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Title

Elevated CO₂ enhances the growth of hybrid larch F₁ (*Larix gmelinii* var. *japonica* × *L. kaempferi*) seedlings and changes its biomass allocation

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Key Message

Elevated CO₂ enhances the photosynthesis and growth of hybrid larch F₁ seedlings. However, elevated CO₂-induced change of tree shape may have risk to the other environmental stresses.

Abstract

The hybrid larch F₁ (*Larix gmelinii* var. *japonica* × *L. kaempferi*) is one of the most promising species for timber production as well as absorption of atmospheric CO₂. To assess the ability of this species in the future high CO₂ environment, we investigated the growth and photosynthetic response of hybrid larch F₁ seedlings to elevated CO₂ concentration. Three-year-old seedlings of hybrid larch F₁ were grown on fertile brown forest soil or infertile volcanic ash soil, and exposed to 500 μmol mol⁻¹ CO₂ in a free air CO₂ enrichment system located in northern Japan for two growing seasons. Regardless of soil type, the exposure to elevated CO₂ did not affect photosynthetic traits in the first and second growing seasons; a higher net photosynthetic rate was maintained under elevated CO₂. Growth of the seedlings under elevated CO₂ was greater than that under ambient CO₂. We found that elevated CO₂ induced a change in the shape of seedlings: small roots, slender-shaped stems and long shoots. These results suggest that elevated CO₂ stimulate the growth of hybrid larch F₁, although the change in tree shape may increase the risk of other stresses, such as strong winds, heavy snow, and nutrient deficiency.

Key words

Acclimation to high CO₂; allometry; dry matter allocation; needle characteristics; photosynthesis; tree shape

1. Introduction

Larix species are distributed widely in cool temperate regions in northern hemisphere (Gower and Richards 1990). The Japanese larch (*Larix kaempferi*) is characterized by its high growth rate, and has been planted in many parts of the northern hemisphere (Ryu et al. 2009). However, some biotic and abiotic difficulties have raised concerns (e.g. Brasier and Webber 2010). To overcome these problems, the hybrid larch was recently developed by crossing Dahurian larch (*Larix gmelinii* var. *japonica*) and pollen of Japanese larch. The selected hybrid larch F₁ grew faster and had a high capacity for carbon accumulation compared to the Japanese larch under present conditions (Miyaki 1990; Kita et al. 2009). In addition, the resistances to strong wind and heavy snow of hybrid larch F₁ is also superior to Japanese larch (Kaji 1983). As a result, the hybrid larch F₁ is one of the most important tree species for timber production as well as for absorption of atmospheric CO₂ as a carbon sink (Ryu et al. 2009). The atmospheric CO₂ concentration has increased dramatically since the industrial revolution and reached to 400 μmol mol⁻¹ (Monastersky 2013). This increase is continuing (e.g. Meehl et al. 2007). An understanding of the ecophysiological responses of the hybrid larch F₁ to future high atmospheric CO₂ concentration is important for sustainable forestry using hybrid larch F₁ plantations.

Growth and photosynthesis of *Larix* species is generally enhanced under elevated CO₂. (Tjoelker et al. 1998a; 1998b; Handa et al. 2005; 2006). However, Yazaki et al. (2004) reported no significant effects of elevated CO₂ on the biomass growth of *L. kaempferi* seedlings in the experiment at environmental control chambers. On the other hand, Yazaki et al. (2001) observed that elevated CO₂ stimulates increase of the stem diameter of *Larix sibirica* under relatively high nutrient conditions, but not under low nutrient conditions. Eguchi et al. (2004) also reported growth enhancement of *L. kaempferi* seedling by elevated

CO₂ only under high nutrient condition. These studies suggest that the responses of *Larix* species to elevated CO₂ differ between species, and are affected by environmental conditions, especially nutrient condition.

