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Photosynthetic response and stomatal behaviour of *Pinus koraiensis* during the fourth year of exposure to elevated CO_2 concentration

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Abstract

4-year-old *Pinus koraiensis*, planted in open-top chambers at Changbai Mountain Station, received three different treatments [700 µmol(CO_2) mol^{-1} = EC, control chamber = CC, and field = F]. Saturated net photosynthetic rate (P_N) was 62 % higher in EC plants compared to CC and F plants as shown by PAR/P_N response curves. Despite the increased P_N, EC plants still showed decreased photosynthetic capacity when compared with CC and F plants at the same measurement CO_2 concentration. EC plants had the highest stomatal conductance (g_s) and ratio of intercellular to ambient CO_2 concentration (C_i/C_a) compared with CC and F plants when measured at the same CO_2 concentration. The C_i/C_a ratio was a sensitive indicator of stomatal behaviour, but not of photosynthesis. The responses of g_s to EC did not correlate in magnitude or direction with responses of P_N to EC. There was no significant difference in the number of stomata lines and stomata between EC and C_a.

Additional key words: net photosynthetic rate; stomatal conductance; stomata number.

Introduction

Plant photosynthetic rate is increased following initial exposure to elevated CO_2, EC (Ward and Strain 1999). However, the high photosynthetic rate is not maintained over long time periods and leads to a reduction in the photosynthetic response to CO_2 (Arp and Drake 1991). Despite significant down-regulation of photosynthesis is observed, light-saturated photosynthetic rate measured at growth conditions is strongly higher at EC than at ambient CO_2 (Medlyn *et al*. 1999).

Photosynthesis is dependent on stomata for its supply of CO_2, therefore, stomatal response to CO_2 is important (Morison 1998). The change of net photosynthetic rate (P_N) and stomatal conductance (g_s) does not always show similar magnitude or consistent direction. Photosynthetic stimulation was maintained despite a 30 % decrease in g_s for perennial ryegrass exposed to 600 µmol(CO_2) mol^{-1} for 10 years using FACE (Ainsworth *et al*. 2003). P_N of *Liquidambar styraciflua* averaged 46 % higher in the +200 µmol(CO_2) mol^{-1} treatment and g_s averaged 14–24 % lower (Gunderson *et al*. 2002).

Increased atmospheric CO_2 concentration causes reduced g_s, and the reduction of g_s varies widely (Bunce 2000, Morison 2001). Trees generally show smaller responses than herbaceous plants (Saxe *et al*. 1998) and deciduous trees often show stronger responses than conifers (Medlyn *et al*. 2001). Stomata aperture and number of stomata per unit leaf area (or stomata density) affect g_s (Weyers and Lawson 1997).

The change of stomata number is a long-term response to EC (Morison 1998). *Quercus myrtifolia* exposed to 700 µmol(CO_2) mol^{-1} showed no change in stomata density (Lodge *et al*. 2001). Poole *et al*. (2000) found that stomata densities in leaves of *Alnus glutinosa* grown at ambient and elevated CO_2 concentration were not significantly different. Scots pine (*Pinus sylvestris*) trees were grown for 4 y under either ambient or elevated [+400 µmol mol^{-1}] CO_2 concentrations in open-top chamber (OTC). A significant reduction in stomata density was observed in the EC treatment as compared with the ambient CO_2 treatment (Lin *et al*. 2001). Contradicting observations, however, were reported for *P. banksiana* (Stewart and Hoddinott 1993) and *P. pinaster* (Guehl

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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; OTC – open-top chamber; PAR – photosynthetically active radiation; P_N – net photosynthetic rate.

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et al. 1994).

At present, the research is focused on the multi-year CO_2 exposure of forest trees grown in the field using OTCs where root and nutrient availability may not be limiting to the growth of seedlings. We examined P_N, g_s, ratio of intercellular to ambient CO_2 concentration (C_i/C_a), and stomata number of current needles of 4-year-old *P. koraiensis* seedlings after exposure to 700 µmol(CO_2) mol^{-1} for four years in the OTCs.

