and modeling studies (Lebourgeois et al., 2010) have illuminated that warm temperature would expand plant growing season due to early onset and later end (Parmentier et al., 2011; Chung et al., 2013). Previous studies about the effect of experimental warming on tree species have focused primarily on net mineralization, net nitrification and denitrification rates, ectomycorrhizal colonization and root exudation in early growing season, peak of growing season and non-growing season (Zhao and Liu, 2009; Yin et al., 2012, 2013, 2014). Global warming affects plant growth through direct effects on photosynthesis and respiration (Albert et al., 2011) and indirect effects on soil nitrogen availability (Sardans et al., 2008) and soil moisture (Holsten et al., 2009). Previous study reported that an increase in autumn temperature did not increase the photosynthetic rate but affects the consumption of excess energy in seedlings of Jack pine (Busch et al., 2007). Based on the available evidence, it is unclear whether photosynthesis rate is enhanced by warming at the late autumn (Lebourgeois et al., 2010; Gimeno et al., 2012), and it deserves further investigation.

It is well known that fine roots of plants play a key role in the acquisition of water and essential nutrients, which make a substantial contribution to biogeochemical cycling at the ecosystem level, and soil resources available for plants are highly variable in space and time (Pregitzer et al., 2002). Plants must respond to the rapid changes in soil resource availability by physiological and structural adjustments of the fine absorbing roots, such as to increase the rate of nutrient uptake per unit root length (Ryel and Caldwell, 1998). Therefore, changes in physiological activity of roots under unstable circumstances would result in corresponding shifts in plant growth and root lifespan (Yin et al., 2014). Although many conifer species are adapted to cool, acidic soils, and are expected to absorb more ammonium in comparison with nitrate (Hawkins and Robbins, 2010), nitrate reductase (NR) is generally considered to be the rate-limiting enzyme in inorganic nitrogen (N) assimilation (Young et al., 2007; Yu and Zhang, 2012) because nitrogen absorption is directly associated with the reduction rate of nitrate to nitrite (Calatayud et al., 2008; Huang et al., 2013). Root activity (as TTC, triphenyltetrazolium chloride, reducing capacity) is commonly used as an important physiological parameter for evaluation of ion and water uptake (Huang et al., 1997; Wang et al., 2006). To date, a great deal of attention is being paid to the root exudation and soil nitrogen transformation under experimental warming (Yin et al., 2012, 2013). These studies reported that experimental warming marked increased exudation rates in *Picea asperata* and *Abies faxoniana*. Few studies, however, have investigated roots N uptake rates and their associated parameters under global warming.

A few studies have focused on plant growth and physiological response of *P. asperata* and *A. faxoniana* seedlings to short-term continuous warming (Yin et al., 2008; Zhao and Liu, 2009; Hou et al., 2011). However, long-term experiments to investigate the effects of artificial warming are definitely needed (Wang et al., 2011). Early developmental stages of plants are expected to be more sensitive to environmental changes than adult stages (Weltzin et al., 2000) and are critical life-stages of plant life cycles, which are tightly associated with modulating forest regeneration and species composition, as well as ecological succession (Lloret et al., 2004). Furthermore, most conifers keep their leaves year-around and are able to photosynthesize when temperatures are warm, the impact of an extended autumn due to warming would likely to be greater for conifer growth than for deciduous trees. Therefore, we used the seedlings of *P. asperata* and *A. faxoniana*, the dominant species of subalpine coniferous forests in the Eastern Tibetan Plateau located at the transition zone between Qinghai-

Tibet plateau and the Sichuan basin (Wang et al., 2003), as materials and investigated the morphological and physiological responses of leaf and root in the late autumn to investigate the effects of global warming on conifer trees. Based on the available information, we hypothesized that: 1) night warming would enhance late autumn leaf photosynthetic rate; 2) night warming would increase N uptake capacity of roots.

2. Materials and methods

2.1. Study site and experimental design

The study was conducted in Maoxian Mountain Ecological Research Station (31°42' N, 103°54' E, 1826 m a.s.l.), Chinese Academy of Sciences, Sichuan province, China, with mean annual temperature, precipitation and evaporation 9.3 °C, 825.2 mm, and 968.7 mm, respectively. In this region, the growing season generally starts in late April and ends in October (Zhu et al., 2012). The current experiment continued the study of Yin et al. (2014), which was designed as a randomized block experiment with two warming treatments (control and night warming) and two species (*P. asperata* and *A. faxoniana*). Each plot was 2 × 2 m in size with a 5 m distance between any two adjacent plots. Each treatment of each species had 4 plots as replicates. The indigenous soil of all plots was replaced, to a depth of 30 cm, by sieved topsoil from a nearby coniferous forest. The soil classified as a mountain brown soil series (Chinese taxonomy), and the soil properties, determined in October 2010, were as follows: pH, 6.8; total N, 4.0 g kg⁻¹; soil organic C, 61 g kg⁻¹; and bulk density, 0.91 g cm⁻³. The warmed plots were heated by MSR-2420 infrared radiators (Kalglo Electronics Inc, Bethlehem, PA, USA) while the control used 'dummy' heaters of the same shape and size as the infrared heater suspended 1.5 m above the middle of the plots. Uniform 4-year-old *P. asperata* and *A. faxoniana* seedlings from a local nursery were selected based on plant height and stem base diameter at the beginning of the experiment. The average plant height and stem basal diameter were 15.6 ± 0.8 cm and 3.7 ± 0.7 mm for *P. asperata*, and 11.8 ± 0.7 cm and 3.5 ± 0.6 mm for *A. faxoniana*, respectively. In each plot, the space between seedlings was 30 × 40 cm in the beginning and 70 × 80 cm since 2013. The experiment layout was conducted in September 2010 and warming treatment was initiated on October 2011, which was 13 months after the seedlings were planted. The warmed plots were heated from local time 7:00 pm to 7:00 am (12 h day⁻¹) year-around, and all the plots were watered with collected precipitation nearby and weeds were removed as frequently as needed.