An increase of CO₂ concentration will stimulate leaf photosynthetic rate in general because CO₂ is the primary substrate for photosynthesis. However, elevated CO₂-induced enhancement of photosynthetic rate occasionally decreases with course of time, known as 'down-regulation of photosynthesis' and growth is then slower than expected (Tissue and Oechel 1987; Tissue et al. 1993; Ellsworth et al. 2004; Ainsworth and Rogers 2007; Eguchi et al. 2008; Tissue and Lewis 2010). Watanabe et al (2011) reported strong photosynthetic down-regulation under elevated CO₂ in seedlings of the hybrid larch F₁ grown under low nutrient availability, although the growth was enhanced by elevated CO₂. Many reports have suggested that decrease of nutrient concentration, especially nitrogen (N), in leaves, and limitation of sink capacity for photosynthate, are the reasons for down-regulation of photosynthesis under elevated CO₂ (Nowak et al. 2004; Ellsworth et al. 2004; Eguchi et al. 2008; Norby et al. 2010; Watanabe et al. 2011).

Limited information is available on photosynthetic responses of woody plant to free-air CO₂ enrichment (FACE) under different soil conditions (e.g. Norby and Zak 2011). Eguchi et al. (2008) reported there was a down-regulation of photosynthesis in the leaves of *Betula maximowicziana* saplings in infertile and immature volcanic ash soil, whereas no such symptom was found in fertile brown forest soil. The volcanic ash soil is common soil in Japan because of many volcanos (Kato 1983).

It has been reported that allocation of photosynthate between organs is one of the most important functions that determine water and nutrient status and tolerance to mechanical stresses such as strong wind and heavy snow (Lambers et al. 2008; Sterck 2005). FACE

experiments are suitable for evaluating changes in allocation induced by elevated CO₂, because there is no limitation of root growth and environmental conditions are natural. Calfapietra et al. (2003) found no elevated CO₂-induced change in biomass allocation between above ground and below ground in three *Populus* species grown in POP/EURO FACE. In FACE experiments with aspen, elevated CO₂ did not induce any difference in biomass allocation among plant organs (King et al. 2005). Handa et al. (2008) found no stimulation in root production by 30-year-old European larches (*Larix decidua*) in response to CO₂ enrichment at the Swiss treeline. Based on these reports, we do not expect any response of biomass allocation to elevated CO₂ in the hybrid larch F₁. In fact, Watanabe et al. (2011) found no significant difference in the biomass ratio of above ground to below ground of hybrid larch F₁ seedlings grown under ambient CO₂ and elevated CO₂ in environmental control chambers.

In the present study, therefore, we hypothesize that hybrid larch F₁ grown in infertile and immature volcanic ash soil shows down-regulation of photosynthesis under elevated CO₂ and not change in biomass allocation. To test these hypotheses, we studied the responses of growth and photosynthesis of hybrid larch F₁ seedlings to elevated CO₂ under different soil conditions.

2. Materials and methods

2.1 Experimental design

We used the FACE system located in Sapporo Experimental Forest, Hokkaido University, Japan (43°06' N, 141°20' E) (Eguchi et al. 2008; Watanabe et al. 2010), which is in a transition zone between cool temperate and boreal forests, and is considered to be sensitive to global climate changes (Matsuda et al. 2002). The present experiment had a split-plot factorial design and employed the randomized block method. The whole-plot

treatment consisted of two levels of CO₂ [ambient (370-380 μmol mol⁻¹ CO₂) and elevated CO₂ (500 μmol mol⁻¹ CO₂)] with three site replications, giving a total of 6 sites for data analysis, including the variance among the 6 sites. The sub-plot treatment consisted of two types of soil (brown forest soil and pumice included volcanic ash soil) at each site. The chemical and physical properties of brown forest soil and volcanic ash soil in the FACE site are described in Table 1 after Eguchi et al. (2005; 2008). Three seedlings were assigned to each CO₂-soil-site combination, giving a total of 36 seedlings.

Three-year-old cutting of hybrid larch F₁ (sp “Gream”) were planted in each soil on 21 May 2008, and were grown for 520 days to 23 October 2009. The average values ± standard deviation of the height and stem base diameter at 3 cm from the ground of the seedlings at the beginning of the experiment were 64.9 ± 8.3 cm and 8.60 ± 0.6 mm, respectively.

