Short-term responses of *Picea asperata* seedlings of different ages grown in two contrasting forest ecosystems to experimental warming

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ABSTRACT

Low temperatures are generally limiting factors in alpine ecosystems. Predicted global warming therefore could have profound impacts on these ecosystems in the future. This study was conducted to compare effects of experimental warming on the phenology, growth and photosynthesis of dragon spruce seedlings of two age classes (2- and 8-year-old seedlings) grown in two contrasting subalpine forest ecosystems (dragon spruce plantation versus spruce-fir dominated natural forest) using the open top chamber (OTC) method in the Eastern Tibetan Plateau of China. The OTCs enhanced daily mean air (30 cm above the soil surface) and soil temperatures (5 cm below the soil surface) by 1.2 °C and by 0.6 °C in two experimental sites, respectively, throughout the growing season. Conversely, soil volumetric moisture declined by 3.8% and 2.8% in the plantation and natural forest. Experimental warming markedly extended the growing season of two age classes in both sites. However, there were no clear differences in phenology between ages or sites. Warming often increased the growth and photosynthesis of dragon spruce seedlings. There were pronounced differences in the morphological and physiological performances between ages or sites. Nevertheless, there were no significant interactions of warming, age and site on phenology, growth and photosynthesis. Irrespective of seedling ages or experimental sites, artificial warming had significant increases in component biomass except the root. The size of warming effect on biomass depended strongly on seedling age and experimental site. Elevated temperatures remarkably increased net N mineralization rates and extractable inorganic N pools in both sites. Both net N mineralization and extractable inorganic N pool were pronouncedly greater in the natural forest than in the plantation. Taken together, our results indicate that warming generally has positive effects on the phenology, growth and photosynthesis for both seedling age classes in any site. Younger seedlings are more sensitive to warming as compared with the older seedlings. Reforestation dramatically affects the responses of soil N turnover and availability to warming. We conclude that both direct and indirect warming effects synchronously modify the seedling responses.

1. Introduction

Global air temperatures are predicted to increase 1.8–4.0 °C over this century, with a greater warming occurring in the higher latitudinal and altitudinal ecosystems (IPCC, 2007). With the temperature effects on almost all biochemical processes, projected global warming can directly affect the physiological performances and growth rates of vegetations (Llorens et al., 2004; Zhao and Liu, 2008). Moreover, global warming can also indirectly influence the plant physiological mechanisms and growth through altering the growing seasons, changing soil water content and increasing

soil N turnover and availability (Wan et al., 2005; Kudernatsch et al., 2008). Low temperatures, low nutrient availabilities and short growing season are generally considered to be the most important limiting factors controlling the performance of alpine plants (Gugerli and Bauert, 2001; Hyvönen et al., 2007). Such projected warming therefore has great potential to influence the establishment, survival, productivity of alpine vegetation.

In general, leaf morphology and physiology vary with tree age (Day et al., 2001; Thomas and Winner, 2002; Mediavilla and Escudero, 2003). Furthermore, many previous studies have demonstrated that juvenile age trees may be more sensitive to environmental changes (i.e., temperature, moisture) than mature trees (Howe et al., 2004; Greenwood et al., 2008), with less research focusing on the ecophysiological performances of younger, and smaller life stages (Reinhardt et al., 2009). Comparative studies of different growth stages may provide essential information for understanding the strategies adopted by the species at different

stages of their life cycle, as well as the selective pressures that operate in each stage (Mediavilla and Escudero, 2003). To our knowledge, to date, little is known about warming effects on the morphological and physiological performances of the trees with different life stages.

The sub-alpine and alpine forest ecosystems in the eastern Tibetan Plateau located at the transitive zone from Qinghai-Tibet plateau to Sichuan basin could be very sensitive to global climate change with important consequences for the global C and N balance (Wang et al., 2003). Last century, natural coniferous forests in southwestern China were deforested and reforested with dragon spruce (*Picea asperata* Mast.). Currently, there are over 1 million hectares of dragon spruce plantation in western Sichuan, accounting for approximately fifty percent of the forest area in this region. The magnitude of warming on the Tibetan Plateau is projected to be large relative to many other regions and the soils on the Tibetan Plateau contain large amounts of soil organic matter (Xu et al., 2003; Jiang et al., 2009). Thus, the soil N turnover and growth of alpine forest in this region could be more susceptible than in other ecosystems under future warmer conditions.

Ecosystem response could depend strongly on initial conditions of ecosystems, such as stocks and initial turnover rates of soil organic matter, the dominant form of available N in the soil, the chemical composition and turnover rates of plant residues (Shaver et al., 2000). Soil responses to warming are likely to be complicated by land-use change (Striegl and Wichland, 1998; Zhang et al., 2005). Reforestation is one of the most important land-use practices. This practice often has profound impacts on the soil properties of forest ecosystems. Thus, reforestation could largely affect the responses of soil ecosystems to projected global warming, and further influence the vegetations growing in these soils. Therefore, it is very important to investigate synchronously soil properties and seedling growth in different land-use types in a warming experiment, particularly in the early stages. In addition, to our knowledge, less emphasis has been placed on the comparison of the eco-physiological performances of different age classes under warming conditions. Such information could be helpful to understand the regeneration and establishment of plants under a warmer world, and to accurately predict responses of terrestrial ecosystems to future global warming. In this study, we used open top chamber (OTC) technique to simulate global warming. This experiment was conducted to investigate the effects of experimental warming on the growth and photosynthesis of dragon spruce seedlings of two age classes grown in two contrasting forest soils (dragon spruce plantation versus spruce-fir dominated natural forest). These data allow us to: (1) examine how seedlings growth and soil N turnover response to experimental warming in both ecosystems; (2) identify whether there are response differences in plant growth and soil N status between sites under the warmer conditions; and (3) examine whether there are differences in plant responses between ages.

