ORIGINAL PAPER

Species-specific and needle age-related responses of photosynthesis in two *Pinus* species to long-term exposure to elevated CO_2 concentration

Yu-Mei Zhou · Cun-Guo Wang · Shi-Jie Han · Xu-Bing Cheng · Mai-He Li · A-Nan Fan · Xiu-Xiu Wang

Abstract There is, so far, no common conclusion about photosynthetic responses of trees to long-term exposure to elevated CO_2. Photosynthesis and specific leaf area (SLA) of 1-year-old and current-year needles in *Pinus koraiensis* and *P. sylvestriformis* grown in open-top chambers were measured monthly for consecutive two growing seasons (2006, 2007) after 8–9 years of CO_2 enrichment in northeastern China, to better understand species-specific and needle age-related responses to elevated CO_2 (500 μmol mol^{-1} CO_2). The light-saturated photosynthetic rates (P_{Nsat}) increased in both species at elevated CO_2, but the stimulation magnitude varied with species and needle age. Photosynthetic acclimation to elevated CO_2, in terms of reduced V_{cmax} (maximum carboxylation rate) and J_{max} (maximum electron transport rate), was found in *P. koraiensis* but not in *P. sylvestriformis*. The photosynthetic parameters (V_{cmax}, J_{max}, P_{Nsat}) measured in different-aged needles within each species responded to elevated CO_2 similarly, but elevated CO_2 resulted in much pronounced variations of those parameters in current-year needles than in 1-year-old needles within each species. This result indicated that needle age affects the magnitude but not the patterns of photosynthetic responses to long-term CO_2 enrichment. The present study indicated that different species associated with different physioecological properties responded to elevated CO_2 differently. As global change and CO_2 enrichment is more or less a gradual rather than an abrupt process, long-term global change experiments with different plant species are still needed to character and better predict the global change effects on terrestrial ecosystems.

Keywords Global environmental change · Photosynthetic acclimation · Photosynthetic parameters · Photosynthetic responses · Specific leaf area

Communicated by U. Luettge.

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Abbreviations
AQE   Apparent quantum yield
P_{Nsat} Light-saturated photosynthetic rate
V_{cmax} Maximum carboxylation rate
J_{max} Maximum electron transport rate
SLA   Specific leaf area

Introduction

Photosynthetic responses to elevated CO_2 concentrations have been widely studied during the past decades (Wang et al. 1995; Curtis et al. 2000; Ghannoum et al. 2010). The

effects of elevated CO_2 concentration on photosynthesis were found to be dependent on plant species, environmental conditions, duration of CO_2 exposure, and growth stage of plants (Tissue et al. 1997; Ainsworth et al. 2002; Sigurdsson et al. 2002; Aranjuelo et al. 2009). After 3 years of CO_2 enrichment (704 μmol mol^{-1} CO_2), no change in photosynthetic capacity of *Quercus myrtifolia* and *Q. chapmanii* was found, but the co-dominant *Q. geminata* showed a photosynthetic down-regulation (Ainsworth et al. 2002). During the first 3 years of 700 μmol mol^{-1} CO_2 exposure, no evidence for photosynthetic down-regulation in *Citrus aurantium* was found, but after 10 years of CO_2 enrichment, the trees became acclimatized to elevated CO_2 showing a decline in photosynthesis (Idso and Kimball 1991, 2001; Adam et al. 2004).

It is still not fully explained whether and why long-term exposure to elevated CO_2 down-regulates plant photosynthesis. Theoretically, increased CO_2 concentration could stimulate C_3 plants' photosynthetic rate, because the current ambient CO_2 concentration is much lower than their CO_2 saturation point (Long et al. 2004). Results gained from experiments with free-air CO_2 enrichment (FACE) or open-top chambers (OTCs) showed that photosynthetic capacity could be stimulated (Sholtis et al. 2004; Rasse et al. 2005; Liberloo et al. 2007), sustained (DeLucia and Thomas 2000; Gielen and Ceulemans 2001; Ainsworth et al. 2003; Calfapietra et al. 2005), or down-regulated (Medlyn et al. 1999; Lee et al. 2001) in plants after long-term exposure to elevated CO_2 concentrations.

Woody plant species grown in FACE or CO_2 springs often show no photosynthetic down-regulation (Stylinski et al. 2000; Springer et al. 2005; Davey et al. 2006; Liberloo et al. 2007) but an increase in photosynthetic rate despite decreased photosynthetic capacity (Ainsworth et al. 2003). For example, there was no photosynthetic down-regulation in *Liquidambar styraciflua* L. trees exposed to elevated CO_2 over 3 years (Herrick and Thomas 2001). After 10 years of CO_2 enrichment (600 μmol mol^{-1} CO_2), *Lolium perenne* L. cv. Bastion still had a 43% increase in light-saturated photosynthetic rate (P_{Nsat}) and 36% stimulation of daily carbon uptake (Ainsworth et al. 2003). However, some tree species (*Picea abies* (L.) Karst., *Betula pendula* Roth and *Prunus avium* L. × *pseudocerasus* Lind.) exhibited a down-regulation of photosynthesis after long-term exposure to elevated CO_2 (Atkinson et al. 1997; Roberntz and Stockfors 1998; Riikonen et al. 2005). The photosynthetic down-regulation or acclimation was found to be associated with a reduction in maximum carboxylation rate (V_{cmax}) and RuBP regeneration capacity mediated by the maximum electron transport rate (J_{max}) (Calfapietra et al. 2005), and also often with limitations of nutrient and sink strength (Gunderson and Wullschleger 1994). Herrick and Thomas (2001) found that the P_{Nsat} in elevated CO_2

treated *L. styraciflua* increased (+48%), and V_{cmax} and J_{max} did not change with CO_2 treatments. Sigurdsson et al. (2002) and Ainsworth et al. (2003) stated that plants growing at elevated CO_2 had an increased P_{Nsat} in spite of whether photosynthetic down-regulation occurred or not.