Fumigation with CO₂ was carried out in the daytime when the photosynthetic photon flux (PPF) exceeded 70 μmol m⁻² s⁻¹, which is the light compensation point of photosynthesis. Fumigation took place each growing season, from leaf emergence to leaf senescence. This fumigation regime was according to the other FACE experiment with woody plants (Karnosky et al. 2005; Liberloo et al. 2009; Norby et al. 2010; Ellsworth et al. 2012). The mean day-time CO₂ concentration in FACE during fumigation periods was 493 μmol mol⁻¹. The value of CO₂ concentration was within 500 ± 50 μmol mol⁻¹ for 46% of the fumigation period, and was within 500 ± 100 μmol mol⁻¹ for 77% of the fumigation period.

2.2 Measurements of plant growth

During the 2008 and 2009 growing seasons, we measured the height and stem diameter at 3 cm from the ground of all seedlings. On 23 October 2009 the seedlings were all harvested, to determine the dry mass of the plant organs. Firstly, we collected 5 long-shoots (a

branch with needles) that had emerged in 2009 from the top of the seedlings, for information on shoot traits. Because seedlings have mostly long-shoots even heterophyllous larch, we focus on long-shoots. After measurement of the total length, the shoots were dried in an oven at 70°C for 1 week. And then, we separated the needles from the shoots and determined the dry mass of needles and branches.

The remained needles, branch, trunk and root were also collected and weighed after drying in an oven. The dry mass of needles from 5 long-shoots mentioned above and that of remained needles were summed to determine the whole-plant needle dry mass. Similarly, whole-plant stem (branch and trunk) dry mass was calculated as a sum of the dry mass of branches from 5 long-shoots and remained branches and trunk. For collecting root, we started digging soil in a place distant 30-40 cm from seedlings by hand shovel and our hands, and carefully dug toward root of the seedlings. Most of the roots were existed in a range 30 cm in diameter and 30 cm in depth, and 6-7 lateral roots extended 50-80 cm to mainly horizontal direction.

2.4 Measurement of needle gas exchange rate and nitrogen content

The gas exchange rates of the fully mature young needles were measured using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (LI-6400-40, Li-Cor Inc., Lincoln, NE, USA). Measurements were made on 18-21 September 2008 and 8-11 September 2009, when a high photosynthetic rate was observed in larch saplings (Kitaoka et al. 2000).

We randomly selected 2 seedlings per CO₂-soil-site combination (6 seedlings per treatment). The needle temperature and PPF during the measurement were maintained at 25.0 ± 1.0°C and 1500 μmol m⁻² s⁻¹. The leaf-to-air vapor pressure deficit was approximately 1.5

kPa. To obtain the intercellular CO₂ concentration (C_i)-response curve of the net photosynthetic rate (A), i.e. the A/C_i curve, A was determined under external CO₂ concentration (C_a) at 60, 140, 220, 300, 370, 500, 800, 1100, 1400 and 1700 $\mu\text{mol mol}^{-1}$. We determined the value of A and the stomatal conductance of needles for water vapour at growth CO₂ concentration (i.e. 370 and 500 $\mu\text{mol mol}^{-1}$ for ambient and elevated CO₂ treatment, respectively); these values were designated as A_{growth} and G_s . The maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were calculated based on 5 pairs of A and C_i under lower CO₂ concentration and those under higher CO₂ concentration, respectively (Farquhar et al. 1980; Long and Bernacchi 2003). We applied least-square method for curve fitting. The Rubisco Michaelis constants for CO₂ (K_c) and O₂ (K_o) and the CO₂ compensation point in the absence of dark respiration (Γ^*) for the analysis of the A/C_i curve were estimated from leaf temperature according to Bernacchi et al. (2001).

After measurement of the gas exchange rate, we determined the projected needle area with an image scanner, and weighed the needles to determine their dry mass after oven-drying at 70°C for 5 days. From the results, the leaf mass per unit area (LMA) was calculated. The N content of the needles was determined using a gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) after combustion with circulating O₂ using a NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Osaka, Japan). A calibration curve was made by using acetanilide (N = 10.36%, C = 71.09, Wako, Osaka, Japan).