2. Materials and methods

2.1. Experimental site

The study was conducted on two sites that were within approximately 300 m distance of each other (Fig. 1). One site was in a dragon spruce plantation (65-year-old) and the other was in a
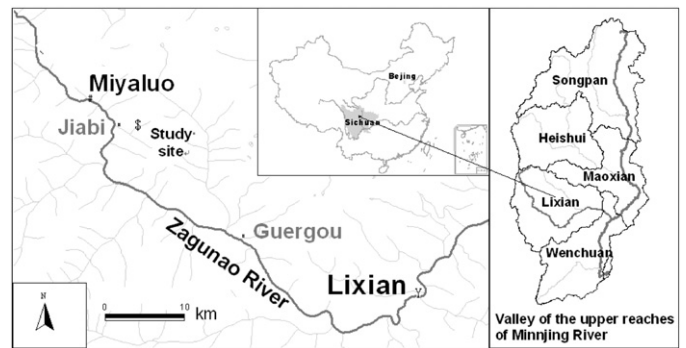

Fig. 1. Location of the study site in Miyaluo area of western Sichuan.

spruce-dominated natural forest (200-year-old). There is approximately 500 m from our study site to tree line. Both experimental sites are located at the Miyaluo Experimental Forest of Lixian County, Eastern Tibetan Plateau (31°35′N; 102°35′E; 3150 m a.s.l). This dragon spruce plantation was originated from the clear-cutting land of 1940s. The climate belongs to a montane monsoon that is humid and rainy in the summer but cold and dry in the winter. The mean annual temperature is 6–10 °C, with a maximum monthly mean air temperature of 12.6 °C in July and a minimum of −8 °C in January. Mean annual precipitation ranges from 600 mm to 1100 mm. Soils at two sites are classified as the mountain brown soil series (Chinese taxonomy). Basic soil properties are shown in Table 1.

2.2. Open top chamber installation

In order to increase the air temperature, in late September 2008, six open top chambers (OTCs) were set up in two contrasting forest sites with similar canopy, respectively. One control plot was randomly established in the vicinity of each OTC. The OTCs used in this study was made of acrylic glass, which transmits approximately 90% of photosynthetically active radiation. The OTCs was hexagonal and 80 cm high, with 2.45 m^2 in the ground area and 1.64 m^2 in open-top area. It was expected that all of the selected plots were similar in microhabitat characteristics.

2.3. Microclimate monitoring

To quantify the environmental factors affected by the OTC, two automatic recording systems were set up in both experimental sites, respectively. Air temperature at 30 cm high and soil temperature 5 cm below the soil surface were measured by alternating among sensors connected to a dataloger (Campbell AR5, Avalon, USA). Temperature monitoring was carried out in three OTCs and three control plots, respectively. Data were taken at 60 min intervals with the automatic recording system from May to October 2009.

2.4. Plant material

In our study region, 2- and 8-year old seedlings are very crucial two stages in the life history of dragon spruce. Therefore,

Forest type	SOC (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	C/N	pH
Plantation	50.24(3.83)a	2.95(0.44)a	0.65(0.03)a	14.16(0.39)a	17.03(1.05)a	6.19(0.47)a
Natural forest	145.02(14.87)b	9.56(1.19)b	0.67(0.07)a	11.31(0.51)b	15.16(0.87)b	5.85(0.65)a

Values within the same column with different letters are significantly different at $P < 0.05$.

uniform and healthy younger (2-year-old) and older (8-year-old) dragon spruce seedlings (*Picea asperata* Mast.) from a local nursery were selected based on plant height, basal diameter and fresh weight. Seedlings were transplanted into the OTCs and control plots. Each OTC or control plot was divided into four 40 cm × 40 cm subplots. Two diagonal subplots in each plot were used for planting 2-year-old seedlings; the other two were used for planting 8-year-old seedlings. Five 8-year-old and nine 2-year-old seedlings were planted in each subplot, respectively. The seedlings were transplanted in late September 2008 and the observations were initiated from late April 2009, providing more than seven months equilibrium period to minimize the transplanting disturbance.

2.5. Phenology observation

In order to observe phenological events, six plants in each age class were randomly chosen in each plot, and marked with small plastic taps before bud burst in April 2009. Bud phenology (e.g., bud break, bud set) was observed at approximately three-day interval. In this study, the beginning of bud burst was calculated at the time of 50% of bud break on the marked plants. Similarly, the beginning of bud set was calculated at the time of 50% of bud coloring on the marked branches. The growing season was defined as the interval ranging from bud break to bud dormancy.

2.6. Growth measurement

After planted, each seedling was tagged and its basal diameter and height were measured and recorded. At the end of growing season, basal diameter and height were measured again. Relative increments in basal diameter or height were then calculated for one growing season separately as (value$_{Oct}$ − value$_{Apr}$)/value$_{Apr}$ × 100. Likewise, we measured the length of current shoot originating from the top bud on tagged plants. At the end of growing season, 30 randomly selected seedlings (5 plants per plot) in each age class were destructively sampled to determine the stem, foliage, and root biomass. Plant fractions were oven-dried separately for 72 h at 80 °C and the dry mass of each fraction were determined. Dried leaves were pooled and ground to determine the total nitrogen content using UDK152 apparatus (Velp Scientifica, Ulpiate, Milan, Italy). Newly produced leaves were digitally scanned into a personal computer and then analyzed with a UTHSCSA ImageTool (IT) analysis system (University of Texas Health Science Center, San Antonio, TX, USA) to determine the leaf area. The leaves were then dried to a constant mass at 70 °C. The specific leaf area (SLA) was calculated after the dry weight was determined.

2.7. Gas exchange

A portable photosynthesis system (Model LI-6400; LI-COR, Inc., Lindoln, Nebraska) in open-circuit mode was used to determine instantaneous gas exchange on fully expanded current-year needles under controlled optimal conditions. The photosynthetic photon flux density (PPFD) was maintained at 1000 μmol (photon) m^{-2} s^{-1} using the LI-6400 artificial light source, and temperature was maintained at 20 °C with a relative humidity of 60% inside the leaf chamber during measurement. The CO_2 concentration within the leaf measurement chamber was maintained at 380 μmol m^{-2} s^{-1}. Net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) were measured. Leaves used for photosynthetic measurements were scanned and analyzed with a UTHSCSA ImageTool (IT) analysis system (University of Texas Health Science Center, San Antonio, TX, USA) to determine the leaf area. All of the measurements were completed over 7 days, from 09:00 to 11:30 each day.

Light response curves of the fully expanded current-year needles were measured at 0, 20, 50, 80, 100, 150, 200, 400, 600, 800, 1000, 1200, and 1500 μmol (photon) m^{-2} s^{-1} of photosynthetic active radiation under the uniform conditions (20 °C, 380 μmol (CO_2) m^{-2} s^{-1}, and 60% RH). The linear regressions of PAR and A over the range of 0–200 μmol (photon) m^{-2} s^{-1} of PAR were applied to determine apparent quantum yield (AQY). Maximum net assimilation rate (A_{max}), light compensation point (LCP) and dark respiration rate (R_d) were estimated according to Ye (2007).

2.8. Soil sampling and analysis

Soil samples were collected from the topsoil (0–15 cm) late in the growing season of 2009. One core (3 cm in diameter, 0–15 cm deep) was randomly taken in each subplot. The four soil cores from each plot were mixed to get one composite sample and delivered immediately to the laboratory. Each composite sample was passed through a sieve (4 mm diameter), and any visible living plant material was manually removed from the sieved soil. The sieved soil was kept in the refrigerator at 4 °C for soil analysis. Extractable inorganic N (ammonium and nitrate) was extracted with a 2 M KCl extracting water solution. Ammonium and nitrate in extract was measured by colorimetry.