Needle age may be an important factor in determining the physioecological adaptation of conifers to environmental change (Turnbull et al. 1998; Zha et al. 2002; Crous and Ellsworth 2004; Crous et al. 2008). Different-aged needles of conifers show differences in eco-physiology (Wang et al. 1995) and, thus, may respond to CO_2 enrichment differently (Crous and Ellsworth 2004). The P_{Nsat} of both 1- and 2-year-old needles in *Pinus sylvestris* increased, but the 2-year-old needles had a smaller P_{Nsat} than the 1-year-old needles at elevated CO_2 condition (Wang et al. 1995), indicating a needle age effect on the photosynthetic responses to elevated CO_2. Similarly, the stimulation effect of elevated CO_2 on photosynthesis was found to be less marked in 1-year-old needles compared to current-year needles in *P. sylvestris*, *P. sitchensis* (Medlyn et al. 1999) and *P. taeda* L. (Crous and Ellsworth 2004). Down-regulation of photosynthesis was evident in 1-year-old but not in current-year needles of *Pinus radiata* in response to elevated CO_2 (Turnbull et al. 1998). These results suggest that relatively older needles are prone to acclimatizing themselves to changes in CO_2 concentration. Some studies stated that photosynthetic acclimation occurred only in older foliage, or earlier in older than in younger leaves (Turnbull et al. 1998; Griffin et al. 2000; Jach and Ceulemans 2000a; Laitinen et al. 2000; Tissue et al. 2001).

Photosynthetic responses of tree species belonging to different genera or families are obviously different. For example, Karnosky et al. (2003) described that 3-year elevated CO_2 (560 μmol mol^{-1} CO_2) increased the upper canopy light-saturated CO_2 assimilation rate in *Populus tremuloides* (+33%) and in *Betula papyrifera* (+64%), but not in *Acer saccharum*. For different species belonging to the same genus, Ainsworth et al. (2002) found that *Q. myrtifolia* and *Q. chapmanii* did not show any effects of elevated CO_2 (704 μmol mol^{-1} CO_2) on photosynthetic capacity, but the co-dominant *Q. geminate* showed significantly negative CO_2 effects on photosynthetic capacity. On the other hand, Bernacchi et al. (2003) reported that light-saturated and daily integrated photosynthesis in three *Populus* species increased similarly at elevated CO_2 (550 μmol mol^{-1} CO_2). Such somewhat contradictory findings mentioned above prompted us to test the long-term CO_2 effects on photosynthesis in other tree species growing in other regions. We examined the photosynthesis of current-year and 1-year-old needles in *P. koraiensis* Sieb. et Zucc. and *P. sylvestris* var. *sylvestriformis* exposed to elevated CO_2 (500 μmol mol^{-1} CO_2) for 8–9 years

(1999–2006/2007) in northeastern China. Our hypothesis are that (1) different species of the same genus respond to long-term elevated CO_2 exposure similarly; (2) photosynthetic stimulation will still be detectable in trees after 8–9 years of CO_2 enrichment; (3) different-aged needles respond to elevated CO_2 differently; and (4) the photosynthetic acclimation responses to elevated CO_2 are greater in older needles than in younger ones since the former exposed longer than the latter to elevated CO_2. The rationale behind the selection of those two *Pinus* species for the study is that the two protected species would be sensitive to global environmental change since they are distributed only in very limited area but with important ecological and economic significance.

Materials and methods

Experimental plants and CO_2 treatments

The experiment was conducted at the research station of Changbai Mountain Forest Ecosystem (42°24′N, 128°05′E, 738 m a.s.l.), Jilin Province, northeastern China. The annual mean air temperature is 3.6°C and mean precipitation is 695 mm (Guan et al. 2006). The mean temperature and precipitation during the growing season (May–October) are 14.9°C and 530 mm, respectively. Small OTCs ($1 \times 0.9 \times 0.9$ m³) were established at the research station in May 1999. Seeds of *P. koraiensis* and *P. sylvestriformis* were sown into uniform local forest soil with a total organic carbon of 5.5% and a total nitrogen of 0.3% in the OTCs in May 1999. Seeds of the two pine species were obtained from a plantation nearby. As plants got larger and denser, eight hexagonal OTCs (4.0 m in both height and diameter) were established. The walls of OTCs were built with clear glass to maximize penetration of solar radiation. An average penetration ratio of solar radiation was ∼70% in sunny days. Sapling of *P. koraiensis* and *P. sylvestriformis* were separately transplanted at 0.5 m × 0.5 m spacing into the new OTCs in September 2003, according to their CO_2 treatments. *P. sylvestriformis* is a fast-growing species, and *P. koraiensis* is a relative slow-growing species. At the beginning of the present study in May 2006, *P. koraiensis* was 8 years old with 60 ± 12 cm in height and 1.8 ± 0.3 cm in base diameter, and *P. sylvestriformis* was also 8 years old with 160 ± 40 cm in height and 3.0 ± 0.9 cm in base diameter.

CO_2 treatments (ambient CO_2 concentration of 370 µmol mol⁻¹ CO_2 for 4 chambers, and elevated CO_2 concentration of 500 µmol mol⁻¹ CO_2 for other 4 chambers) began in the small OTCs in June 1999, and continued

in the big OTCs till now. Elevated CO_2 has been supplied to the chambers by pipes connected to an industrial CO_2 tanks. The concentrated CO_2 was pumped into the chambers from a height of 1.6 m and was diffused. A concentration of 500 µmol mol⁻¹ CO_2 was automatically controlled by CO_2 sensors (SenseAir, Sweden) installed in the center of each chamber. Elevated CO_2 was supplied day and night (24 h day⁻¹) during the growing season (May–October) from 1999 to 2005. Thereafter, CO_2 has been added only for the daytime during the growing season since 2006. All chambers accepted natural rainfall during the experimental period. The mean growing season temperature in OTCs was higher (+2.4°C) compared to the unchambered field (Li et al. 2009), but there was no significant difference in air and soil temperature between OTCs treated with elevated CO_2 and ambient CO_2 during the experimental period. Hence, the present study can compare the CO_2 effects among the OTCs.