Air temperature (at 5 cm aboveground) and soil temperature (at 5 cm depth) were measured using DS1923G temperature/humidity iButton data loggers and DS1921G Thermochron iButton data loggers (DS1921G-F5, Maxim Integrated Products; Dallas Semiconductor Inc., Sunnyvale, CA, USA) in each plot, respectively. Compared to the control plots (Yin et al., 2014), the half-hourly air temperature (at 5 cm aboveground) and soil temperature (at 5 cm depth) within the warmed plots elevated by an average of 1.95 °C and 3.10 °C, respectively. The soil water content was not significantly different ($P = 0.409$) between the warmed plots (25.4 ± 2.3%) and the control plots (26.2 ± 1.7%). Please refer to Yin et al. (2014) for further information about the microclimate of the experiment site.

2.2. Gas exchange and chlorophyll fluorescence

The fully expanded leaves, which were randomly selected from 3 plants of each species in each treatment, were measured for photosynthesis light response curve under controlled optimal

conditions using an open-mode portable photosynthesis system (Model LI-6400, Li-Cor, Inc., Lincoln, NE, USA). All the measurements were performed from 08:00 to 11:30 each day in April, July and late October 2014, which represent early, middle and late growing season for the plants, respectively. Afterward, the measured leaves were collected and their projected areas were determined with a scanner and WinRHIZO image analysis software (Régent instruments, Quebec, QC, Canada). Subsequently, these leaves were dried at 70 °C for 48 h to constant weight. Specific leaf area (SLA) was calculated as the ratio of projected area to dry weight.

Responses to photosynthetically active radiation (PAR) were measured at 0, 20, 50, 80, 100, 200, 300, 400, 600, 800, 1000, 1200, 1600, 1800, and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with temperature of 24–26 °C and relative humidity of 36–55% inside the leaf chamber. This curve of photosynthesis was fitted with a non-rectangular hyperbola according to Hirose and Werger (1987).

$$P_n = \frac{AQE \times PAR + P_{\max} - \sqrt{(AQE \times PAR + P_{\max})^2 - 4 \times AQE \times PAR \times P_{\max} \times K}}{2K} - R_d$$

where P_n is net photosynthesis rate, AQE is apparent quantum efficiency, P_{\max} is light-saturated rate of photosynthesis, K is convexity and R_d is dark respiration rate. First, from linear regression of PAR at 0–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, AQE and R_d were obtained as the slope and y-intercept of these regressions, respectively. Then, a non-rectangular hyperbola was fitted to the whole curve using the AQE and R_d values to obtain P_{\max} (Hikosaka et al., 2004).

In late October of 2014, fully expanded and exposed current-year leaves were randomly selected from 8 plants of each treatment. The leaves have been dark-adapted for 30 min prior to the measurement and then they were used for chlorophyll fluorescence measurement using a modulated fluorometer (PAM 2100, Walz, Effeltrich, Germany) according to Yin et al. (2009). The maximum quantum efficiency of PS II (Fv/Fm) was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Rosenqvist and Van, 2003).

2.3. Leaf chemical analysis

Fully expanded and exposed current-year leaves of plants were randomly selected from 3 plants of each species in each treatment in late October of 2014. The leaf samples were divided into two portions, one of which was used for chlorophyll (Chl) determination using a spectrophotometer (Unicam UV-330, USA) (Wellburn, 1994), and the other was dried at 60 °C for 72 h. The oven-dried samples were ground and homogenized using a SPEX 8000-D mixer mill (SPEX, Edison, NJ, USA) for soluble sugar and starch concentrations determination according to Chow and Landhausser (2004). The standard curve was established by using a series of diluted solutions of glucose. The final absorbance of total soluble sugar and starch in samples was determined spectrophotometrically at 620 nm.

2.4. Root sampling

In late October of 2014, eight seedlings selected randomly from each treatment were sampled by excavating the intact soil block of 60 cm × 60 cm × 50 cm (depth) around the seedlings to maintain

the integrity of the root system, of which five root systems were well washed with deionized water, and they were wrapped in lab tissue paper to remove water on the root surface, and then transported to the laboratory in iceboxes within 5 h. They were stored at –20 °C until later processing. The remainder of the samples was taken intact to the laboratory for ion flux determination.

2.5. Determination of root morphology and physiology

Root morphology, i.e. total root length, total surface area and average diameter, was determined using a scanner and WinRHIZO image analysis software (Régent instruments, Quebec, QC, Canada) according to the method of Nan et al. (2013). The roots were then dried at 70 °C for 48 h to a constant weight. Specific root length (SRL) and specific root surface area (SRA) were calculated as the ratio of length to biomass and the ratio of surface area to biomass, respectively. The fresh root samples were used immediately to

assess root vigor using the triphenyltetrazolium chloride (TTC) method as described by Ruf and Brunner (2003). Root activity was expressed as the amount of TTC deoxidized to triphenylformazan (TF). NR (EC 1.7.99.4) activity of roots was determined according to Corzo and Niell (1991). Five seedlings of each species randomly selected from each treatment were destructively sampled to determine final shoot, coarse and fine root (diameter ≤ 2 mm) biomass. Root/shoot mass ratio and coarse root/fine root biomass ratio, were derived based on the measured data.