Soil N mineralization rates were measured from *in situ* incubations using the buried bag technique (Adams et al., 1989). The incubations were performed using perforated PVC tubes (15 cm in height and 5 cm in diameter). Parafilm covered the top of each tube to avoid leaching of nitrate. The technique prevents plant uptake of mineralized nutrients but allows uptake by the microorganisms. The seasonal net N mineralization was expressed as the difference in inorganic N (nitrate and ammonium) in the soil before and after incubation in the bags.

2.9. Statistical analysis

Three-way analysis of variance (ANOVA) was used to examine the effects of warming, site, age and their interactions for phenological events (bud break, bud dormancy and growing season), growth parameters (specific leaf area, relative increment in basal diameter and height), and photosynthetic parameters (A, E, g_s, A_{max}, LCP, R_d, AQY and leaf N content). For specific parameter noted in this study, Student t-tests were used to compare the effect of the experimental warming. In order to assess age and site effects on plant biomass, the effect size (ES) is defined as: ES = ln(Mean$_{OTC}$/Mean$_{control}$), where Mean$_{OTC}$ is the mean for one OTC (five plant), Mean$_{control}$ is the mean for one control plot (five plant). Values above zero indicate a positive effect (warming caused an increase in the biomass). Two-way analysis of variance (ANOVA) was used to examine the effects of age, site and their interactions for effect size. Two-way analysis of variance (ANOVA) was also conducted to examine the effects of site, warming and their interaction for soil N mineralization and inorganic N pool. Before analysis, all data were tested for the assumptions of ANOVA. If data were heterogeneous, they were ln-transformed before analysis. The statistical tests were considered significant at the $P < 0.05$ level. All statistical tests were performed using the SPSS 13.0.

3. Results

3.1. Microclimate

The OTCs caused expected warming effects in both experimental sites. In contrast to the control plot, air temperature and soil temperature in the OTCs were on average increased by 1.2 and 0.6 °C in both experimental sites throughout the growing season

Table 2
Monthly mean air temperature (°C), soil temperature (°C) and soil moisture (%) in the open top chambers and control plots of two experimental sites.

Forest type	Month	Air temperature		Soil temperature		Soil moisture	
		OTC	Control plot	OTC	Control plot	OTC	Control plot
Natural forest	May	10.8	9.7	9.6	9.2	25.5	28.7
	June	11.8	10.6	10.8	10.3	28.0	29.7
	July	14.2	12.9	13.0	12.2	29.9	33.5
	August	14.3	13.1	13.1	12.5	27.3	30.8
	September	13.3	12.2	12.8	12.2	29.6	31.4
	October	6.3	5.1	7.9	7.5	36.2	39.1
Plantation	May	10.5	9.4	9.6	9.1	25.1	31.9
	June	11.9	10.6	10.9	10.3	25.8	30.0
	July	14.8	13.3	13.4	12.6	26.9	30.4
	August	14.3	13.1	13.6	12.9	28.1	31.2
	September	13.3	12.1	13.1	12.7	30.4	33.3
	October	6.5	5.3	7.5	6.8	35.5	37.8

(Table 2). Conversely, soil volumetric moisture at 10 cm depth declined 3.8% in the plantation and 2.8% in the natural forest, respectively, throughout the growing season (Table 2). Moreover, there was no obvious seasonality in soil moisture in both forest ecosystems (Table 2).

3.2. Bud phenology

Irrespective of experimental sites or seedling ages, artificial warming had remarkable effects on the phenological process of the dragon spruce seedlings (Tables 3 and 4). There was significant difference in bud break between warming regimes regardless of sites or ages (Table 3). Either 2- or 8-year-old seedlings grown in the OTCs showed significantly later bud dormancy in both experimental sites. Warming significantly extended the growing season length of the dragon spruce seedlings (Table 3). Statistical analyses show that seedling age and experimental site had no clear effects on bud phenology (Table 4). Likewise, no any interactive effect on bud phenology was detected (Table 4).

3.3. Growth parameters

Regardless of seedling ages or experimental sites, elevated temperatures significantly increased the relative growth of plant height and basal diameter, current shoot length and specific leaf area (Fig. 2; Table 4). Additionally, there was significant difference in relative increment of plant height and basal diameter, and specific

leaf area between 2- and 8-year-old seedlings (Table 4). Similarly, experimental site also pronouncedly affected the relative growth in plant height and basal diameter, but did not influence the specific leaf area (Table 4). Statistical analyses indicate that there was no any interactive effect of warming, site and age on growth parameters (Table 4).

3.4. Biomass accumulation

Irrespective of seedling ages or experimental sites, artificial warming tended to cause significant increases in component biomass (Fig. 3A–D). Experimental warming significantly increased leaf and total biomass for both seedling age classes at both sites (Fig. 3A–D). Similarly, elevated temperatures induced a significant increase in stem biomass except the younger seedlings grown in the plantation site (Fig. 3A–D). Conversely, artificial warming did not obviously influence root biomass for 2- and 8-year old seedlings in any site (Fig. 3A–D). Statistical analyses indicate warming effects on biomass, to some extent, depended on experimental site or seedling age (Fig. 4). Additionally, site and age alone significantly influenced the performances of stem and total biomass under the warming conditions (Fig. 4).

3.5. Photosynthetic parameters

Irrespective of seedling ages, artificial warming tended to stimulate the gas exchange of dragon spruce seedlings in both

Table 3
The differences (days) of phenological events between the open top chambers and control plots in both dragon spruce and natural forest.

Forest type	Seedling age	Bud break	Bud dormancy	Growing season
Plantation	2	+6.8[**]	−6.1[**]	↑12.9[**]
	8	+8.2[**]	−5.6[*]	↑13.8[**]
Natural forest	2	+7.3[**]	−5.8[**]	↑13.1[**]
	8	+7.7[**]	−4.8[*]	↑12.5[**]

"+" advanced; "−"delayed; "↑"extended.
[*] $P < 0.05$.
[**] $P < 0.01$.

Table 4
Results of three-way ANOVA showing the P values for responses of bud break, bud dormancy, growing season, relative growth rate (RGA) in diameter and height and specific leaf area to warming (W), site (S), and age (A).