Materials and methods

Plants: Experimental site was at Changbai Mountain Station, Chinese Academy of Sciences (42°N, 128°E) in 1999. Here, four-year-old *P. koraiensis* seedlings were planted directly into the ground of OTCs with aluminum frame covered with clear glass. Chambers with dimensions of 1.2×0.9×0.9 m were open on the top. There were about 140 trees in each chamber. The experiment involved three treatments [700 µmol(CO_2) mol^{-1} chambers = EC, ambient control chamber = CC, and field = F]. The ambient air averaged approximately 350 µmol(CO_2) mol^{-1} in the daytime. Blowers pushed mixed air (industrial CO_2 and atmosphere) into a perforated plastic pipe at the bottom of chamber. Industrial high CO_2 was provided by liquid CO_2 tanks stored outside the OTCs. The desired CO_2 concentration was maintained by varying the rate of air flow. CO_2 concentration was monitored using the *CI-301* gas analyzer. The standard deviation of CO_2 concentration was about 50 µmol mol^{-1}. Treatments were applied continuously (24 h d^{-1}) for the duration of each growing season (May to September) from 1999 to 2002. The CO_2 concentration in chambers was checked and adjusted regularly to ensure target value.

g_s **and** P_N were measured between 08:00–10:30 on clear days. Three trees were selected on the edge and centre of the chamber at random to measure photosynthesis. Mature, fully irradiated and attached needles were measured using a *Li-COR 6400* portable photosynthetic analyzer. Leaf chamber temperature and relative humidity were measured with thermocouples linked to the *LI-6400* analyzer. To maintain environmental conditions at ambient levels in chambers, air temperature and humidity were controlled by cooling and dehumidifying the air prior to entering the chamber, respectively. The leaf chamber temperature was about 27 °C and relative humidity was about 50 % during measurements. P_N responses to photosynthetically active radiation (PAR) were determined at their respective growth CO_2 concentration. In order to examine if the photosynthetic capacity loses in response to 4-y-exposure to 700 µmol(CO_2) mol^{-1}, plants grown in both high and ambient CO_2 concentration were compared at the same measurement CO_2 concentration [ambient, 500 and 700 µmol(CO_2)mol^{-1}] under saturating PAR (PAR>1 000 µmol m^{-1} s^{-1}), respectively. In parallel, g_s and C_i/C_a ratio of plants grown at three different conditions were also measured. Needles were arranged in the chamber such that self-shading was minimized and all needles were parallel to the plane of the leaf chamber. Needles were assumed to be flat and leaf area was measured with a portable leaf area meter (*LI-3000*; *Li-Cor*). Prior to each measurement, needles were equilibrated in the chamber at certain PAR and CO_2 concentration. Needles were considered equilibrated if the gas exchange parameters were stable for 0.5 min.

Stomata number: There are three sides of needle of *P. koraiensis,* but only two sides have stomata line and stomata. The stomata occur in a number of straight lines running along the length of the needle on two sides of a needle. 20 current needles were collected at random from 20 plants per treatment and 3 mm long epidermis was cut along the needle from two sides and viewed with a microscope. The number of stomata lines and stomata per line were counted.

Statistics: Mean values of P_N, g_s, C_i/C_a, stomata line, and stomata number were compared separately using one-way analysis of variance. All statistical tests were performed using *SPSS 11.5* software. The conclusions were reached by using LSD tests.

Results

The relationship between P_N and PAR at the CO_2 concentration at which the plants were grown (Fig. 1) showed there was a significant increase of 62 % in saturated maximum P_N in EC plants compared with CC and F plants. There was no significant difference in saturated P_N between CC and F plants. There was also no significant difference between EC and CC or F plants in P_N when PAR was less than about 200 µmol m^{-2} s^{-1}. EC substantially increased P_N when PAR was over 400 µmol m^{-2} s^{-1}. But the saturation irradiance of EC plants approximated those of CC plants.