2.6. Analysis of net fluxes of NH_4^+ , NO_3^- , H^+ and O_2 in roots

To investigate net fluxes of NH_4^+ , NO_3^- , H^+ and O_2 at the root surface, three white fine roots were randomly selected in the soil depth from 1 to 7 cm and excised from the intact root system of each plant from each treatment. The excised roots were immersed in measuring solution. Net fluxes of these ions were measured non-invasively using a scanning ion-selective electrode technique (SIET system, BIO-IM-XY, Younger USA Science and Technology Corp. MA, USA). A detailed description of the method has been presented elsewhere (Xu et al., 2006; Li et al., 2010; He et al., 2011). The ion-selective microelectrode with 2–4 μm aperture and the oxygen-microelectrode were manufactured and silanized by Xuyue (Beijing) Sci. & Tech. Co., Ltd., Beijing, China with a backfilling solution and an ion-selective liquid cocktail as reported by Luo et al. (2013a, 2013b). Prior to the flux measurements, the ion-selective electrodes were calibrated by a standard solution (1 mM KCl, 0.1 mM CaCl_2 and 0.1 mM NH_4NO_3) with two extra NH_4NO_3 concentrations of 0.05 and 0.5 mM for NH_4^+ and NO_3^- , pH 5.5 and pH 6 for H^+ , and O_2 concentrations of 0% and 0.21% for oxygen-microelectrode, respectively. We only used electrodes with Nernstian slopes of 58 ± 5 mV for NH_4^+ and H^+ , and -58 ± 5 mV for NO_3^- per tenfold concentration difference. The measuring solution contained 0.1 mM NH_4NO_3 , 0.1 mM CaCl_2 and 1 mM KCl. The pH of the measuring solution was 5.5. All the measurements were performed at room temperature (24–26 °C).

To monitor the positions along the root where the maximal ion influx of NH_4^+ and NO_3^- occur, a preliminary experiment was

performed according to the method of Luo et al. (2013b). The maximal root net ion uptake of *P. asperata* and *A. faxoniana* were detected in the region of 16.5–18 and 11–12 mm, respectively. Therefore, net fluxes of NH_4^+ and NO_3^- were measured in this region of both species. For the positions where the maximal net influxes of NH_4^+ and NO_3^- take place in roots, net fluxes of H^+ and O_2 were further investigated in this region as well.

2.7. Statistical analysis

Net flux data were exported and calculated using JCal V3.2.2 (a free MS Excel spreadsheet, youngerusa.com or xuyue.net). All the experimental data were tested for normality before further analyses. The effect of species and warming on variables were analyzed by two-way ANOVAs and the differences between different treatments were performed by LSD-test. Statistical significance was considered to be $P < 0.05$. Statistical package SPSS for Windows, version 17.0 (SPSS Inc, Chicago, Ill), was used for statistical analysis.

3. Results

3.1. Growth traits responses of both species to night warming

Night warming affected root morphology significantly (Table 1), both *P. asperata* and *A. faxoniana* exhibited higher total fine root length, total fine root surface area, average diameter, SRL and SRA under night warming than under control. No significant interaction of species and night warming treatment was detected in these root morphology parameters. As for the species difference, it was only found in total root length, SRL and SRA, *P. asperata* had higher total root length, SRL and SRA than *A. faxoniana*. Additionally, night warming significantly increased root and shoot biomass in the two coniferous species, but it only significantly enhanced the root/shoot mass ratio (R/S) in *A. faxoniana* (Fig. 1A, B and C). In contrast, night warming decreased the coarse root/fine root mass ratio (C/F) of both species, but significant differences were only observed in *P. asperata* (Fig. 1D). Also, night warming had no significant effect on average plant height and stem base diameter of both species (data not shown).

3.2. Physiological parameters of roots

Night warming significantly increased root TTC reducing capacity and NR activity in both species (Fig. 2). There was no species difference detected in TTC reducing capacity (Fig. 2A). Root NR activity was similar between *P. asperata* and *A. faxoniana* in control plots, but root NR activity of *P. asperata* was higher than that of *A. faxoniana* under night warming (Fig. 2B). Compared to control group, root soluble sugar and starch concentrations of both species were lower in the night warming groups (Fig. 3).

3.3. Net fluxes of NH_4^+ , NO_3^- , H^+ and O_2 of roots

Net fluxes of NH_4^+ and NO_3^- were measured along the root tips in both species, large variation at different positions were observed, and the maximal net influxes of NH_4^+ and NO_3^- occurred in the region of 16.5–18 mm in *P. asperata* and 11–12 mm in *A. faxoniana* from the root apex (data not shown). Night-warming increased the influxes of NH_4^+ , NO_3^- and O_2 and the efflux of H^+ in both species (Fig. 4). However, the effect of warming on net influxes of NH_4^+ and NO_3^- in *A. faxoniana* was not significant (Fig. 4A and B). As for the species effect, *P. asperata* had higher NH_4^+ , NO_3^- , O_2 influxes and H^+ efflux than *A. faxoniana* (Fig. 4).