Factor	Bud break	Bud dormancy	Growing season	RGR in diameter	RGR in height	Specific Leaf area
Age	0.195	0.168	0.187	<0.001[*]	<0.001[*]	0.007[*]
Site	0.368	0.327	0.348	0.008[*]	0.012[*]	0.483
Warming	<0.001[*]	<0.001[*]	<0.001[*]	0.002[*]	<0.001[*]	<0.001[*]
A × S	0.323	0.286	0.308	0.365	0.323	0.839
A × W	0.349	0.278	0.317	0.647	0.526	0.740
S × W	0.290	0.236	0.252	0.479	0.428	0.819
A × S × W	0.447	0.335	0.385	0.959	0.756	0.872

[*] $P < 0.05$.

□ 2-year-old seedlings grown in the OTCs ▨ 2-year-old seedlings grown in the control plots
■ 8-year-old seedlings grown in the OTCs ⊟ 8-year-old seedlings grown in the control plots
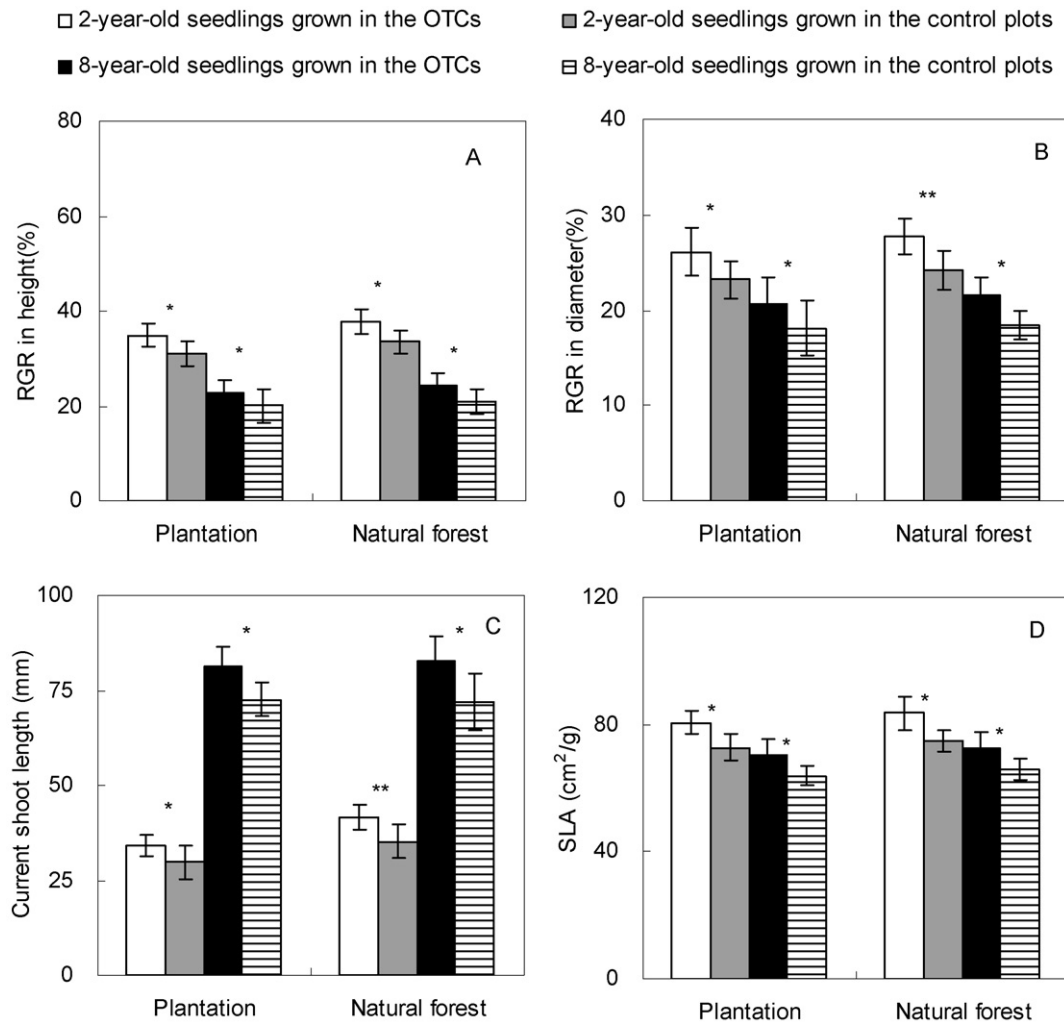

Fig. 2. Effects of warming on relative increments in plant height and basal diameter, current shoot length, and specific leaf area of 2- and 8-year-old dragon spruce seedlings grown in the plantation and natural forest soils. Student t-test, $^*P < 0.05$, $^{**}P < 0.01$; values are means ± S.D.

experimental sites (Figs. 5A–D and 6A–D). Elevated temperatures significantly increased the instantaneous net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), maximum net photosynthetic rate (A_{max}), apparent quantum yield (AQY), dark respiration rate (R_d), photosynthetic light compensation point (LCP) and leaf N concentration for both age seedlings in each experimental site (Figs. 5A–D and 6A–D). Furthermore, seedling age had significant effects on g_s, E, LCP, R_d, AQY and marginally significant effects on A, A_{max} (Table 5). Similarly, the effects of experimental site were significant on E, AQY, and leaf N concentration, but not significant on A, g_s, and LCP

(Table 5). Whereas, no any interactive effect of warming, site and age on gas exchange and leaf N concentration was detected (Table 5).

3.6. Soil N mineralization and availability

In the incubation experiments with the absence of plants, warming induced a remarkably higher net N mineralization rate in the plantation. Likewise, similar tendency was also observed in the natural forest (Fig. 7A). In addition, there was significant difference in soil N mineralization between two contrasting sites. However, the

Table 5
Results of three-way ANOVA showing the P values for responses of leaf N concentration, net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), apparent quantum yield (AQY), maximum net assimilation rate (A_{max}), light compensation point (LCP) and dark respiration rate (R_d) to warming (W), site (S), and age (A).