P_N of both EC and ambient CO_2 plants was compared at 350, 500, and 700 µmol(CO_2) mol^{-1}, respectively. P_N increased with the increase of measurement CO_2 concentration for all plant types (Fig. 2). At the specific measurement CO_2 concentration, P_N of CC plants was the highest. P_N in EC plants was 23, 9, and 16 % lower compared to CC plants when measured at 350, 500, and 700 µmol(CO_2) mol^{-1}, respectively. Compared with F plants, EC plants did not show significantly increased P_N

when measured at 350 μmol(CO_2) mol^{-1} (p=0.846). P_N of EC plants was higher than that of F plants when measured at 500 and 700 μmol(CO_2) mol^{-1}.

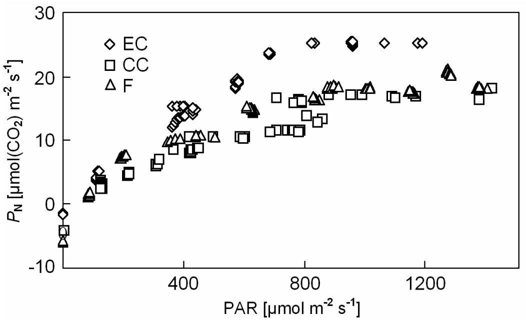

Fig. 1. Relation of net photosynthetic rate (P_N) to photosynthetically active radiation (PAR) at respective growth CO_2 concentrations.

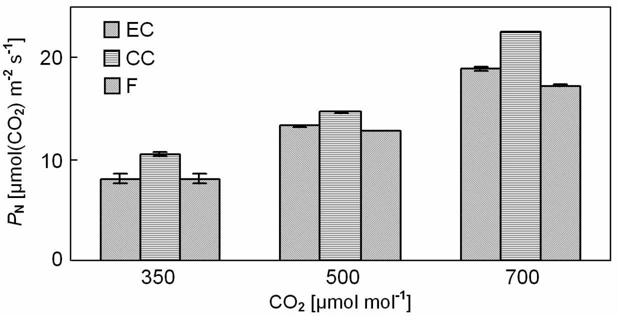

Fig. 2. Net photosynthetic rate (P_N) of plants grown under different conditions (EC, CC, F) and measured at 350, 500, and 700 μmol(CO_2) mol^{-1}.

g_s decreased with the increase of measurement CO_2 concentration for plants grown at EC and ambient CO_2 (Table 1). When measured at the same CO_2 concentration, g_s of EC and CC plants was the highest and the lowest, respectively. When measured at 350, 500, and 700 μmol(CO_2) mol^{-1}, g_s was 44, 39, and 43 % higher in EC plants than in CC plants, respectively. Similarly, g_s increased by 16, 19, and 15 % compared to F plants, respectively.

The C_i/C_a ratio decreased with the increase of measurement CO_2 concentration for ambient CO_2 and EC plants similar to g_s (Table 1). C_i/C_a of EC plants was the highest compared to ambient CO_2 plants, and it was 8, 2, and 8 % higher compared to CC plants when measured at 700, 500, and 350 μmol(CO_2) mol^{-1}, respectively. Similarly, C_i/C_a ratio increased by 6, 8, and 6 % compared to that of F plants, respectively. C_i/C_a of CC plants was significantly higher than that of the F plants when measured at 500 μmol(CO_2) mol^{-1}, opposite to measurements at 350 and 700 μmol(CO_2) mol^{-1}.

Table 1. Stomatal conductance (g_s) and ratio of intercellular to ambient CO_2 concentration (C_i/C_a) at different measurement CO_2 concentrations [mol m^{-2} s^{-1}]. Means±the standard error. Means followed by the different letter in the same row are significantly different (p<0.05).

