DOI: 10.1007/s11099-012-0049-z PHOTOSYNTHETICA 50 (3): 422-428, 2012

Photosynthesis and nitrogen allocation in needles in the sun and shade crowns of hybrid larch saplings: effect of nitrogen application

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Abstract

We studied the effects of applying 50 kg(N) ha^{-1} $year^{-1}$ of nitrogen (N) on needle photosynthesis, N allocation and nutrient content in the sun- and shade crowns of the hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*). The light-saturated net photosynthetic rate (P_{Nmax}) was not significantly affected by N application or crown position, although the contents of N, P, K, and chlorophyll (Chl), and the maximum rates of carboxylation and electron transport were lower in needles of the shade crown than of the sun crown. This difference was mainly due to an increase in the intercellular CO_2 concentration (C_i) in the needles of the shade crown. Analysis of N allocation in photosynthetic systems revealed that more N was allocated to functions related to electron transport and ribulose-1,5-bisphosphate (RuBP) regeneration in needles of the shade crown. N allocation in needles of the hybrid larch F_1 was regulated mainly by the light conditions, rather than by N application.

Additional key words: foliar nutrient; hybrid larch; nitrogen deposition; sun- and shade needles.

Introduction

To maintain a high growth rate of trees, photosynthesis should take place throughout the entire crown, with efficient use of N resources (Šesták 1985, Matyssek *et al.* 2008). This is because N is believed to be a primary limiting resource in northern forests (Schulze *et al.* 2005, Braun *et al.* 2010, Anten and During 2011). It is therefore important to know the N allocation to the crown according to the environment (Marek *et al.* 2002, Lambers *et al.* 2008).

Recently, we develop a new hybrid larch species F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*). It is a fast growing, light-demanding species and has good tolerance against both biotic and abiotic stresses (Ryu *et al.* 2009). It is recognized as one of the most promising species for moderating atmospheric CO_2 throughout northeast Asia because of its high storage capacity of carbon in the stem with high specific gravity (Koike *et al.* 2000, Qu *et al.* 2004). The response of the hybrid larch to nutrient availability is not well known, in particular the allocation traits of N (Koike 2009).

Received 17 September 2011, *accepted* 2 May 2012.
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Abbreviations: C_a – external CO_2 concentration; C_i – intercellular CO_2 concentration; Ca – calcium; Chl – chlorophyll; g_s – stomatal conductance of water vapor; J_{max} – maximum rate of electron transport; K – potassium; K_c – value of Rubisco Michaelis constants for CO_2; K_o – value of Rubisco Michaelis constants for O_2; LHCP – light-harvesting chlorophyll complex protein; LMA – leaf mass per area; Mg – magnesium; N – nitrogen; N_1 – nitrogen allocated in light-harvesting chlorophyll complex protein and photosystems; N_2 – nitrogen allocated in bioenergetics (electron carriers except for photosystems, coupling factor and Calvin cycle enzymes except for Rubisco); N_3 – nitrogen allocated in Rubisco; N_4 – nitrogen allocated in other components in needle; N_m – N content per unit leaf mass; P – phosphorus; P_{max} – net assimilation rate at 1,700 μmol mol^{-1} CO_2, P_N – net assimilation rate; P_{Nmax} – light-saturated net photosynthetic rate; PNUE – photosynthetic nitrogen-use efficiency; PPF – photosynthetic photon flux; V_{cmax} – maximum rate of carboxylation; V_{cr} – the specific activity of Rubisco, Γ* – CO_2 compensation point in the absence of dark respiration.
Acknowledgements: We thank Dr. N.P.R. Anten and Prof. Ü. Niinemets for their encouragment in this study. Thanks are also due to Dr. Anthony Garrett of Scitext Cambridge, U.K. for linguistic comments. We acknowledge financial support in part *via* a Grant-in-Aid from the Japan Society for the Promotion of Science through Scientific Research on Innovative Areas (to T. Koike). Research Fellowships for Young Scientists program (to M. Watanabe and Y.S. Kim), and Young Scientists B (to M. Watanabe), and also a project study grant from Development of Mitigation and Adaptation Techniques to Global Warming in the Sectors of Agriculture, Forestry, and Fisheries (to K. Kita).

We usually plant larch seedlings at a fairly high density in plantations for high quality timber. As a result, after several years, the crown of these larches will suffer from self-shading or between-tree shading (Kuuluvainen and Pukkala 1989, Marek *et al.* 2002). N is allocated within the foliage of the crown according to the light conditions in each part of the plant (Hirose and Werger 1987, Hirose *et al.* 1988, Niinemets 1997, Schulze *et al.* 2005). More N is generally allocated to foliage in the upper sun crown than to the lower shade crown (Rosati *et al.* 2000, Lambers *et al.* 2008).