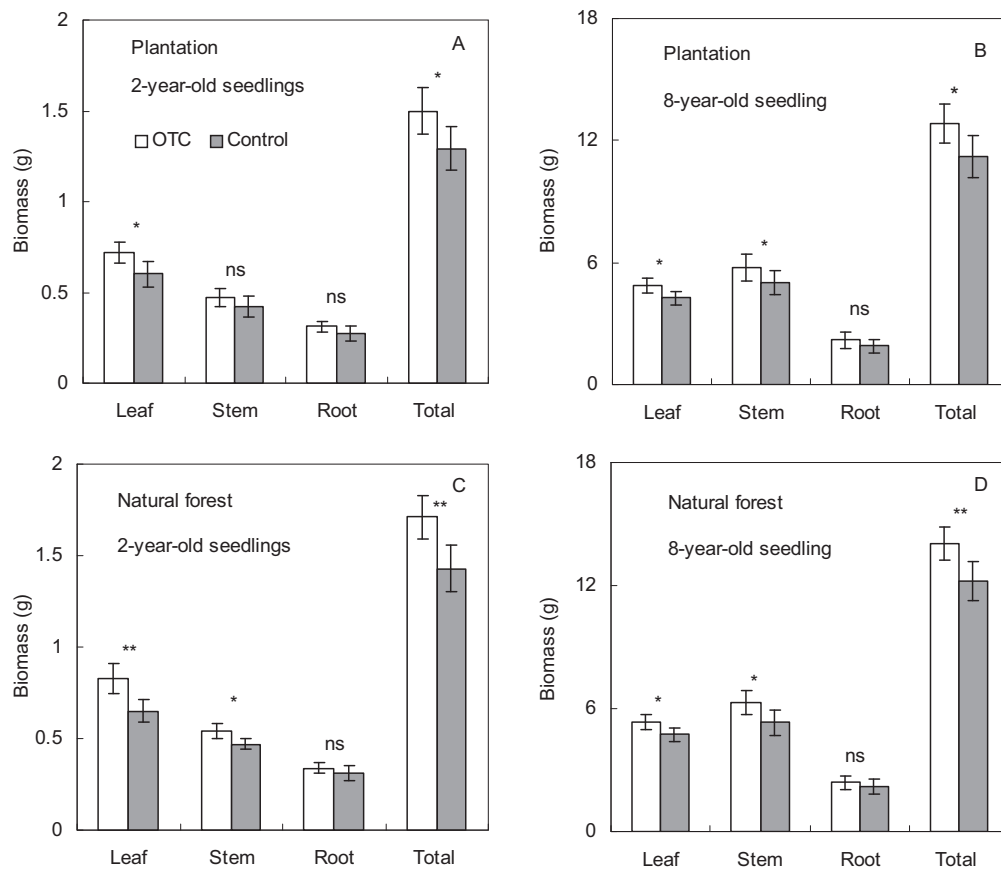| Factor	Leaf N	A	g_s	E	A_{max}	LCP	R_d	AQY
Age	0.121	0.088[**]	0.003[*]	0.001[*]	0.073[**]	0.002[*]	0.019[*]	<0.001[*]
Site	0.002[*]	0.117	0.132	0.035[*]	0.091[**]	0.148	0.063[**]	0.048[*]
Warming	0.013[*]	0.008[*]	0.001[*]	<0.001[*]	0.011[*]	0.006[*]	<0.001[*]	<0.001[*]
A × S	0.973	0.880	0.582	0.707	0.557	0.616	0.822	0.221
A × W	0.363	0.398	0.551	0.549	0.878	0.96	0.686	0.933
S × W	0.837	0.318	0.742	0.626	0.637	0.799	0.643	0.650
A × S × W	0.995	0.599	0.945	0.941	0.930	0.944	0.988	0.978

[*] $P < 0.05$.
[**] $P < 0.1$.

Fig. 3. Effects of warming on biomass (dry mass) accumulation of 2- and 8-year-old dragon spruce seedlings grown in the plantation and natural forest soils. Student t-test, *P < 0.05, **P < 0.01; values are means ± S.D.
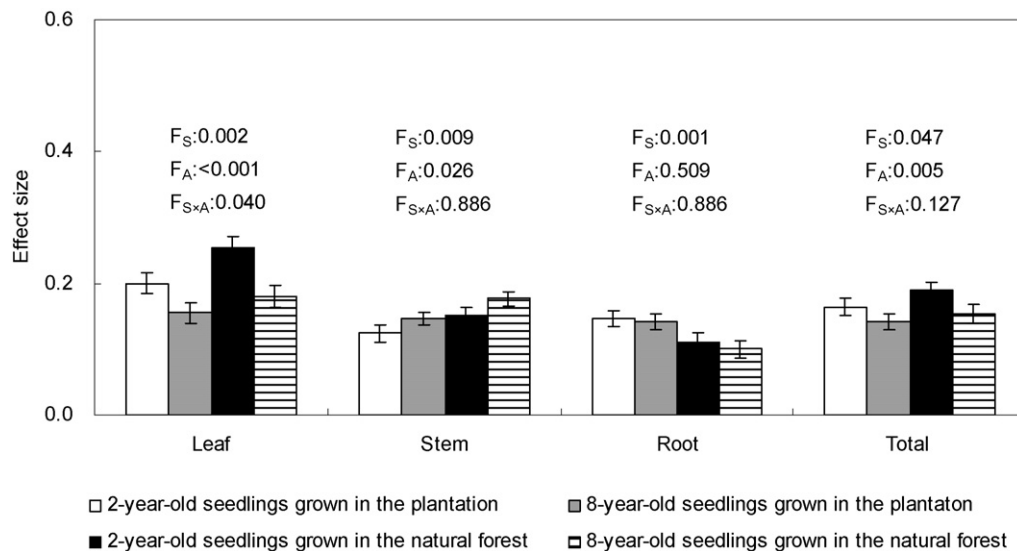

Fig. 4. Effect size in the biomass of 2- and 8-year-old dragon spruce seedlings grown in the plantation and natural forest soils. Two-way ANOVA; F_S: experimental site effect, F_A: seedling age effect, $F_{S \times A}$: interaction effect of experimental site × seedling age.

interactive effect of warming × site was not significant on soil N mineralization (Fig. 7A).

Experimental warming significantly increased the extractable inorganic N pool in both sites (Fig. 6B). Extractable inorganic N pool was markedly higher in the natural forest than in the plantation (Fig. 6B). Furthermore, the interaction of site × warming was marginally significant on the extractable inorganic N pool (Fig. 6B).