Growth type	g_s			C_i/C_a		
	Measurement CO_2 conc. [μmol(CO_2) mol^{-1}]					
	350	500	700	350	500	700
EC	0.240±0.002^a	0.216±0.001^a	0.203±0.001^a	0.734±0.007^a	0.675±0.001^a	0.653±0.001^a
CC	0.169±0.001^b	0.155±0.001^b	0.142±0.001^b	0.679±0.001^b	0.663±0.001^b	0.606±0.001^b
F	0.207±0.003^c	0.182±0.004^c	0.176±0.001^c	0.691±0.004^c	0.627±0.001^c	0.617±0.001^c

Table 2. Stomata lines and stomata number of current needles. SLLN: number of stomata lines on left side of needle; NSLN: number of stomata per 1 mm long stomata lines on left side; SLRN: number of stomata lines on right side of needle; NSRN: number of stomata per 1 mm long stomata lines on right side; SLN: number of stomata lines of whole needle; NSN: number of stomata per 1 mm long stomata lines of whole needle.

Index	Growth CO_2 concentration [μmol(CO_2) mol^{-1}]		
	EC	CC	F
SLLN	4.66±0.29	4.56±0.32	4.65±0.27
NSLN	14.27±0.51	14.09±0.31	14.56±0.41
SLRN	5.33±0.23	5.28±0.38	5.30±0.22
NSRN	14.31±0.40	13.61±0.29	14.52±0.28
SLN	9.99±0.41	9.84±0.65	9.95±0.41
NSN	14.29±0.41	13.85±0.27	14.54±0.31

Needle of *P. koraiensis* has three sides, but only two sides have stomata lines and stomata. We called them left and right side according to the node, respectively. The number of stomata lines on right side was higher than that on left side for EC, CC, and F plants, but the stomata number was approximate equal. The stomata number per unit length of stomata line on the right side of needle of CC plants was less than that of EC and F plants (Table 2), but the difference was not significant (p>0.3). The number of stomata lines and stomata on left side, right side, and whole needle was approximately equal among the three treatments. The difference was not significant.

Discussion

Photosynthesis: According to the PAR/P_N response curve, *P. koraiensis* seedlings exhibited increased saturated P_N in response to EC when measured at growth condition in this study. Despite the increased P_N, EC plants still showed evidence of decreased photosynthetic capacity when compared with ambient plants at the same measurement CO_2 concentration. Down-regulation of photosynthesis did not imply that the P_N measured at EC was lower than that at ambient CO_2. It was consistent with results previously reported for arctic tussock tundra (Tissue and Oechel 1987, Grulke *et al.* 1990) and one-year-old shoots of *Picea sitchensis* (Barton and Jarvis 1999). In a study of 15 field-based experiments on European forest tree species, P_N measured at a common CO_2 concentration was 10–20 % lower in trees grown at EC (Medlyn *et al.* 1999). However, other studies on tree species reported a lack of photosynthetic acclimation to EC (Idso and Kimball 1992, Teskey 1995). Increase in P_N by 12–144 % and no down-regulation in photosynthetic capacity occurred in *Liriodendron tulipifera* and *Quercus alba* throughout a 3-year field exposure (Gunderson *et al.* 1993).

Stomatal conductance and stomata number: Generally, g_s declines when exposed to a transient increase in atmospheric CO_2 concentration (Medlyn *et al.* 2001). However, does long-term exposure to EC result in a similar reduction in g_s, particularly in woody species? In our study, plants grown at EC had the highest g_s compared with those grown at ambient CO_2 concentration. g_s is affected primarily by stomatal aperture but also by the number of stomata per unit leaf area (Weyers and Lawson 1997). Since the stomata number of *P. koraiensis* was not affected by EC, the increase in g_s at EC was mainly related to stomata aperture. Stomata aperture was not only sensitive to CO_2 concentration but also sensitive to some other environmental stimuli, such as irradiance, humidity (Morison 1998), temperature, water potential of needle, *etc*. The result also suggests that g_s responses to CO_2 need not correlate in magnitude or direction with stomata number responses to CO_2 (Maherali *et al.* 2002).