2.5 Statistical analysis

Two-way analysis of variance (two-way ANOVA) with the SPSS software (SPSS, Inc, Chicago, IL, USA) was applied to test the effects of CO₂ and soil, and their interaction. Hypothesis concerning CO₂ effects (1 d.f.) were tested against whole-plot site variation (4

d.f.), whereas the main effects of soil and CO₂ × soil interaction (1 d.f.) were tested against the soil × site/CO₂ error term (4 d.f.). To analyse effects of CO₂ on the relation between growth parameters, tests for standardized major axes (SMA; model II type regression) were run on a freeware program, (S) MATR (Version 2; see <http://www.bio.mq.edu.au/ecology/SMATR/>). When there was no significant difference in slopes of the regression lines between treatments, we applied a common slope and analysed difference in intercept of the regression lines.

3. Results

3.1 Growth traits

Height and diameter increments of seedlings of the hybrid larch F₁ were significantly greater under elevated CO₂ (Figure 1). Stimulation of growth was observed mainly in the second growing season. Neither significant effect of soil nor significant interaction between elevated CO₂ and soil for height and diameter increments was found during experimental period. **[Figure 1]**

The elevated CO₂ significantly increased the stem and whole-plant dry mass of the seedlings (Table 2). There was no significant effect of soil condition of dry mass. Although we found sylleptic shoots from September 2009, no significant effect of elevated CO₂ and soil, or significant interaction between elevated CO₂ and soil types, was found in the dry masses of needle and stem in the sylleptic shoots. **[Table 2]**

Table 3 shows shoot traits of the seedlings of the hybrid larch F₁. Elevated CO₂ significantly increased the shoot-length, the needle and branch dry mass per shoot, and the branch dry mass per unit shoot length. Only the needle dry mass per unit shoot-length was unaffected by elevated CO₂. Neither significant effect of soil nor significant interaction

between elevated CO₂ and soil for all parameters in shoot traits was found. There was a positive correlation between length and dry mass of branch per unit length of hybrid larch F₁ throughout all treatments (Fig. 2). [Table 3, Figure 2]

3.2 Relationship between growth parameters

Figure 3 shows the relations between the whole-plant dry mass and: needle dry mass (a), stem-and-branch dry mass (b) and root dry mass (c); and between diameter increment and height increment during the second growing season (d), and between diameter increment and length of shoot elongation during the second growing season (e). Height and diameter increments during the second growing season were calculated as the difference between values on 22 September 2008 and on 23 October 2009. As no significant effect of soil or of interaction of CO₂ and soil for all parameters was found, regression analyses throughout the two soil types were conducted in each CO₂ treatment. Elevated CO₂ did not change the slope of the regression line in any of these relationships. The intercept of the regression line between dry mass of roots and the whole-plant under elevated CO₂ was significantly lower than for controls (Fig. 3c) when we applied a common slope to both CO₂ treatments, indicating that the ratio of root dry mass to whole-plant dry mass was less under elevated CO₂. There was no significant difference between control and elevated CO₂ in the slope and intercept of the regression lines between other organs (i.e. needle, and stem and branches) and the whole-plant dry mass. Height increment and length of shoot growth in 2009 as compared to given diameter increment under elevated CO₂ were significantly higher than those for control (Fig. 3d, e). Consequently, the shape of seedlings of hybrid larch F₁ under elevated CO₂ was characterized as small roots, slender-shaped stems and long shoots. [Figure 3]

3.3 Photosynthetic traits

The value of A_{growth} in 2008 and 2009 was significantly increased by elevated CO_2 (Table 4). There was no significant effect of elevated CO_2 on G_s , V_{cmax} , J_{max} or LMA. We found significant reduction in N_{area} and a trend of reduction in N_{mass} under elevated CO_2 in 2009; in contrast, these N content were not affected by elevated CO_2 in 2008. In the seedlings grown on volcanic ash soil, there was a trend of decrease of J_{max} in 2008. On the other hand, significant increase of J_{max} in volcanic ash soil was found in 2009. The N_{mass} in volcanic ash soil was significantly higher than that in brown forest soil in 2008. No significant interaction between CO_2 and soil was found in the values of any parameter. [Table 4]

4. Discussion

Opposite to our hypothesis, we found that elevated CO_2 induced a change in the dry matter allocation between plant organs of hybrid larch F_1 seedlings, leading to small roots, slender-shaped stems and long shoots (Fig. 3). *Larix* species are light-demanding species and regenerate in areas denuded by natural or man-made disturbances (Gower and Richards 1990; Ryu et al. 2009). Light demanding species usually undergo rapid increase in height to occupy space in order to obtain light, especially during the seedling phase (Sterck 2005). Additional carbohydrate gained under elevated CO_2 might enhance this growth trait. These changes may help the initial growth of the planting stocks in a plantation, because there is competition with the other plants grown in the forest floor, especially the genus *Sasa* in northern Japan (e.g. Makita et al. 2004).