4. Discussion

4.1. Direct warming effects

4.1.1. Direct warming effect through stimulating growth rate

Temperature almost affects all biochemical processes in terrestrial ecosystems. Therefore, global warming is likely to influence
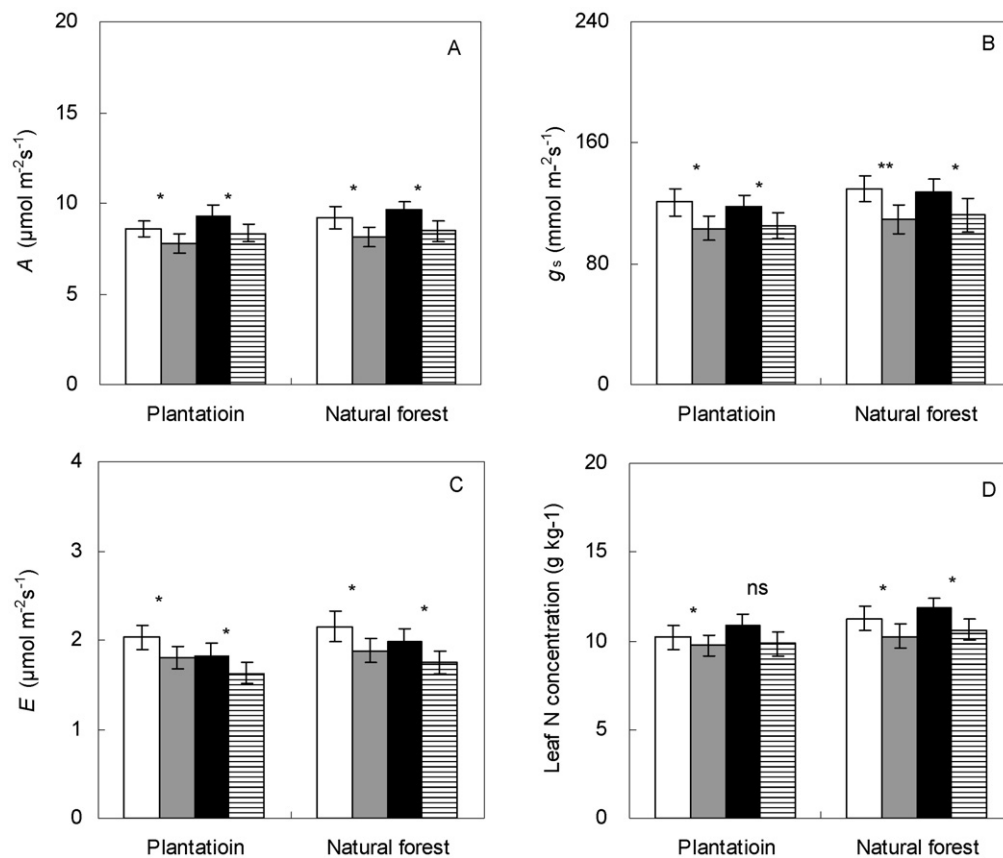
Fig. 5. Effects of warming on net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and leaf N concentration of 2- and 8-year-old dragon spruce seedlings grown in the plantation and natural forest soils. Student t-test, *$P < 0.05$, **$P < 0.01$; values are means ± S.D.; blank bar: 2-year-old seedlings grown in the OTCs; gray bar: 2-year-old seedlings grown in the control plots; black bar: 8-year-old seedlings grown in the OTCs; stripe bar: 8-year-old seedlings grown in the control plots.

plant growth through direct effects on metabolic rates, such as photosynthesis, respiration, and nutrient and water uptake (Llorens et al., 2004). Additionally, temperature is a key factor that regulates ontogenetic development rate (Morison and Lawlor, 1999). Previous studies have reported that experimental warming accelerated organ initiation and expansion rates (Wada et al., 2002; Llorens et al., 2004; Xu et al., 2009). In the present case, artificial warming stimulated the growth of 2- and 8-year-old seedlings regardless of experimental sites, judged from the increased plant height, basal diameter, current shoot length and biomass in the OTCs. Irrespective of experimental sites, elevated temperatures produced significant increases in the SLA for two age classes. Similar response was also found in other coniferous tree seedlings (Zha et al., 2002; Zhao and Liu, 2008). However, this result was different from the performances of rose hyvrida and dwarf shrubs in a middle-latitude alpine region (Suzuki and Kudo, 2000; Pandey et al., 2007). Additionally, warming pronouncedly increased the current shoot length regardless of seedling ages or experimental sites. Our results were consistent with those from other warming experiments (Wada et al., 2002; Llorens et al., 2004; Xu et al., 2009). The increased current shoot length and SLA in the warmed plots suggest that warming treatment leaded to an increase in cell number or size, or both. Biomass accumulation is an important plant response to environmental changes. Irrespective of seedling ages or experimental sites, warming tended to cause significant increases in component biomass except for root biomass. This response was possibly because the magnitude of soil warming caused by the OTCs was relatively small in both sites. Moreover, transplanting stress may, to some extent, mask the warming effects on root growth.