Gas exchange measurements

Gas exchange of 1-year-old and current-year needles in *P. koraiensis* and *P. sylvestriformis* was measured in situ at mid-month from May to October during the eighth and ninth growing season (2006, 2007) of CO_2 enrichment, using a portable open-system gas analyzer (Li-6400, LiCor Inc., Lincoln, NE, USA). Three plants were randomly selected in each chamber for the measurements. 1-year-old and current-year needle fascicles from non-shaded top shoots in each selected plant were carefully, separately put into the conifer chamber. Measurements were made at ambient temperature on clear days between 0830 and 1130 hours at each measurement date. Only 1-year-old needles were measured in May because the current-year needles just emerged.

After the photosynthesis measurement, the needles used for gas exchange analysis were removed from the shoots to determine the needle area and mass. Needle area was measured using a leaf area analyzer (Li-3000, LiCor Inc., Lincoln, NE, USA). Needle mass was weighed to the nearest 0.001 g after oven-drying at 80°C for 24 h. Specific leaf area (SLA) is defined as the ratio of needle area to dry mass.

The response curves (P_N/C_i) of net photosynthetic rate (P_N) to intercellular CO_2 concentration (C_i) were generated by controlling external CO_2 concentrations at 9 levels from 0 to 1,000 µmol mol⁻¹ CO_2 under a constant photon flux density of 1,300 µmol m⁻² s⁻¹ (saturating or near-saturating light) provided by a LED light source. Water vapor pressure deficit was generally held between 1.0 and 1.5 kPa. The response curves (P_N/PAR) of P_N to photosynthetically active radiation (PAR) were measured at

growth CO_2 concentrations (370 μmol mol^{-1} CO_2 for ambient and 500 μmol mol^{-1} CO_2 for enrichment) by increasing irradiance at 12 levels from 0 to 1,300 μmol m^{-2} s^{-1} provided by a LED light source. Since P_N/C_i and P_N/PAR curves measured in different months showed similar pattern, only the P_N/C_i curve measured in July 2006 and the P_N/PAR curve measured in August 2006 as representative curves were presented (shown in Figs. 1, 2).

Both P_N/C_i and P_N/PAR curves were calculated using the Photosyn Assistant software (Dundee Scientific, Dundee, UK). Two important parameters that can potentially limit photosynthesis, V_{cmax} (maximum carboxylation rate of Rubisco) and J_{max} (RuBP regeneration capacity mediated by maximum electron transport rate), were modeled by analyzing P_N/C_i curves. Light-saturated photosynthetic rate (P_{Nsat}) and apparent quantum yield (AQE) were determined by analyzing P_N/PAR curves.

Statistical analyses

The normality test and Levene's test to check the equality of variances were carried out on datasets prior to statistical analyses to verify a normal distribution and the homogeneity of the variances. We used paired-samples t test to test the differences in photosynthetic parameters and SLA between elevated and ambient CO_2 for each tree species within each measurement date, but only the annual mean values (±SE) of the parameters were presented in Table 1 to character the two species. Repeated-measures analyses of variance (RM ANOVAs) were used to analyze the effects of CO_2 treatment, needle age, and their interaction within each species, and the effects of species, CO_2 treatment, needle age, and their two-way and three-way interactions across species (results shown in Table 2). All statistical analyses were performed at 0.05 level with SPSS 13.0 software (SPSS Inc, Chicago, IL).
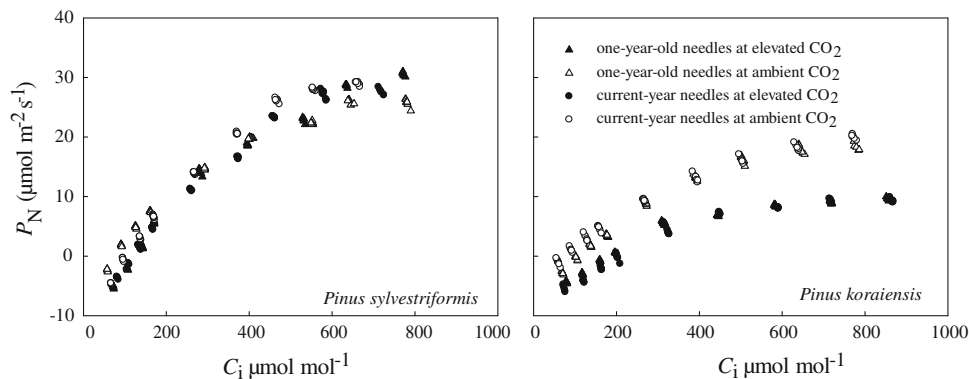

Fig. 1 Responses of net photosynthetic rate (P_N) to intercellular CO_2 concentration (C_i) of 1-year-old (*closed triangles*, *open triangles*) and current-year needles (*closed circles*, *open circles*) of *Pinus sylvestriformis* and *P. koraiensis* exposed to ambient (370 μmol mol^{-1}, *open circles and open triangles*) and elevated (500 μmol mol^{-1}, *closed circles and closed triangles*) CO_2 concentrations for eight growing seasons (1999–2006) in northeastern China. Measurements were carried out on mid-July 2006
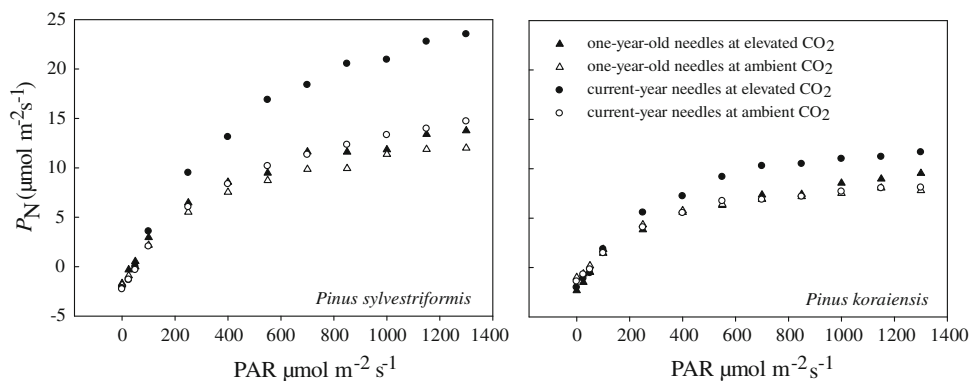