3.4. Leaf physiological characteristics

Night warming significantly increased P_{max} of both species in July and October (Fig. 5). In contrast, the positive effect of night warming on P_{max} in April was observed only in *P. asperata*. We also measured AQE, Rd, Fv/Fm and Chl contents in late October, and these parameters were enhanced by night warming in both species (Table 2, Fig. 6). It was obvious that Rd and Fv/Fm were similar in both species under night warming and control (Table 2, Fig. 6). The contents of foliar soluble sugar and starch in *P. asperata* and *A. faxoniana* were significantly decreased by night warming (Table 2).

4. Discussion

4.1. The root physiological activity, morphological traits and biomass allocation in response to night warming

In the present study, the net influxes of NH_4^+ and NO_3^- were increased by night warming in both species (Fig. 4A and B), but a significant effect was observed only in *P. asperata*. Hence, the response of root N uptake rates to experimental warming, to some extent, may be species-specific. Moreover, when the measurements were made at the same temperature, roots of warmed plots had higher N uptake than that of the control plots. Therefore, *P. asperata* had acclimated to the warm temperature and the higher N uptake was not merely a result of warmed roots. Additionally, it should be noted that uptake rates of NH_4^+ were higher than that of NO_3^- in *P. asperata*, which is consistent with a previous study (Hawkins et al., 2008). However, there was no significant difference in the uptake rates of NH_4^+ and NO_3^- in *A. faxoniana*. Hawkins and Robbins (2010) reported that relative uptake rates of NH_4^+ versus NO_3^- were strongly influenced by pH. Uptake rates of NH_4^+ in Douglas-fir and lodgepole pine changed little between pH 4 and pH 7, while uptake rates of NO_3^- increased significantly between pH 4 and pH 7. All measurements in our study were carried out at pH 5.5 different from the in situ soil pH of 6.8, which would generate positive effects on rates of NO_3^- uptake, but this effect may not be detected in NH_4^+

Table 1
Root morphological characteristics of *P. asperata* and *A. faxoniana* under experimental warming and control condition at late October of 2014.

Species	Treatment	Total root length (m)	Total root surface area (cm ²)	Average diameter (mm)	Specific root length (m g ⁻¹)	Specific root surface area (cm ² g ⁻¹)	Root moisture content (%)
<i>P. asperata</i>	Control	34.85 ± 0.45 b	94.14 ± 3.85 c	0.33 ± 0.03 c	25.35 ± 2.58 b	68.44 ± 2.69 b	55.9 ± 3.2 b
	Warming	37.14 ± 1.29 a	102.26 ± 1.74 a	0.45 ± 0.04 a	29.67 ± 1.57 a	81.65 ± 2.95 a	68.2 ± 4.7 a
<i>A. faxoniana</i>	Control	29.83 ± 0.15 d	90.94 ± 0.94 c	0.41 ± 0.01 b	12.97 ± 1.29 c	39.51 ± 0.68 d	61.6 ± 5.2 b
	Warming	32.60 ± 0.38 c	96.41 ± 1.32 b	0.46 ± 0.04 a	14.83 ± 1.43 c	43.89 ± 1.73 c	73.4 ± 4.9 a
P-values	Fs	***	ns	ns	***	**	ns
	Fw	**	*	**	**	***	**
	Fs × w	ns	ns	ns	ns	ns	*

Data are mean ± SE (n = 3). Data within columns followed by different letters are significantly different. P-values of the ANOVAs of warming (Fw), species (Fs), and their interaction (Fs × w) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