4.1.2. Direct warming effect through enhancing photosynthetic capacity

Low temperature stress produces a decrease in leaf gas exchange. In contrast, raising temperatures may increase the leaf size, carbon assimilation and transpiration rate in regions where low temperatures are limiting the physiological activities of plants. Additionally, global warming may induce ecophysiological changes in plants which could affect their long-term performances (Michelsen et al., 1996). Both photosynthesis and respiration are temperature dependent and among the most sensitive processes in response to global warming. Photosynthesis and respiration are interdependent, with respiration relying on photosynthesis for substrate, whereas photosynthesis depends on respiration for a range of compounds, such as carbon skeletons for protein synthesis and ATP for sucrose synthesis and repair of photosynthetic proteins (Atkin et al., 2000). The temperature-mediated differences in leaf respiration are tightly linked to concomitant differences in leaf photosynthesis (Atkin et al., 2005). A change in temperature might result in an immediate alternation in the rate of each process. In this study, elevated temperatures significantly enhanced A, A_{max} and R_d. This was possibly because that warming provided more optimal temperature conditions for gas exchange (Wang et al., 1995). Moreover, in the present case, artificial warming significantly increased the leaf N concentrations for two age classes regardless of experimental sites. The increased leaf N concentration in the OTCs may, to some extent, contribute the increased leaf photosynthesis. Because leaf N concentrations are associated closely with Rubisco content, since Rubisco constitutes about 50% of leaf protein in which 70–80% of leaf N is invested (Field and Mooney, 1986). AQY is the efficiency of light utilization in photosynthesis. In this study, experimental
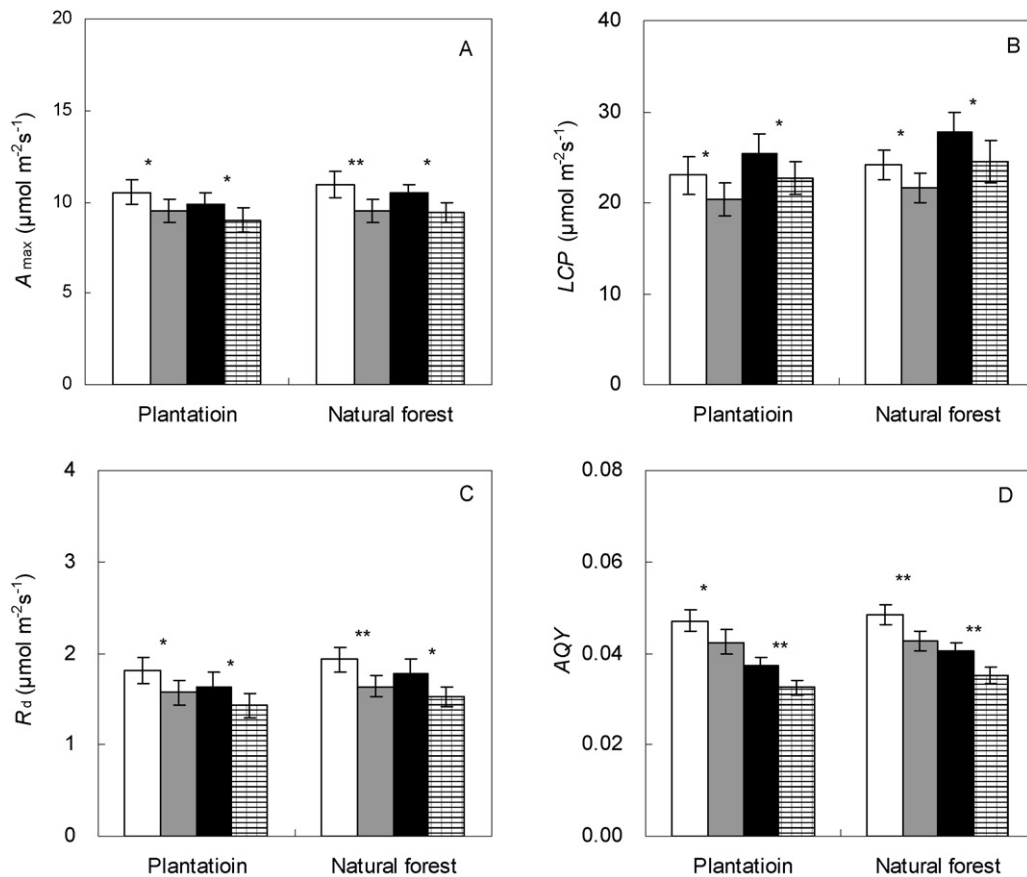
Fig. 6. Effects of experimental warming on apparent quantum yield (AQY), maximum net assimilation rate (A_{max}), light compensation point (LCP) and dark respiration rate (R_d) of 2- and 8-year-old dragon spruce seedlings grown in the plantation and natural forest soils. Student t-test, *$P < 0.05$, **$P < 0.01$; values are means ± S.D.; blank bar: 2-year-old seedlings grown in the OTCs; gray bar: 2-year-old seedlings grown in the control plots; black bar: 8-year-old seedlings grown in the OTCs; stripe bar: 8-year-old seedlings grown in the control plots.

warming also significantly increased the AQY of 2- and 8-year-old dragon spruce seedlings. Such positive effects of experimental warming on AQY have also been reported by other studies (Saxe et al., 2001; Zhao and Liu, 2008).

4.2. Indirect warming effects

4.2.1. Indirect warming effect through altering plant phenology

In combination with warming effects (e.g., extended growing season, soil nutrient availability), changes in these factors may

also influence the growth, photosynthetic performance of plants (Wan et al., 2005; Kudernatsch et al., 2008). Growing season can be extended by early bud break, late dormancy, or combination. In this study, as a result of warming effects, bud break was significantly advanced and bud dormancy was pronouncedly delayed regardless of experimental sites or seedling ages. Therefore, growing season was substantially extended by experimental warming. On the other hand, the number of days with daily mean air temperature above 10 °C was greater in the OTCs than in the control plots, also implying a longer growing season in the OTCs. The phenological
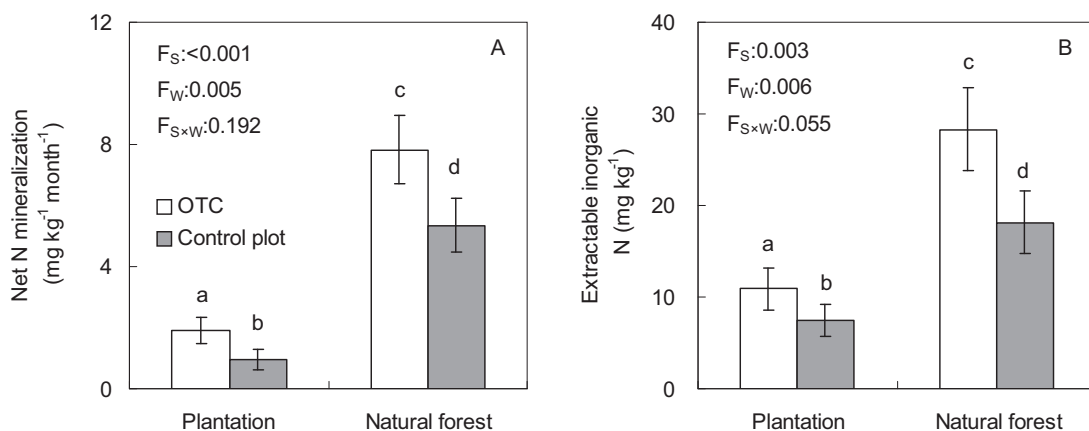

Fig. 7. Effects of experimental warming on net soil N mineralization and extractable inorganic N in two contrasting forest soils. Two-way ANOVA, Values are means ± S.D. The bars with different letters are significantly different form each other. F_W: warming effect, F_S: experimental site effect, $F_{S×W}$: interaction effect of experimental site × warming.

responses noted in this study were in good agreement with many other similar studies (Arft et al., 1999; Wada et al., 2002; Norby et al., 2003; Xu et al., 2009). The phenological differences caused by warming could benefit gross photosynthesis and plant growth (Norby et al., 2003; Wan et al., 2005). Warming-induced phenological changes would lead to a longer favorable period for plant photosynthesis and growth, resulting in increased leaf area index and green biomass during the growing season. In this study, the extended growing season could, at least partially, contribute the increased growth of dragon spruce seedlings. On the other hand, warming-induced changes in spring phenology may increase the risk of frost damage (Bokhorst et al., 2010; Taulavuori et al., 2004). However, we did not found any obvious frost injury in this study.