Fig. 2 Responses of net photosynthetic rate (P_N) to photosynthetically active radiation (PAR) of 1-year-old (*closed triangles*, *open triangles*) and current-year needles (*closed circles*, *open circles*) of *Pinus sylvestriformis* and *P. koraiensis* exposed to ambient (370 μmol mol^{-1}, *open circles* and *open triangles*) and elevated (500 μmol mol^{-1}, *closed circles* and *closed triangles*) CO_2 concentrations for eight growing seasons (1999–2006) in northeastern China. Measurements were carried out on mid-August 2006

Table 1 Photosynthetic parameters and specific leaf area (mean values ± SE) in two needle age classes of *Pinus sylvestriformis* and *P. koraiensis* exposed to elevated (500 μmol mol⁻¹ CO_2) and ambient CO_2 (370 μmol mol⁻¹ CO_2) for nine growing seasons (1999–2007) in northeastern China

	Pinus sylvestriformis			*Pinus koraiensis*		
	Elevated CO_2	Ambient CO_2	P	Elevated CO_2	Ambient CO_2	P
One-year-old needles						
V_{cmax} (μmol m⁻² s⁻¹)	57.9 ± 5.4	65.5 ± 7.7	ns	31.3 ± 4.5	37.6 ± 3.0	0.021
J_{max} (μmol m⁻² s⁻¹)	140.9 ± 12.8	137.5 ± 12.1	ns	80.2 ± 10.9	92.5 ± 9.5	0.021
AQE (μmol photons m⁻² s⁻¹)	0.046 ± 0.005	0.043 ± 0.005	ns	0.041 ± 0.005	0.036 ± 0.004	ns
P_{Nsat} (μmol m⁻² s⁻¹)	15.6 ± 1.4	11.8 ± 1.0	<0.001	12.5 ± 1.5	10.6 ± 1.4	ns
SLA (cm² g⁻¹)	49.0 ± 1.5	48.8 ± 1.0	ns	50.3 ± 1.4	50.0 ± 1.1	ns
Current-year needles						
V_{cmax} (μmol m⁻² s⁻¹)	53.4 ± 8.2	61.6 ± 12.3	ns	25.1 ± 2.1	31.9 ± 2.7	<0.001
J_{max} (μmol m⁻² s⁻¹)	129.4 ± 15.7	149.1 ± 28.9	ns	75.1 ± 5.5	93.8 ± 9.8	0.004
AQE (μmol photons m⁻² s⁻¹)	0.064 ± 0.010	0.060 ± 0.011	ns	0.053 ± 0.007	0.048 ± 0.008	ns
P_{Nsat} (μmol m⁻² s⁻¹)	22.2 ± 1.8	16.7 ± 1.4	0.002	15.8 ± 1.6	11.7 ± 1.7	<0.001
SLA (cm² g⁻¹)	64.9 ± 4.2	64.0 ± 2.3	ns	58.1 ± 1.2	62.9 ± 1.6	0.017

V_{cmax}, maximum carboxylation efficiency (μmol m⁻² s⁻¹); J_{max}, maximum electron transport rate (μmol m⁻² s⁻¹); *AQE*, apparent quantum yield (μmol photons m⁻² s⁻¹); P_{Nsat}, light-saturated photosynthetic rate (μmol m⁻² s⁻¹); *SLA*, specific leaf area (cm² g⁻¹)

Statistically significant difference ($p < 0.05$) was given between elevated and ambient CO_2 treatments within each category and species. *ns* non-significant ($p > 0.05$)

Table 2 Effects of CO_2 treatments (elevated CO_2 of 500 μmol mol⁻¹ CO_2 and ambient CO_2 of 370 μmol mol⁻¹ CO_2), needle age (1-year-old needles and current-year needles), and their interactions on photosynthetic parameters and specific leaf area for *Pinus* *koraiensis* and *P. sylvestriformis* exposed to CO_2 treatments for nine growing seasons (1999–2007) in northeastern China, tested using repeated-measures ANOVA

	V_{cmax}		J_{max}		AQE		P_{Nsat}		SLA	
	F	P	F	P	F	P	F	P	F	P
Pinus sylvestriformis										
CO_2 (C)	1.081	ns	0.985	ns	0.492	ns	11.042	0.01	0.097	ns
Age (A)	41.722	0.003	4.488	ns	6.996	0.029	8.166	0.021	85.217	<0.001
C × A	2.308	ns	0.029	ns	0.040	ns	0.107	ns	0.123	ns
Pinus koraiensis										
CO_2 (C)	13.366	0.011	32.171	0.001	6.386	0.035	4.416	ns	0.907	ns
Age (A)	4.331	ns	6.548	0.043	15.725	0.004	9.344	0.016	51.486	<0.001
C × A	0.383	ns	4.258	ns	3.887	ns	0.285	ns	0.533	ns

V_{cmax}, maximum carboxylation efficiency (μmol m⁻² s⁻¹); J_{max}, maximum electron transport rate (μmol m⁻² s⁻¹); *AQE*, apparent quantum yield (μmol photons m⁻² s⁻¹); P_{Nsat}, light-saturated photosynthetic rate (μmol m⁻² s⁻¹); *SLA* specific leaf area (cm² g⁻¹)

F and *P* values are given. *ns* non-significant ($p > 0.05$)

Results

Light- and CO_2-saturated photosynthesis after long-term CO_2 enrichment

After 8–9 years of CO_2 enrichment, CO_2-saturated photosynthesis of the two species differed in responses to intercellular CO_2 concentration. *P. sylvestriformis* had higher photosynthetic rate than *P. koraiensis* when compared at the same intercellular CO_2 concentration (Fig. 1).