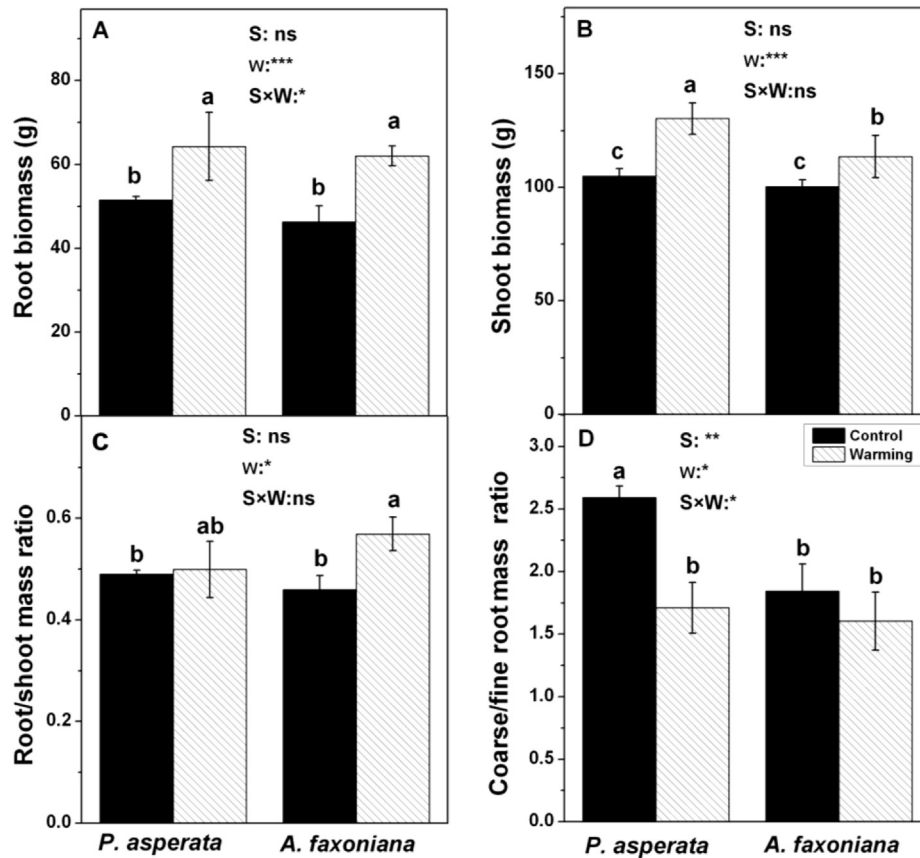


Fig. 1. The responses of root biomass (A), shoot biomass (B), root/shoot mass ratio (C) and coarse root/fine root mass ratio (D) in *P. asperata* and *A. faxoniana* at late October of 2014 to experimental warming. Data indicate mean \pm SE ($n = 5$). Bars with different letters are significantly different. *P*-values of the ANOVAs of warming (W), species (S), and their interaction ($S \times W$) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

uptake. Additionally, the extraction most probably cuts off a part of the ectomycorrhizal hyphae, which might be the reason that *A. faxoniana* have near-equal uptake rates of NH_4^+ and NO_3^- . Although the exact mechanisms behind these variations due to night warming are not well known, possible underlying mechanisms are primarily the difference in root physiological activity (Calatayud et al., 2008), morphological traits (Wan et al., 2002) and

belowground C allocation (Johnson, 2006).

Our results showed that NR activity and TTC reducing capacity of roots in both species were significantly elevated by night warming (Fig. 2A and B), which indicates that the elevated N uptake rates is related to the increased NR activity and TTC reducing capacity by experimental warming because higher NO_3^- uptake rates can be related to an increases in NR activity (Calatayud et al., 2008). TTC

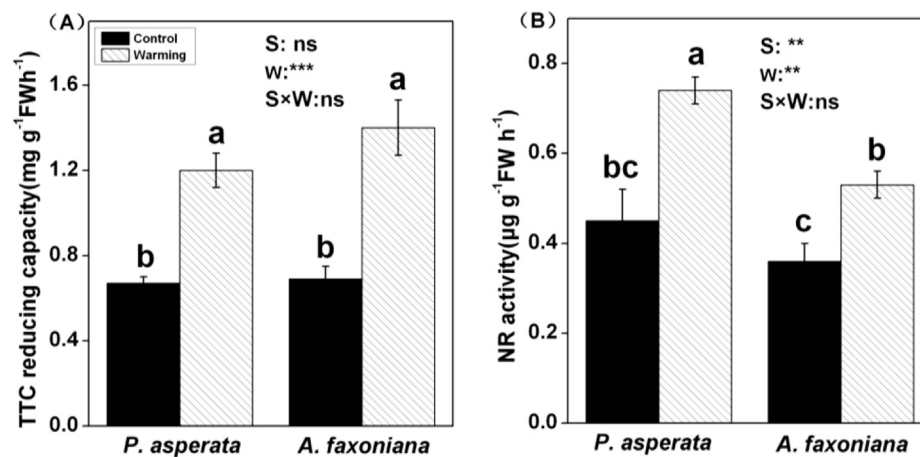


Fig. 2. Root TTC reducing capacity (A) and nitrate reductase activities (NR) (B) of *P. asperata* and *A. faxoniana* at late October of 2014 under experimental warming and control conditions. Data indicate mean \pm SE ($n = 3$). Bars with different letters are significantly different. *P*-values of the ANOVAs of warming (W), species (S), and their interaction ($S \times W$) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

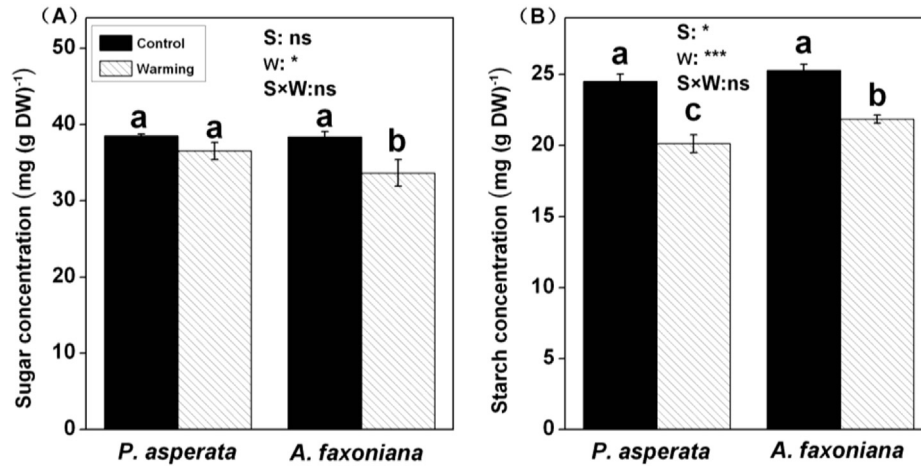


Fig. 3. Root soluble sugar (A) and starch concentration (B) of *P. asperata* and *A. faxoniana* at late October of 2014 under experimental warming and control conditions. Data indicate mean \pm SE ($n = 3$). Bars with different letters are significantly different. *P*-values of the ANOVAs of warming (W), species (S), and their interaction ($S \times W$) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