On the other hand, these changes in tree shape would give rise to high sensitivity to strong wind and heavy snow even in foliage-less period. The ratio of height to diameter at breast height of a main tree (H/DBH ratio) is an index of slenderness. Close relation between H/DBH ratio and windthrow damage are reported in many tree species including *Larix*

species (e.g. Mitchell 2000; Shibuya et al. 2011). Actually, the H/DBH ratio of Japanese larch in severely damaged stands by typhoon was 28% higher than that in the stands without damage (Shibuya et al. 2011). In the present study, the H/D ratio (we employed stem basal diameter for calculating slenderness because the size of the seedlings were relatively small, Yamanobe 2006) of the hybrid larch F_1 at the end of the second growing season were 82.2 in control site and 103.9 in elevated CO_2 site as averages of two soil conditions, and the difference was significant. Although the values in the present study were obtained from young seedlings, the 26% of enhancement in H/D ratio is considered to be significant in the risk for strong wind.

Based on the prediction of climate change (Christensen et al. 2007), snowfall and air temperature during winter will increase in northeastern Asia, including northern Japan. As mention above, hybrid larch F_1 have better resistance to heavy snow as compared to Japanese larch (Kaji 1983). One of the important reasons of high resistance is lower number and length of branch, leading lower snow receiving (Kaji 1983). Longer branch under elevated CO_2 (41% increase, Table 3) would increase in the area for snow receiving and therefore may enhance the risk of damage by heavy snow even in hybrid larch F_1 .

The dry mass of branches per unit shoot length increased significantly under elevated CO_2 , but there was no significant effect on the dry mass of needles per unit shoot length (Table 3). We found a positive correlation between length and dry mass of branch per unit length in the hybrid larch F_1 throughout all treatments (Fig. 2). In general, a thicker branch is needed to hold a longer shoot. We therefore suspect that the increase in branch dry mass per unit shoot length under elevated CO_2 is not due to changes in the shape of the shoot, but represents simple growth stimulation of the shoot length.

We found different responses of traits for main stem and branch to elevated CO_2

(Figs. 2 and 3d), although we unfortunately did not determine the diameter of branch for the analyses of shoot traits (Table 3). Main stem became slender under elevated CO₂ but branch did not. This might indicate main stem has a flexibility of safety ratio for bending or breaking. And relatively low velocity of wind in our experimental site (0.67 m s⁻¹ as average of the two growing seasons) might allow main stem to elongate with low margin of safety ratio. On the other hand, because branch elongated horizontally is received a certain amount of force by gravity, there may be no margin for decreasing safety ratio.

We did not observe photosynthetic down-regulation in gas exchange parameters (Table 4). The stimulation rate of A_{growth} of 45% by elevated CO₂ was similar to or higher than values in previous studies of woody plants (Nowak et al. 2004; Ainsworth and Long, 2007). However, we cannot be sure that elevated CO₂ does not induce photosynthetic down-regulation of hybrid larch F₁. Watanabe et al. (2011) reported down-regulation of photosynthesis of hybrid larch F₁ seedlings by elevated CO₂ (720 μmol mol⁻¹) with low nutrient availability. The value of N_{mass} in our study was in the range 2.02-2.53%, whereas 0.93-1.32% of N_{mass} was reported in Watanabe et al. (2011). Reduction in nutrient uptake may also occur in the seedlings grown in elevated CO₂ treatment because of the reduction in the root dry mass relative to the whole-plant dry mass of the seedlings (Fig. 3). Actually we found elevated CO₂-induced reduction in N content of needles in 2009 (Table 4). If this reduction in N content would continue, it might lead to down-regulation of photosynthesis in the hybrid larch F₁ (Crous et al. 2008; Norby et al. 2010).