4.2.2. Indirect warming effect through increasing soil N availability

Warming-induced changes in the release of nutrients may affect plant response indirectly (Verburg and Breemen, 2000). Low temperature and N availability are generally considered to be the most important limiting factors controlling the performance of alpine plants (Gugerli and Bauert, 2001). Temperature is key factor that regulates the N turnover and transformation of soil ecosystems (Hart and Perry, 1999). Thus, projected global warming is likely to stimulate soil N mineralization at large. Some studies have demonstrated that soil N availability increased with soil warming. Nevertheless, these studies were mainly conducted in high-latitude ecosystems (Rustad et al., 2001; Biasi et al., 2008; Sardans et al., 2008). In this study, experimental warming caused significant increases in soil N availabilities in both forest ecosystems. Additionally, irrespective of experimental sites, net soil N mineralization rate was much higher in the OTCs versus in the control plots. Therefore, the increased N availability could be the result of the higher N mineralization induced by warming. Our result was consistent with those from other warming experiments conducted in arctic and alpine ecosystems (Rustad et al., 2001; Biasi et al., 2008). In addition, the effect size of warming on N availability was relatively great in the natural forest in comparison with the plantation. This may be explained by the differences in soil N pools and soil physical and chemical properties (relatively large bulk density, small porosity and slow decay of SOM in the plantation) between two contrasting ecosystems (Liu et al., 2002). Warming-Increased N mineralization is likely to favor net primary production (NPP) especially in areas where N is limiting NPP (Verburg and Breemen, 2000). Therefore, the increased soil N availability observed in this study could, to some extent, benefit the N uptake and growth of the seedlings.

4.3. Differences in growth and photosynthesis between age classes

Many aspects of leaf morphology and physiology may vary with tree age (Thomas and Winner, 2002; Mediavilla and Escudero, 2003). Furthermore, most previous studies have demonstrated that juvenile age classes are likely to be more sensitive to environmental stress (e.g., temperature and moisture) than mature trees (Howe et al., 2004; Greenwood et al., 2008), with less research focusing on the ecophysiological performances of younger, and smaller life stages. In this case, experimental warming stimulated the growth in both age classes. Whereas, compared with the older seedlings, warming-induced increases in growth and gas exchange were relatively great for 2-year-old seedlings, indicating superior warming sensitivity of younger seedlings. On the other hand, irrespective of warming treatments, 2-year-old seedlings often exhibited stronger performances in growth rate and photosynthesis parameters (e.g., RGA in height and diameter, SLA, *A*, *AQY*) compared to the 8-year-old seedlings in both experimental sites. Comparing the growth and ecophysiological performances among different seedling ages

may help to identify potential life-stage bottlenecks and enable early predictions about ultimate regeneration. Compared with the 8-year-old seedlings, 2-year-old seedlings allocated more assimilation to leaf growth. This was probably because very young seedlings generally lack fully expanded lateral branches and may devote more assimilation to new needle growth (Kohyama, 1983). Comparative studies of different growth stages may provide essential information for understanding the strategies adopted by the species at different stages of their life cycle, as well as the selective pressures that operate in each stage (Mediavilla and Escudero, 2003). In this case, there were clear differences in photosynthetic performances (e.g., *LCP*, *AQY* and *g*$_s$) between two age classes. This may be due to the differences in sunlight interception among the age classes or may be indicative of developmental or physiological differences between age classes. Additionally, faster growth during the early establishment may make germinant seedlings more competitive, and help them to enhance their survivals. Seedling establishment and juvenile growth are critical periods in the life cycle of tree species (Reinhardt et al., 2009). In our study site, in the natural conditions, younger seedlings often face larger stress (e.g., low light, low temperature and animal feed) as compared to the older seedlings. Because very young seedlings were much few in our studied forests (Yin et al., 2007). Thus, this stage may be a key period for dragon spruce establishment and regeneration. However, the seedlings with higher SLA in the warmed plots would be more susceptible to disease or pathogens attack. In our study, elevated temperatures resulted in stronger responses in younger seedlings as compared with the older seedlings. Therefore, projected global warming may, to some degree, favor the regeneration of dragon spruce forest.

4.4. Response differences in plant and soil between sites

Ecosystem response could depend strongly on initial conditions of ecosystems, such as stocks and initial turnover rates of soil organic matter, the relative size of the plant and soil C pools, the dominant form of available N in the soil (Shaver et al., 2000). Soil responses to warming are likely to be complicated by land-use change (Striegl and Wichland, 1998; Zhang et al., 2005). Nutrients availability, particularly N availability, is the primary limiting factor for plant productivity in most high-altitude and high-latitude ecosystems (Shaver and Chapin, 1980). In this case, N availability was much higher in the natural forest than in the plantation. Moreover, warming-induced increases in inorganic N pools were relatively great in the natural forest as compared to the plantation. In the present case, irrespective of warming treatments, the growth and physiological parameters were significantly greater in the natural forest than in the plantation. Many previous studies have demonstrated that increasing soil N availability can enhance the growth and photosynthetic mechanisms of plants (Mo et al., 2008). Therefore, the differences in seedling growth between two contrasting soils may be partly explained by the differences in the soil N availability.

5. Conclusion

First, present study demonstrated that experimental warming generally increased the growth and gas exchange of dragon spruce seedlings in both experimental sites. Also, seedling age and experimental site alone often had pronounced effects on ecophysiological performances of dragon spruce seedlings regardless of warming regimes. Nevertheless, warming effects on the growth and photosynthetic parameters noted in this study did not depend on experimental site or seedling age. On the other hand, irrespective of experimental sites and seedling ages, artificial warming generally

produced significant impacts on the component biomass of dragon spruce seedlings. Moreover, responses of component biomass to artificial warming depended partly on seedling ages and experimental sites.

Second, elevated temperatures greatly influenced the phenological events of both seedling age classes grown in both forest soils but no clear difference in phenology was detected between experimental sites or seedling ages. Obviously, phenological activities of understory dragon spruce were not associated to life stages or soil properties. Phenological events of understory dragon spruce may be mainly controlled by temperature.

Third, this study has also demonstrated that warming significantly increased net soil N mineralization in both forest ecosystems. Additionally, higher exactable inorganic N pool was also observed in the warmed plots, indicating that experimental warming increased soil N turnover rate and N availability in two forest ecosystems. The responses of N transformation to experimental warming depended partly on forest types. Moreover, significant differences in N pools between two contrasting soils may, to some degree, explain the differences in growth and physiological performances of dragon spruce seedlings between sites.

Finally, one growing season study could not thoroughly reflect the responses of these seedlings to the artificial warming. However, short-term treatment can, at least, elucidate any response patterns between sites and ages at early stage of experimental warming. Although a seedling study may not well-predict the performance of mature tree, physiological and morphological responses of different age classes to warming may indicate the observed results from seedlings may, to some extent, overestimate the positive effects of projected global warming on forest productivity. Evidence of an inter-relation between growth and experimental site suggests that warming impacts on tree growth could be related closely to soil properties, especially soil N status under future warming conditions. Apparently, responses of forest ecosystems to projected global warming could be greatly complicated by land-use changes.

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