reducing capacity is commonly used as an important physiological parameter for evaluation of nutrient uptake (Huang et al., 1997; Wang et al., 2006). It has been reported that protons play a crucial role in plant uptake of NH_4^+ and NO_3^- since the H^+ gradient maintained by PM H^+ -ATPases accelerate absorption of these ions (Miller and Cramer, 2005; Britto and Kronzucker, 2006). In the current study, higher net H^+ efflux under experimental warming

than control plot was observed in both species (Fig. 4C), which is consistent with the uptake of NH_4^+ and NO_3^- . Similar results were also reported in Douglas-fir, lodgepole pine (Hawkins et al., 2008), soybean (Hawkins and Robbins, 2010) and *Populus* (Luo et al., 2013b). In addition, higher net O_2 influx (as root respiration) under artificial warming was observed (Fig. 4D), which corresponds well with the influxes of NH_4^+ , NO_3^- and H^+ efflux. Since root

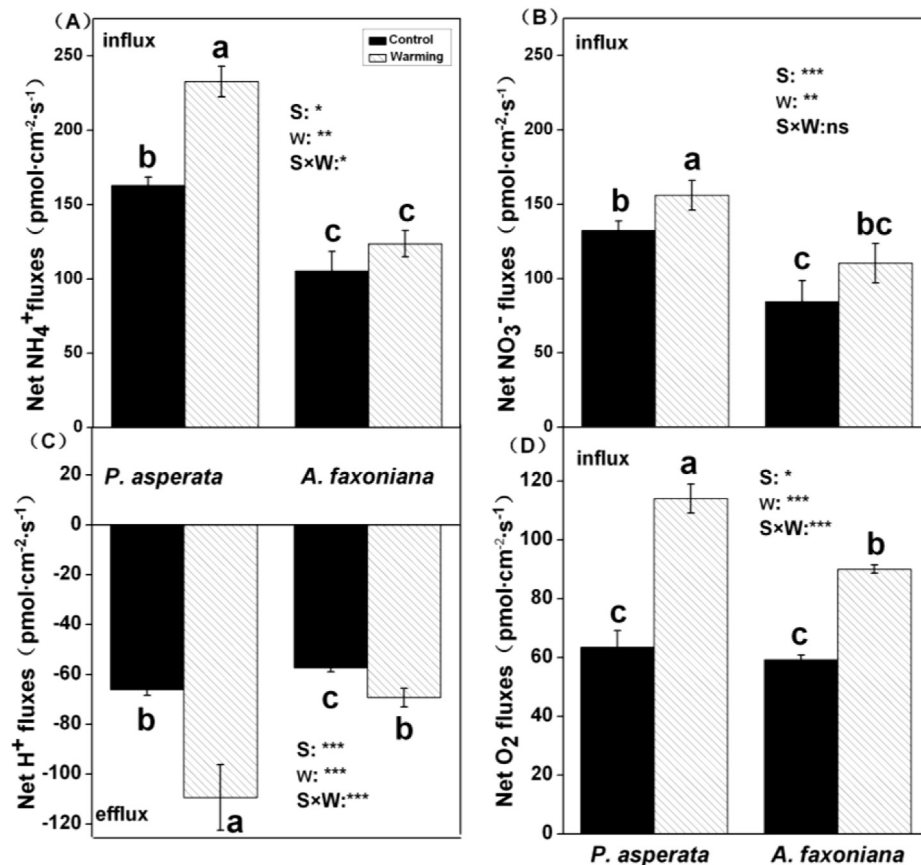


Fig. 4. Net flux of NH_4^+ (A), NO_3^- (B), H^+ (C) and O_2 (D) of *P. asperata* and *A. faxoniana* fine roots at late October of 2014. Data indicate mean \pm SE ($n = 3$). Bars with different letters are significantly different. *P*-values of the ANOVAs of warming (W), species (S), and their interaction ($S \times W$) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

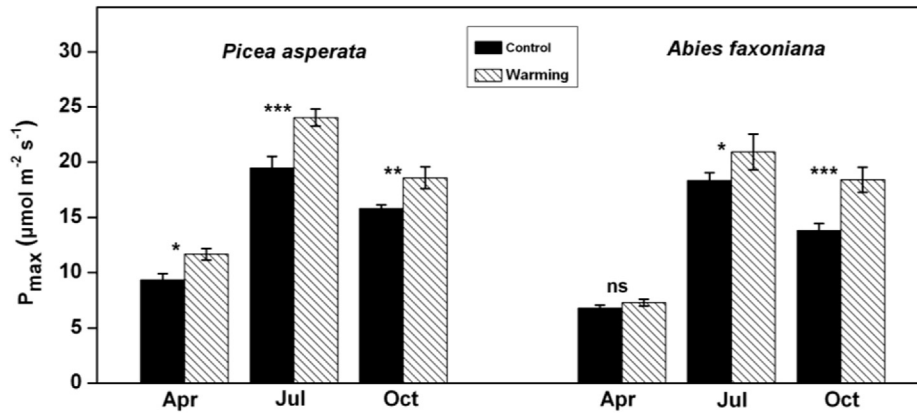


Fig. 5. The effects of experimental warming on the maximum net photosynthetic rate (P_{max}) at April, July and October. Data indicate mean \pm SE ($n = 3$). P -values of the ANOVAs of warming (W), species (S), and their interaction (S \times W) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

Table 2

Leaf photosynthesis parameters, stored photosynthate (soluble sugar and starch), specific leaf area and Fv/Fm of *P. asperata* and *A. faxoniana* under experimental warming and control conditions at late October of 2014.