The magnitude and shape of P_N/C_i response curves did not vary with needle age and CO_2 treatments in *P. sylvestriformis* (Fig. 1). Needle age had no effects on P_N/C_i responses in both species (Fig. 1). However, *P. koraiensis* at elevated CO_2 had lower photosynthetic rates than at ambient CO_2 (Fig. 1).

The net photosynthetic rate in both species showed similar needle age- and CO_2 treatment-related responses to PAR, but *P. sylvestriformis* had higher photosynthetic rate than *P. koraiensis* at the same radiation level (Fig. 2). For

both the species, the current-year needles at elevated CO_2 had the highest photosynthetic rate (Fig. 2). Elevated CO_2 stimulated photosynthetic rate within each needle age class in *P. sylvestriformis* at any given light radiation level (Fig. 2). But only the photosynthetic rate of the current-year needles in *P. koraiensis* was stimulated by elevated CO_2 (Fig. 2).

Seasonal dynamics of photosynthetic parameters

There were pronounced seasonal fluctuations in photosynthetic parameters for both species during the measurement period (Fig. 3). P_{Nsat}, AQE, V_{cmax}, and J_{max} of different-aged needles in both species exhibited similar patterns of change over time at either elevated or ambient CO_2 conditions, although *P. sylvestriformis* had often higher values of P_{Nsat}, V_{cmax}, and J_{max} than *P. koraiensis* within each measurement date (Fig. 3). CO_2 treatments did not affect the seasonal patterns of the parameters in both species, but stimulated the magnitude of the parameters (Fig. 3). The P_{Nsat} of both species peaked at the same time, i.e. in September for current-year needles and in June for 1-year-old needles (Fig. 3). The peaks of V_{cmax} and J_{max} in *P. sylvestriformis* occurred in the middle growing season (July or August), whereas V_{cmax} and J_{max} in *P. koraiensis* were found to be relatively stable over time (Fig. 3).

Effects of elevated CO_2 on photosynthetic parameters

Elevated CO_2 stimulated P_{Nsat} of the 1-year-old and the current-year needles in both species, although the difference in P_{Nsat} of the 1-year-old needles in *P. koraiensis* between elevated CO_2 and ambient CO_2 did not reach a significance level of $p < 0.05$ (Table 1, see also Table 3). The percent

stimulation of P_{Nsat} by elevated CO_2 for 1-year-old and current-year needles reached 32.2 and 32.9% in *P. sylvestriformis*, and 17.9 and 35.0% in *P. koraiensis*, respectively. Elevated CO_2 significantly suppressed V_{cmax} and J_{max} in 1-year-old and current-year needles in *P. koraiensis* but did not affect those in *P. sylvestriformis* (Tables 1, 2, see also Table 3). Elevated CO_2 resulted in a decline of 16.8% in V_{cmax} for 1-year-old needles and 21.3% for current-year needles in *P. koraiensis*. The J_{max} in 1-year-old needles and current-year needles of *P. koraiensis* decreased by 13.3% and 19.9% at elevated CO_2 compared to ambient CO_2, respectively. Elevated CO_2 led to a significant decrease in SLA by 8% in current-year needles of *P. koraiensis*, whereas SLA in *P. sylvestriformis* was not affected by elevated CO_2 (Table 1).

Responses of different-aged needles to long-term CO_2 enrichment

Overall, no interaction between needle age and CO_2 treatment was detected for each species (Table 2). AQE, P_{Nsat}, and SLA of needles varied significantly with needle age for each species after 8–9 years of CO_2 enrichment (Table 2). The pooled data of the two species showed significant needle age effects on all photosynthetic parameters (V_{cmax}, J_{max}, P_{Nsat} and AQE) in trees after 8–9 years of CO_2 enrichment (Table 3).

Current-year needles had significantly higher P_{Nsat} than 1-year-old needles when compared at the same CO_2 treatments for both species (Table 1, see also Fig. 3). The P_{Nsat} in current-year needles was about 42% higher than that in 1-year-old needles for *P. sylvestriformis* at elevated and ambient CO_2. For *P. koraiensis*, the P_{Nsat} in current-year needles was 10.4% higher at ambient CO_2 and 26.4%

Table 3 Effects of CO_2 treatments (elevated CO_2 of 500 μmol mol^{-1} CO_2 and ambient CO_2 of 370 μmol mol^{-1} CO_2), needle age (1-year-old needles and current-year needles), species (*Pinus koraiensis* and *P. sylvestriformis*), and their interactions on photosynthetic parameters and specific leaf area for trees exposed to CO_2 treatments for nine growing seasons (1999–2007) in northeastern China, tested using repeated-measures ANOVA

	V_{cmax}		J_{max}		AQE		P_{Nsat}		SLA	
	F	P	F	P	F	P	F	P	F	P
CO_2 (C)	1.274	ns	180.5	<0.001	3.037	ns	15.257	0.001	0.890	ns
Species (S)	745.2	<0.001	1,216.9	<0.001	9.332	0.008	9.241	0.008	0.480	ns
Age (A)	309.7	<0.001	461.9	<0.001	16.982	0.001	17.166	0.001	128.373	<0.001
C × S	8.047	0.03	53.05	<0.001	0.233	ns	1.61	ns	0.315	ns
C × A	0.008	ns	97.12	<0.001	1.099	ns	0.007	ns	0.626	ns
S × A	235.4	<0.001	350.4	<0.001	0.395	ns	0.094	ns	0.472	ns
C × S × A	0.336	ns	146.2	<0.001	0.473	ns	0.348	ns	0.131	ns