Photosynthesis and g_s are tightly coupled (Gunderson *et al.* 2002). Recent studies introduced a question whether P_N and g_s acclimate to EC in parallel or independently? Drake *et al.* (1997) reported that g_s and photosynthesis acclimate in tandem. However, the results with Douglas-fir seedlings suggest that g_s and photosynthesis acclimate in parallel to EC (Lewis *et al.* 2002). g_s responses to EC did not correlate in magnitude or direction with photosynthesis responses to EC in this study. This CO_2 concentration increased g_s but decreased P_N compared to the CC plants at the same measurement CO_2 concentrations. When compared with F plants, EC increased the g_s and P_N, but both did not show a similar magnitude. Therefore, the relationship between g_s and photosynthetic capacity for *P. koraiensis* exposed to EC for 4 years was neither completely independent nor parallel.

C_i/C_a increased for *Q. myrtifolia* grown in high CO_2 concentration upon exposure to 350 μmol(CO_2) mol^{-1} (Lodge *et al.* 2001). The C_i/C_a ratio increased with the decrease of measurement CO_2 concentration for both EC and ambient CO_2 plants in our study. The C_i/C_a value was not always constant and CO_2 concentration could affect this ratio. The change of C_i/C_a was consistent with g_s and opposite to P_N for *P. koraiensis* EC plants. Therefore, the C_i/C_a ratio should be regarded as the sensitive indicator of stomata behaviour, but not of photosynthesis.

There was significant difference in the eco-physiological response between CC and F plants at both used CO_2 concentrations. Environmental factors such as temperature and irradiance were similar during the measurement. The change of structure and function of *P. koraiensis* resulting from the microenvironment in OTC could be related to the difference in photosynthesis and stomatal behaviour.

References

Ainsworth, E.A., Davey, P.A., Hymus, G.J., Osborne, C.P., Rogers, A., Blum, H., Nösberger, J., Long, S.P.: Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO_2 Enrichment (FACE). – Plant Cell Environ. **26**: 705-714, 2003.

Arp, W.J., Drake, B.G.: Increased photosynthetic capacity of *Scirpus olneyi* after 4 years of exposure to elevated CO_2. – Plant Cell Environ. **14**: 1003-1006, 1991.

Barton, C.V.M., Jarvis, P.G.: Growth response of branches of *Picea sitchensis* to four years exposure to elevated atmospheric carbon dioxide concentration. – New Phytol. **144**: 233-243, 1999.

Bunce, J.A.: Responses of stomatal conductance to light, humidity and temperature in winter wheat and barley grown at three concentrations of carbon dioxide in the field. – Global Change Biol. **6**: 371-382, 2000.

Drake, B.G., Gonzàlez-Meler, M.A., Long, S.P.: More efficient plants: A consequence of rising atmospheric CO_2? – Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 609-639, 1997.

Grulke, N.E., Riechers, G.H., Oechel, W.C., Hjelm, U., Jaeger, C.: Carbon balance in tussock tundra under ambient and elevated atmospheric CO_2. – Oecologia **83**: 485-494, 1990.

Guehl, J.M., Picon, C., Aussenac, G., Gross, P.: Interactive effects of elevated CO_2 and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. – Tree Physiol. **14**: 707–724, 1994.

Gunderson, C.A., Norby, R.J., Wullschleger, S.D.: Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. – Plant Cell Environ. **16**: 797-807, 1993.

Gunderson, C.A., Sholtis, J.D., Wullschleger, S.D., Tissue, D.T., Hanson, P.J., Norby, R.J.: Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. – Plant Cell Environ. **25**: 379-393, 2002.