Species	Treatments	Chl (a+b) concentration (mg g ⁻¹)	Specific leaf area (cm ² g ⁻¹)	Soluble sugars concentration (mg (g DW) ⁻¹)	Starch concentration (mg (g DW) ⁻¹)	Fv/Fm
<i>P. asperata</i>	Control	1.41 \pm 0.06 c	44.07 \pm 0.61 b	80.99 \pm 1.05 a	18.41 \pm 0.75 a	0.64 \pm 0.06 b
	Warming	1.63 \pm 0.05 a	58.15 \pm 4.02 a	74.70 \pm 0.42 b	16.02 \pm 0.12 c	0.80 \pm 0.03 a
<i>A. faxoniana</i>	Control	1.08 \pm 0.04 d	44.90 \pm 1.95 b	64.49 \pm 0.61 c	17.24 \pm 0.33 b	0.67 \pm 0.02 b
	Warming	1.46 \pm 0.03 b	45.24 \pm 1.54 b	52.96 \pm 0.83 d	14.94 \pm 0.11 d	0.78 \pm 0.02 a
P -values	Fs	**	*	***	*	ns
	Fw	***	*	***	**	**
	Fs \times w	ns	*	***	ns	ns

Data are mean \pm SE ($n = 3$). Data within columns followed by different letters are significantly different. P -values of the ANOVAs of warming (Fw), species (Fs), and their interaction (Fs \times w) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

respiration produces energy for nutrient uptake, an increase in respiration rate is correlated with a raise of the uptake rates (Baldi et al., 2010). Furthermore, our previous study (Yin et al., 2013) has reported that experimental warming increased ectomycorrhizal colonization in both species. Hence, we suggested that ectomycorrhizal colonization may become one of the driving forces contributing to the elevated root N uptake rates.

In addition to altered root physiological activity, higher root N uptake rates of both species under experimental warming might be associated with warming-induced changes in root morphological

traits and belowground C allocation. The roots of plants are very sensitive to variations in soil condition, and plants accommodate this change by regulating root morphological characteristics (Nibau et al., 2008; Wang et al., 2009). Our results showed that night warming had positive effects on morphological traits such as total roots length, surface area, specific root area and specific root length of both species (Table 1), which is consistent with previous studies (Luo et al., 2001; Wan et al., 2002). Furthermore, night warming resulted in higher tissue water content of root samples (the differences of fresh- and dry-weight/dry weight) than that of control

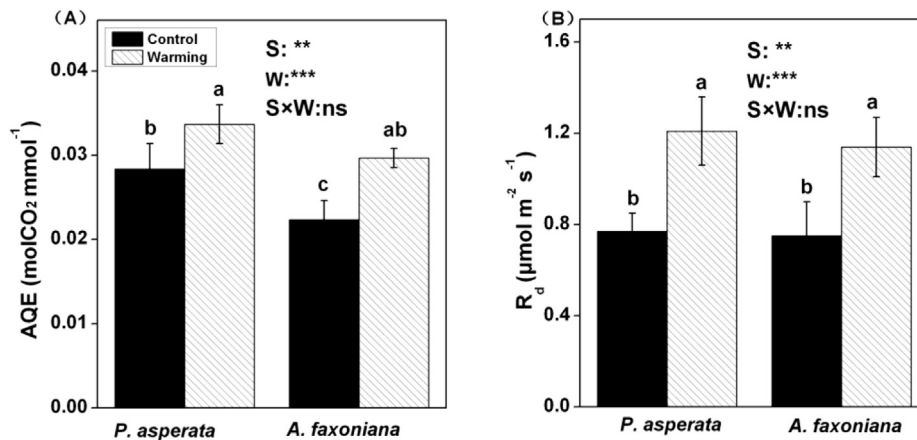


Fig. 6. Apparent quantum efficiency (AQE) (A), and dark respiration rate (R_d) (B) of *P. asperata* and *A. faxoniana* at late October of 2014 under experimental warming and control conditions. Data indicate mean \pm SE ($n = 3$). Bars with different letters are significantly different. P -values of the ANOVAs of warming (W), species (S), and their interaction (S \times W) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

plots in both species (Table 1). Therefore, although night-warming increased average diameter, dry weight per unit length in the warmed plots was still lower than that of the control plots. In addition, root samples used for the determination of root morphology were fresh samples, while root biomasses used for the calculation of specific root length and specific root area were dry weights. Therefore, night warming still increased the specific root length and specific root area when the average root diameter was greater. Also, artificial warming significantly increased the root/shoot mass ratio (R/S) in *A. faxoniana* seedlings and decreased the coarse root/fine root mass ratio (C/F) of the *P. asperata* seedlings, resulting in an elevated C partitioning to the belowground biomass and to the fine roots in response to night warming (Fig. 1). The ratios of C/F and R/S for the *A. faxoniana*, however, were not affected by artificial warming. Hence, the effects of night warming on the C allocation of plants may be largely species-specific. All in all, trees are likely to increase their belowground C allocation and fine root production to better exploit adequate nutrient resources in the rhizosphere for growth under experimental warming, which is in line with previous studies (Johnson, 2006; Bai et al., 2010). Overall, the root N uptake rates of roots acclimated by experimental warming were mainly correlated with the changes of morphological traits, elevated physiological activity and increased belowground C allocation and fine root production.