V_{cmax}, maximum carboxylation efficiency (μmol m^{-2} s^{-1}); J_{max}, maximum electron transport rate (μmol m^{-2} s^{-1}); *AQE*, apparent quantum yield (μmol photons m^{-2} s^{-1}); P_{Nsat}, light-saturated photosynthetic rate (μmol m^{-2} s^{-1}); *SLA* specific leaf area (cm^2 g^{-1})

F and P values are given. *ns* non-significant ($p > 0.05$)
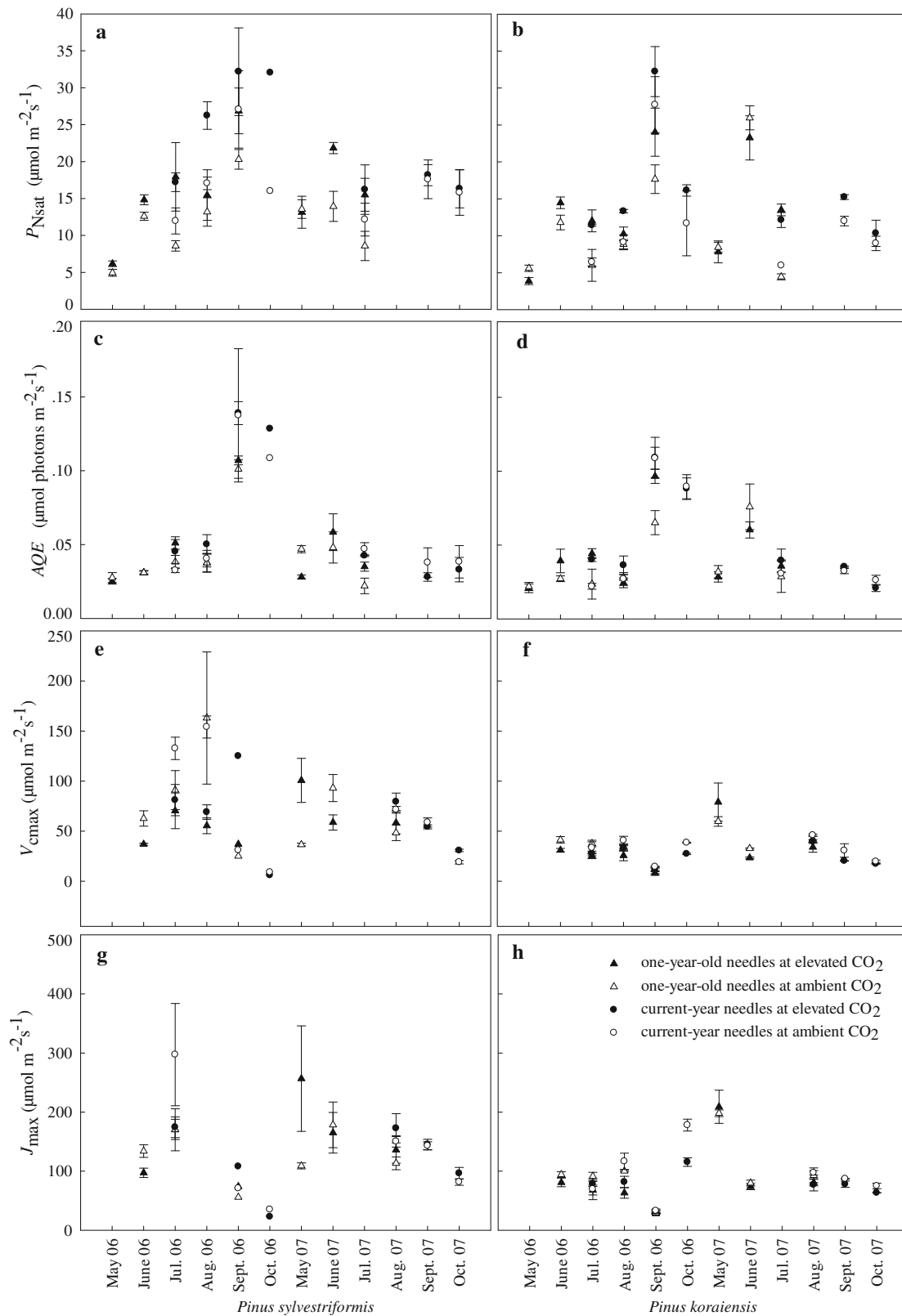
Fig. 3 Seasonal dynamics of light-saturated photosynthetic rate (P_{Nsat}) (**a**, **b**), apparent quantum yield (AQE) (**c**, **d**), maximum carboxylation efficiency (V_{cmax}) (**e**, **f**) and maximum electron transport rate (J_{max}) (**g**, **h**) in 1-year-old (*open triangles*, *closed triangles*) and current-year needles (*open circles*, *closed circles*) of *Pinus sylvestriformis* (*left*) and *P. koraiensis* (*right*) exposed to ambient (370 μmol mol^{-1}, *open triangles*, *open circles*) and elevated CO_2 (500 μmol mol^{-1}, *closed triangles*, *closed circles*) for nine growing seasons (1999–2007) in northeastern China

higher at elevated CO_2 than that in 1-year-old needles (Table 1).

Similar to P_{Nsat}, current-year needles had significantly higher AQE than 1-year-old needles for both species ($p = 0.029$ for *P. sylvestriformis*, and $p = 0.004$ for *P. koraiensis*, Table 2) when compared at the same CO_2 concentrations (Table 1). The AQE in current-year needles of *P. sylvestriformis* was 39.1 and 39.5% higher than those in 1-year-old needles at elevated and ambient CO_2, respectively. The AQE in current-year needles of *P. koraiensis* was 29.3% higher at elevated CO_2 and 33.3% higher at ambient CO_2 than those in 1-year-old needles, respectively (Table 1).