We found little effect of soil on the growth and photosynthetic activity, and response of these to elevated CO₂ by hybrid larch F₁ seedlings (Tables 2, 3 and 4, Fig. 1). This is contrary to our expectation. Larch can grow in areas with relatively poor nutrient condition as found in natural condition (Kayama et al. 2009; Ryu et al. 2009; Watanabe et al. 2012).

The low availability of nutrients in the pumice included volcanic ash soil in the present study would not therefore affect hybrid larch F₁ seedlings, whereas it significantly affects the responses of photosynthetic activity to elevated CO₂ of *Betula platyphylla* var. *japonica* and *B. maximowicziana* (Eguchi et al. 2008). The soil N concentration of volcanic ash soil at our experimental site was 0.14 % (Eguchi et al. 2008), whereas that of Watanabe et al. (2011), which demonstrated photosynthetic down-regulation by elevated CO₂, was nearly half that amount, at 0.08%. If the soil nutrient availability is less than in the present study, down-regulation of photosynthesis might be observed even in the hybrid larch F₁ in FACE experiments. Another possible explanation is change in composition of ectomycorrhizal fungus. The composition of ectomycorrhizal fungus can alter in response to soil nutrient condition (e.g. Lilleskov et al. 2002). Well adapted species of ectomycorrhizal fungus might be dominant in volcanic ash soil. The study of ectomycorrhizal fungus is important future work to understand the nutrient acclimation of hybrid larch F₁ under elevated CO₂.

In conclusion, elevated CO₂ stimulates the initial growth of seedlings of the hybrid larch F₁ without down-regulation of photosynthesis even in infertile soil in the present study. Continuousness of the growth enhancement with special attention to nutrient status and ectomycorrhiza is an important future work. In addition, the resulting change to tree shape, with small roots, slender-shaped stems and longer shoots, may increase the risk from other stresses such as strong wind, heavy snow and nutrient deficiency. We propose next to determine the effects of elevated CO₂ on tolerance of hybrid larch F₁ to climatic stresses.

Author Contribution Statement

MW promoted this study, determined photosynthetic traits, analysed the all data, and wrote the all manuscript. MQ and EN worked for determination of biomass, and discussed data

interpretation. KK prepared seedlings of hybrid larch F₁ for this study, and discussed data interpretation. KT and FS managed the experimental system for CO₂ fumigation. TK is representative person of this project, and proposed the concept of this study, and discussed data interpretation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Chemical and physical analysis of brown forest soil and volcanic ash soil in the present study

	Brown forest soil	Volcanic ash soil
Nitrogen content (mg g ⁻¹)	0.3 (0.02)	0.14 (0.02)
Phosphorus content (mg 100 g ⁻¹)	4.48 (0.25)	0.58 (0.17)
Potassium content (mg 100 g ⁻¹)	21.32 (3.15)	1.55 (1.54)
Fine soil density (×10 ⁶ g m ⁻³)	1.88 (0.20)	2.42 (0.16)
Coarse soil density (×10 ⁶ g m ⁻³)	2.07 (0.11)	1.82 (0.16)
Maximum soil water content (%)	35.7 (2.8)	31.5 (1.4)
Solid phase (%)	52.6 (3.6)	40.8 (2.9)
Liquid phase (%)	35.7 (1.3)	18.5 (2.2)
Gaseous phase (%)	11.7 (4.2)	40.7 (3.3)

Data source: Eguchi et al. (2008)

Each value is the mean of six replications; standard error is shown in parentheses

Table 2. Dry mass of plant organs of hybrid larch F₁ seedlings grown under ambient CO₂ and elevated CO₂ (500 μmol mol⁻¹ CO₂) in combination with different soil types on 23 October 2009

	Brown forest soil		Volcanic ash soil		Two-way ANOVA		
	Ambient CO ₂	Elevated CO ₂	Ambient CO ₂	Elevated CO ₂	CO ₂	Soil	CO ₂ × Soil
Needle	48.5 (8.4)	50.3 (5.2)	39.5 (2.7)	47.3 (8.5)	n.s.	n.s.	n.s.
Stem	148.7 (19.7)	169.9 (13.9)	139.6 (4.8)	165.3 (25.4)	*	n.s.	n.s.
Root	51.8 (8.4)	54.7 (4.1)	54.4 (8.3)	54.6 (14.2)	n.s.	n.s.	n.s.
Whole-plant	236.8 (26.2)	274.8 (20.9)	233.5 (15.0)	267.2 (43.1)	*	n.s.	n.s.