4.2. Night warming effect on photosynthesis in late growing season

Temperature is a key factor that regulates the photosynthetic processes of plants (Yordanov et al., 2000). In the present study, experimental warming significantly enhanced P_{max} in April, July and October, which represent early, middle and late growing season for the plants, respectively (Fig. 5). The results indicated that photosynthesis of both coniferous species could be accelerated by night warming across all growing season, which is in agreement with previous studies (Turnbull et al., 2004; Niu et al., 2008). We also measured AQE, R_d , Fv/Fm and Chl contents in late October. These parameters were enhanced by the experimental warming in both species, which indicated that night warming could accelerate photosynthesis by increasing Chl concentration and AQE (Awada et al., 2003).

Photosynthesis and respiration are interconnected: respiration relying on photosynthesis for substrate, and photosynthesis depends on respiration for a range of compounds, such as carbon skeletons for protein synthesis and ATP for sucrose synthesis (Xu et al., 2012b). As a result, a positive effect of experimental warming on R_d has also been found in the current study (Fig. 6B). In addition, photosynthesis that occurs during the daytime and thus is more sensitive to daily maximum temperature, whereas plant respiration occurs throughout the whole day (Atkin et al., 2013), and is therefore influenced by both daily maximum and minimum temperature (Peng et al., 2013). Leaf carbohydrates synthesized during the daytime tend to be consumed more quickly under warmer nights because of elevated leaf respiration (Griffin et al., 2002), which may induce a rebound effect of compensatory stimulated photosynthesis during the following day (Wan et al., 2009). This mechanism could partly explain the positive effect of night warming on both P_{max} and R_d in the present study.

Additionally, Fv/Fm was obviously stimulated by warming manipulation in both species (Table 2), which indicated that artificial warming enhanced the quantum efficiency of PS II for the two species. This finding is in line with previous studies (Wang et al., 2003; Yin et al., 2008). Besides, night warming had positive effect on SLA of both species in the current study (Table 2). Pyl et al. (2012) reported that lower temperature could increase leaf thickness, and therefore decrease SLA. In the current study, the air temperature (at

5 cm aboveground) and soil temperature (at 5 cm depth) within the warmed plots increased by an average of 1.95 °C and 3.10 °C, respectively, compared to the control plots. Hence, one possible explanation for the differences in SLA may be the temperature variations between warmed and control plots. Furthermore, SLA is considered to be one of the most important factor driving variation in plants relative growth rate as well as the photosynthetic ability (Yin et al., 2008). The result suggested that these seedlings had a greater ability to capture light more effectively, which is consistent with one previous study (Loveys et al., 2002).

4.3. The associations between aboveground and belowground NSC and gas exchange

Non-structural carbohydrate (NSC) is the sum of free sugars, such as sucrose, glucose, fructose, and starch (Shi et al., 2006). In the present study, the NSC of leaves and roots in both species were decreased by experimental warming (Table 2, Fig. 3). Meanwhile, positive effect of warming on R_d of leaves (Fig. 6B) and O_2 influxes of root were observed (Fig. 4D). Hence, one possible explanation for the decrease of NSC concentrations is the enhancements of leaf and root respiration which could result in increased consumption of NSC. Similar results have also been reported in Scots pine (Zha et al., 2001), Douglas-fir (Tingey et al., 2003), *Arabidopsis thaliana* (Jin et al., 2011), *A. faxoniana* (Wang et al., 2011) and *Eucalyptus sideroxylon* (Smith et al., 2012). Besides, in order to absorb water and nutrient effectively, concentration of NSC generally decreases in plants grown under warm circumstances due to the accelerated root respiration. For instance, it was reported that an increase in ion uptake efficiency will doubtlessly lead to additional energy cost (Löhmus et al., 1989). Although photosynthesis in both coniferous species was accelerated by artificial warming, the concentration of NSC was decreased in the current study supposedly as a result of the evaluated R_d of leaves and O_2 influx (as root respiration) (Griffin et al., 2002), which could, in turn, enhance the photosynthesis (Wan et al., 2009). In contrast, several studies found that warming may increase (Djanaguiraman et al., 2011) or have little effect on the concentration of NSC in plant leaves (Xu et al., 2012a). Hence, we suggested that the effects of artificial warming on the concentration of NSC are species-specific and/or dependent on the ontogenetic stage of the trees, the warming method used in experiment, and the degree of warming.

5. Conclusions

Taken together, night warming has positive effect on root and leaf physiological activity of *P. asperata* and *A. faxoniana* in the late growing season. Total root length, total root surface area, average diameter, SRL and SRA were increased by warming. Night-warming also caused significant increase in root TTC reducing capacity, NR activity, net influxes of NH_4^+ and NO_3^- , net H^+ efflux and net influx of O_2 of roots. In addition, warming enhanced foliar gas exchange such as P_n , AQE, R_d and Fv/Fm. In contrast, warming decreased NSC concentrations in leaves and roots of both species. Therefore, we conclude that: 1) night warming would enhance late autumn leaf photosynthetic rate, and 2) warming would increase nutrient uptake capacity of roots.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 31370495, 31070533 and 31400424). We would like to thank Prof. Roger Ardit, Dr. Franz Badeck and anonymous reviewers for their helpful suggestions and valuable comments.

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