The V_{cmax} was found to be needle age-dependent for *P. sylvestriformis* ($p = 0.003$) and age-independent for *P. koraiensis* ($p > 0.05$), while J_{max} was needle age-dependent in *P. koraiensis* ($p = 0.043$) but needle age-independent in *P. sylvestriformis* (Table 2). SLA of the both tree species was significantly affected by needle age ($p < 0.001$ for both tree species) but not by CO_2 treatments ($p > 0.05$ for both species) (Table 2). Current-year needles had greater SLA than 1-year-old needles in the two *Pinus* species (Table 1). The averaged SLA of the current-year needles was 32.4 and 31.1% higher than that of the 1-year-old needles in *P. sylvestriformis* growing at elevated CO_2 and ambient CO_2, respectively (Table 1). For *P. koraiensis*, the averaged SLA of the current-year needles was 15.5% higher at elevated CO_2 and 25.8% higher at ambient CO_2 compared to those in 1-year-old needles, respectively (Table 1).

Responses of the two *Pinus* species to long-term CO_2 enrichment

The two species, *P. sylvestriformis* and *P. koraiensis*, seem to have different photosynthetic sensitivity to CO_2 enrichment. Significant effects of CO_2 treatments on V_{cmax}, J_{max}, and AQE were found in *P. koraiensis* but not in *P. sylvestriformis* (Table 2), whereas significant CO_2 effects on P_{Nsat} were detected in *P. sylvestriformis* but not in *P. koraiensis* (Table 2). The pooled data of the two species also showed significant species effects on all photosynthetic parameters (V_{cmax}, J_{max}, P_{Nsat} and AQE) in trees after 8–9 years of CO_2 enrichment (Table 3).

P_{Nsat} of 1-year-old needles in *P. sylvestriformis* was 24.8% higher at elevated CO_2 and 11.3% higher at ambient CO_2 than that in *P. koraiensis*, respectively. The current-year needles of *P. sylvestriformis* exhibited an increase in P_{Nsat} of 40.5% at elevated CO_2 and 42.7% at ambient CO_2 than those of *P. koraiensis*, respectively (Table 1). V_{cmax} in 1-year-old needles of *P. sylvestriformis* was 1.85-fold higher at elevated CO_2 and 1.74-fold higher at ambient CO_2 than that of *P. koraiensis*. For current-year needles, V_{cmax} of *P. sylvestriformis* was 2.13-fold higher at elevated

CO_2 and 1.93-fold higher at ambient CO_2 than that in *P. koraiensis* (Table 1).

P. sylvestriformis had significantly ($p = 0.008$) higher (ranging from +12 to +25%) AQE both in 1-year-old and current-year needles than *P. koraiensis* at the same CO_2 treatment (Tables 1, 3). Significant difference in SLA between current and 1-year old needles ($p < 0.001$) but not between *P. sylvestriformis* and *P. koraiensis* ($p > 0.05$) was found (Table 3). Only V_{cmax} and J_{max} were significantly affected by two-way and three-way interactions (Table 3).

Discussion

Photosynthetic acclimation to long-term CO_2 enrichment

After 8–9-year CO_2 enrichment, an increase in P_{Nsat} was still detectable in both *P. koraiensis* and *P. sylvestriformis* (Tables 1, 2, 3), but the CO_2 effect did not reach a $p < 0.05$ level in 1-year-old needles of *P. koraiensis* (Tables 1, 2). Similarly, V_{cmax} and J_{max} in needles were significantly suppressed in *P. koraiensis* but not in *P. sylvestriformis* (Tables 1, 3). These results indicated species-dependent responses of photosynthesis to elevated CO_2 within the same genus. Hence, our findings are consistent with our hypothesis 2 but do not fully support our hypothesis 1 (see "Introduction"). Previous studies have already reported that the photosynthetic responses depend on species, treatments and site conditions (Medlyn et al. 1999; Norby et al. 1999; Sholtis et al. 2004; Liberloo et al. 2007).

Both *P. sylvestriformis* and *P. koraiensis* showed an increased P_{Nsat} in spite of whether photosynthetic acclimation occurred (*P. koraiensis*) or not (*P. sylvestriformis*). Hence, photosynthetic acclimation does not mean that CO_2 assimilation in trees at elevated CO_2 is lower than at ambient CO_2 (Medlyn et al. 1999). An acclimation of photosynthetic apparatus in *P. radiata* (Turnbull et al. 1998; Griffin et al. 2000), *P. taeda* (Rogers and Ellsworth 2002), and other perennial trees (Hymus et al. 2002; Riikonen et al. 2005) has been reported. Generally, photosynthetic acclimation has been attributed to limitation in carbohydrate sink strength (Turnbull et al. 1998; Tissue et al. 2001), loss of Rubisco contents or activity (Moore et al. 1999; Rogers and Ellsworth 2002), and decreased nitrogen concentration (Ceulemans and Mousseau 1994; Luomala et al. 2003) or reallocation of foliage nitrogen (Sage 1994).

A carbohydrate accumulation in needles of trees at elevated CO_2 could decrease the needle SLA (Jach and Ceulemans 2000b). A decrease in SLA in current-year needles of *P. koraiensis* at elevated CO_2 was paralleled by

an increase in P_{Nsat} (Table 1), which may imply an accumulation of photosynthate in current-year needles. Both P_{Nsat} and SLA in 1-year-old needles were not affected by elevated CO_2 (Table 1), which may indicate that the photosynthetic acclimation of *P. koraiensis* was not likely caused by sink limitations or a source–sink imbalance. The marked decline in V_{cmax} in both aged needles of *P. koraiensis* (Tables 1, 2) may imply loss of Rubisco based on a significantly positive correlation between V_{cmax} and Rubisco content (Hymus et al. 2002). Data from a wide range of species including *P. taeda* also supported that photosynthetic acclimation at elevated CO_2 was caused by decreased Rubisco levels (Rogers and Humphries 2000; Rogers and Ellsworth 2002).