Idso, S.B., Kimball, B.A.: Effects of atmospheric CO_2 enrichment on photosynthesis, respiration, and growth of sour orange trees. – Plant Physiol. **99**: 341-343, 1992.

Jarvis, A.J., Mansfield, T.A., Davies, W.J.: Stomatal behaviour, photosynthesis and transpiration under rising CO_2. – Plant Cell Environ. **22**: 639-648, 1999.

Lewis, J.D., Lucash, M., Olszyk, D.M., Tingey, D.T.: Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the third and fourth years of exposure. – Plant Cell Environ. **25**: 1411-1421, 2002.

Lin, J., Jach, M.E., Ceulemans, R.: Stomatal density and needle anatomy of Scots pine (*Pinus sylvestris*) are affected by elevated CO_2. – New Phytol. **150**: 665–674, 2001.

Lodge, R.J., Dijkstra, P., Drake, B.G., Morison, J.I.L.: Stomatal acclimation to increased CO_2 concentration in a Florida scrub oak species *Quercus myrtifolia* Willd. – Plant Cell Environ. **24**: 77-88, 2001.

Maherali, H., Reid, C.D., Polley, H.W., Johnson, H.B., Jackson, R.B.: Stomatal acclimation over a subambient to elevated CO_2 gradient in a C_3/C_4 grassland. – Plant Cell Environ. **25**: 557–566, 2002.

Medlyn, B.E., Badeck, F.-W., De Pury, D.G.G., Barton, C.V.M., Broadmeadow, M., Ceulemans, R., De Angelis, P., Forstreuter, M., Jach, M.E., Kellomäki, S., Laitat, E., Marek, M., Philippot, S., Rey, A., Strassemeyer, J., Laitinen, K., Liozon, R., Portier, B., Roberntz, P., Wang, K., Jarvis, P.G.: Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. – Plant Cell Environ. **22**: 1475-1495, 1999.

Medlyn, B.E., Barton, C.V.M., Broadmeadow, M.S.J., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S.B., Kellomäki, S., Laitat, E., Rey, A., Robertnz, P., Sigurdsson, B.D., Strassmeyer, J., Wang, K., Curtis, P.S., Jarvis, P.J.: Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. – New Phytol. **149**: 247-264, 2001.

Morison, J.I.L.: Stomatal response to increased CO_2 concentration. – J. exp. Bot. **49**: 443-452, 1998.

Morison, J.I.L.: Increasing atmospheric CO_2 and stomata. – New Phytol. **149**: 154-156, 2001.

Poole, I., Lawson, T., Weyers, J.D.B., Raven, J.A.: Effect of elevated CO_2 on the stomatal distribution and leaf physiology of *Alnus glutinosa*. – New Phytol. **145**: 511-521, 2000.

Saxe, H., Ellsworth, D.S., Heath, J.: Tree and forest functioning in an enriched CO_2 atmosphere. – New Phytol. **139**: 395-436, 1998.

Stewart, J.D., Hoddinott, J.: Photosynthetic acclimation to elevated atmospheric carbon dioxide and UV irradiation in *Pinus banksiana*. – Physiol. Plant. **88**: 493–500, 1993.

Teskey, R.O.: A field study of the effects of elevated CO_2 on carbon assimilation, stomatal conductance and leaf and branch growth of *Pinus taeda* trees. – Plant Cell Environ. **18**: 565-573, 1995.

Tissue, D.T., Oechel, W.C.: Response of *Eriophorum vaginatum* to elevated CO_2 and temperature in the Alaskan tussock tundra. – Ecology **68**: 401-410, 1987.

Ward, J.K., Strain, B.R.: Elevated CO_2 studies: past, present and future. – Tree Physiol. **19**: 211-220, 1999.

Weyers, J.D.B., Lawson, T.: Heterogeneity in stomatal characteristics. – Adv. Bot. Res. **26**: 317-352, 1997.