Each value is the mean of three replications; standard deviation is shown in parentheses

Two-way ANOVA: * $P < 0.05$; n.s. not significant.

Table 3. Shoot traits of hybrid larch F₁ seedlings grown under ambient CO₂ and elevated CO₂ (500 μmol mol⁻¹ CO₂) in combination with different soil types on 23 October 2009

	Brown forest soil		Volcanic ash soil		Two-way ANOVA		
	Ambient CO ₂	Elevated CO ₂	Ambient CO ₂	Elevated CO ₂	CO ₂	Soil	CO ₂ × Soil
Length (cm)	29.8 (5.2)	47.4 (7.8)	34.3 (2.2)	43.1 (2.5)	**	n.s.	n.s.
Dry mass per shoot (g)							
Needle	0.95 (0.16)	1.31 (0.29)	0.95 (0.09)	1.24 (0.20)	**	n.s.	n.s.
Branch	1.71 (0.28)	2.42 (0.69)	1.88 (0.15)	2.25 (0.46)	**	n.s.	n.s.
Dry mass per unit shoot length (g cm ⁻¹)							
Needle	0.025 (0.002)	0.028 (0.003)	0.025 (0.002)	0.028 (0.003)	n.s.	n.s.	n.s.
Branch	0.039 (0.002)	0.050 (0.006)	0.042 (0.003)	0.049 (0.002)	*	n.s.	n.s.

Five long-shoots were corrected from the top of the each seedling to evaluate shoot traits.

Each value is the mean of three replications; standard deviation is shown in parentheses

Two-way ANOVA: * $P < 0.05$; ** $P < 0.01$; n.s. not significant

Table 4. Photosynthetic traits in needles of hybrid larch F₁ seedlings grown under ambient CO₂ and elevated CO₂ (500 μmol mol⁻¹ CO₂) in combination with different soil types in September of 2008 and 2009

	Brown forest soil		Volcanic ash soil		Two-way ANOVA		
	Ambient CO ₂	Elevated CO ₂	Ambient CO ₂	Elevated CO ₂	CO ₂	Soil	CO ₂ × Soil
2008							
<i>A</i> _{growth}	10.7 (2.3)	16.5 (2.6)	9.5 (0.6)	15.7 (1.2)	**	n.s.	n.s.
<i>G</i> _s	0.10 (0.03)	0.13 (0.03)	0.11 (0.01)	0.12 (0.05)	n.s.	n.s.	n.s.
<i>V</i> _{cmax}	98.7 (4.1)	86.8 (7.1)	81.8 (6.5)	90.1 (13.7)	n.s.	n.s.	n.s.
<i>J</i> _{max}	171.5 (25.5)	170.1 (15.9)	157.9 (31.7)	155.8 (5.0)	n.s.	0.064	n.s.
LMA	87.9 (11.5)	93.3 (8.6)	96.8 (2.2)	83.7 (8.4)	n.s.	n.s.	n.s.
<i>N</i> _{mass}	2.02 (0.05)	2.08 (0.08)	2.32 (0.08)	2.21 (0.19)	n.s.	*	n.s.
<i>N</i> _{area}	1.77 (0.22)	1.93 (0.10)	2.24 (0.07)	1.86 (0.32)	n.s.	n.s.	n.s.
2009							
<i>A</i> _{growth}	12.8 (2.0)	15.8 (1.3)	12.9 (1.3)	18.4 (1.6)	**	n.s.	n.s.
<i>G</i> _s	0.12 (0.01)	0.11 (0.02)	0.13 (0.02)	0.13 (0.03)	n.s.	n.s.	n.s.
<i>V</i> _{cmax}	106.8 (11.8)	115.9 (4.4)	126.9 (24.6)	132.9 (23.3)	n.s.	n.s.	n.s.
<i>J</i> _{max}	191.4 (11.9)	217.0 (20.0)	246.1 (20.5)	246.3 (20.4)	n.s.	**	n.s.
LMA	95.5 (7.5)	102.1 (9.2)	108.4 (6.2)	95.3 (18.3)	n.s.	n.s.	n.s.
<i>N</i> _{mass}	2.40 (0.18)	2.15 (0.21)	2.53 (0.10)	2.28 (0.15)	0.069	n.s.	n.s.
<i>N</i> _{area}	2.30 (0.31)	2.19 (0.03)	2.74 (0.19)	2.16 (0.26)	*	n.s.	n.s.