Species-specific responses of photosynthesis to CO_2 enrichment

The present study, inconsistent with our hypothesis 1 (see "Introduction"), revealed that the two species responded to long-term elevated CO_2 concentration widely differently (Table 3, see also Tables 1, 2, and Figs. 1, 2, 3). Trees, due to their long life span, have potential to acclimate changes in environmental factors such as CO_2 enrichment (Sigurdsson et al. 2002). The difference in responses of photosynthetic capacity to long-term elevated CO_2 concentration between *P. sylvestriformis* and *P. koraiensis* may be partly resulted from differences in their growth behavior. The averaged height of trees (9 years old) growing at ambient CO_2 reached 194 cm for *P. sylvestriformis* (fast-growing) and only 76 cm for *P. koraiensis* (slow-growing) in 2007 (Zhou et al., unpublished data). According to Adam et al. (2004), there was a strongly positive correlation between wood biomass increment and photosynthesis enhancement. Ainsworth et al. 2002 found that *Q. myrtifolia* and *Q. chapmanii* showed an increase in biomass but without any changes in photosynthetic capacity after 3 years of 704 μmol mol^{-1} CO_2 treatment, while the co-dominant *Q. geminata* had a decrease in photosynthetic capacity but no negative CO_2 effects on biomass. Hence, it is possible that *P. koraiensis*, due to the inherent growth limitation, does not need to produce more photosynthate for a small growth rate, showing no stimulation of photosynthetic capacity in 1-year-old needles at elevated CO_2 condition (Table 1). In other words, the photosynthesis of *P. koraiensis* at elevated CO_2 condition was down-regulated by a growth limitation (sink limitation). On the other hand, the fast-growing *P. sylvestriformis* did not show photosynthetic acclimation since it needs higher rates of photosynthesis to support its higher growth rate at elevated CO_2 condition. Therefore, we suggest that occurrence of photosynthetic acclimation of trees at elevated CO_2 is related to the growth rate associated with

trees' physioecological properties. Similarly, the fast-growing trees of *Populus* species acclimate seldom to long-term CO_2 enrichment (Calfapietra et al. 2005; Liberloo et al. 2007).

No significant difference in SLA between *P. koraiensis* and *P. sylvestriformis* was found in the present study (Table 2), indicating that the characteristics of mesophyll cells between the both species are very similar. However, the photosynthetic apparatus of *P. koraiensis* and *P. sylvestriformis* had different use efficiency of low light irradiance because of significant difference in AQE between the two species (Tables 2, 3).

Long-term CO_2 enrichment caused a stimulated photosynthesis in *P. sylvestriformis* and a down-regulated photosynthesis in *P. koraiensis* found in the present study, which indicates that *P. koraiensis* (the dominant tree species in the mixed needle- and broad-leaved forest in the region of Changbai Mountain) rather than *P. sylvestriformis* (an endemic species in the area of Changbai Mountain) may be endangered by a CO_2-rich world. At least, the dominant status of *P. koraiensis* in the mixed forest of Changbai Mountain may be changed due to the decreased photosynthetic capacity and changed competition ability in the future.

Needle age-dependent responses to CO_2 enrichment

Inconsistent with our hypothesis 3 (see "Introduction"), the photosynthetic parameters measured in different-aged needles within each species responded to elevated CO_2 similarly (Table 1; Fig. 3). Again, inconsistent with our hypothesis 4 (see "Introduction"), elevated CO_2 resulted in much stronger decreases in V_{cmax} and J_{max} in current-year needles than in 1-year old needles within each species (Table 1). These findings suggest that needle age does affect the magnitude but not the patterns of photosynthetic responses to long-term CO_2 enrichment (Fig. 3).

Needle age-related differences in the responses of photosynthesis to high CO_2 have been observed previously in *P. radiata* (Turnbull et al. 1998; Griffin et al. 2000), *P. taeda* (Rogers and Ellsworth 2002), and *P. sylvestris* L. (Wang et al. 1995; Jach and Ceulemans 2000a). The P_{Nsat} was highly related to needle age for either *P. sylvestriformis* or *P. koraiensis* (Table 2). P_{Nsat} decreased with increasing needle age in both species (Table 1). Similar to this finding, Tissue et al. (2001) found that an increased demand for photosynthate for supporting rapid growth and development of current-year needles may result in higher P_{Nsat} in current-year needles. The needle age-dependent photosynthetic acclimation was attributed to the difference in sink strength (Turnbull et al. 1998) or biochemical efficiency of photosynthesis (Tissue et al. 2001) with leaf aging and senescence. But Luomala et al. (2003) found that acclimation was not always greater in older

needles than in younger needles although the former exposed to elevated CO_2 longer than the latter.

Conclusion

After 8–9 years of CO_2 enrichment, *P. koraiensis* and *P. sylvestriformis* responded differently to elevated CO_2. Photosynthetic acclimation was found in the slow-growing species *P. koraiensis* but not in the fast-growing species *P. sylvestriformis*. Hence, different species associated with different physioecological properties may play an important role in their responses to elevated CO_2 conditions. The photosynthetic parameters measured in different-aged needles within each species responded to elevated CO_2 similarly, but elevated CO_2 resulted in much pronounced variations of photosynthetic parameters in current-year needles than in 1-year old needles within each species, indicating that needle age affects the magnitude but not the patterns of photosynthetic responses to long-term CO_2 enrichment. As global change such as CO_2 enrichment is more or less a gradual rather than an abrupt process, and different species respond to CO_2 enrichment (or other global change factor) differently, long-term global change experiments with different plant species are still needed to character and predict the global change effects on terrestrial ecosystems.

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