Each value is the mean of three replications; standard deviation is shown in parentheses

Two-way ANOVA: * $P < 0.05$; ** $P < 0.01$; n.s. not significant. Actual P values were shown when $0.05 < P < 0.1$

*A*_{growth}: net photosynthetic rate at growth CO₂ concentration (μmol m⁻² s⁻¹), *G*_s: stomatal conductance to water vapor (mol m⁻² s⁻¹), *V*_{cmax}: maximum rate of carboxylation (μmol m⁻² s⁻¹), *J*_{max}: maximum rate of electron transport (μmol m⁻² s⁻¹), LMA: leaf mass per area (g m⁻²), *N*_{mass}: mass-based N content (%), *N*_{area}: area-based N content (g m⁻²)

Figure captions

Fig. 1 Height and diameter increment of hybrid larch F₁ seedlings grown under ambient and elevated CO₂ (500 μmol mol⁻¹ CO₂) in combination with different soil types (BF: brown forest soil, VA: volcanic ash soil). Each value is the mean of three replications. An asterisk indicates a significant increase in height and diameter increments with elevated CO₂ (results of two-way ANOVA with the factors of CO₂ and soil, $P < 0.05$)

Fig. 2 Relation between shoot length and branch dry mass per unit shoot length of hybrid larch F₁ seedlings. BF: brown forest soil, VA: volcanic ash soil. Each plot indicates the value in each replication

Fig. 3 Relations between the whole-plant dry mass and dry mass of each component (a - c), and diameter increment and height increment (d), and length of shoot elongated (e) during the second growing season. BF: brown forest soil, VA: volcanic ash soil. Solid line: ambient CO₂, dashed line: elevated CO₂. Each plot indicates the value in each replication. The P values of the difference in the intercepts of the regression lines are (a) $P = 0.201$, (b) $P = 0.763$, (c) $P = 0.003$, (d) $P = 0.001$ and (e) $P = 0.003$

Figure 1

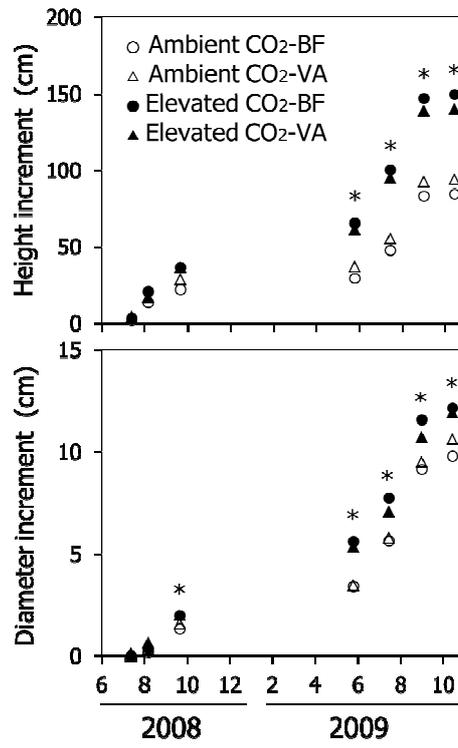


Figure 2

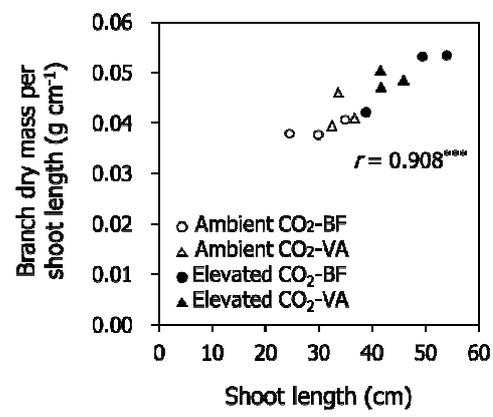


Figure 3