Nitrogen allocation between foliage was also influenced by the incident light conditions at the forest floor. Kitaoka and Koike (2004) observed N allocation between photosynthetic systems in the leaves of four deciduous broad-leaved tree species regenerated in the forest floor. N allocation changed according to the changing light conditions during the growing season. There was also a clear difference in N allocation traits depending on the specific leaf phenology of the tree species.

Atmospheric N deposition has recently been increasing rapidly, especially in eastern Asia, and this trend is expected to continue (Galloway *et al.* 2004, Magnani *et al.* 2007). Previous studies of how the N supply affects responses to irradiance have concentrated on herbaceous plants, and there is little information on tree species (Kitaoka *et al.* 2009). The main effect of increased N availability is an increase in the photosynthetic capacity of partially shaded leaves, although there is no increase in the maximum capacity of leaves exposed to strong light (DeJong *et al.* 1989). Posch *et al.* (2008) found that application of N fertilizer affected mainly the biochemistry and light-use physiology of lower- and mid-canopy needles of *Pinus radiata*, increasing the amount of Chl and hence the amount of the harvested light. We therefore expect increasing N deposition to affect N allocation in shaded foliage. However, it is not known whether deciduous conifers respond differently.

How does the hybrid larch deal with changes in irradiation by means of efficient N allocation between differing positions within the crown, and within needles? A better understanding of N allocation to photosynthetic systems is needed. In the present study we compared photosynthetic traits in lower- and upper-crown needles of hybrid larch saplings grown with and without N application, to assess the photosynthetic acclimation of needles in relation to N allocation. We also discuss the interaction between N application, and the differing conditions of irradiation experienced by different crown positions, in photosynthesis of the hybrid larch.

Materials and methods

Experimental site and plants: On 21st May 2008, planting stocks of 3-year-old clonal seedlings of the hybrid larch F_1 (sp. "Gream" as a nickname; hereafter 'hybrid larch') were planted in the Sapporo Experimental Forest of Hokkaido University, Japan (43.07°N, 141.38°E, 15 m a.s.l.). The hybrid larch was originally developed by crossing female Dahurian larch (*L. gmelinii* var. *japonica* Pilg.) from the Kurile Islands and Sakhalin with male Japanese larch (*L. kaempferi* Carr. = *L. leptolepis* Gordon). The hybrid had tolerance not only to shoot blight disease, grazing by Redback voles and deer, but also to environmental stresses such as drought, low temperature and heavy snow (Ryu *et al*. 2009). Larches have two types of shoot: short shoots and long shoots (Kitaoka *et al*. 2000). Short shoots flush their needles once in spring, and long shoots develop needles from early July until late August. We focus on long-shoot needles because of their high photosynthetic capacity as compared with short-shoot needles (Kitaoka *et al*. 2000).

The soil in the nursery is classified as brown forest soil, which is common in Japan (Nakaji *et al.* 2002, Kayama *et al.* 2009). The planting density for experimental use was 4,200 ha^{-1}. The average ± SD values of height (from soil surface to apical point) and diameter (at 3-cm height from soil surface) at the beginning of experiment in May 2008 were 79.6 ± 8.5 cm and 0.97 ± 0.10 cm in the N-treatment plots, and 77.5 ± 7.5 cm and 0.97 ± 0.09 cm in the control plots.

The experimental site has a cool temperate climate, with adequate rainfall (annual rainfall was 843 mm in 2008; 1,147 mm in 2009 and 1,325 mm in 2010; reported by Japan Meteorological Agency, 2012). The snow-free period is late April to early November. From May to October, the growing season average air temperature at the study site was 19.5°C. Maximum and minimum temperatures from May to October were 35.2 °C in August and 8.3°C in October. The monthly average photosynthetic photon flux (PPF) converted from light intensity by *HOBO*, (Pendant Temperature/ Light Data Logger, *64K-UA-002-64*, Onset, USA) during the three years was shown in Fig. 1. The highest PPF was found in June (over 350 μmol m^{-2} s^{-1}) and gradually decreased until October (around 170 μmol m^{-2} s^{-1}).

Experimental design: We set two levels of N, 0 and 50 kg(N) ha^{-1} $year^{-1}$, referred to as N0 and N50. The plots were replicated 3 times for each N level. Each plot (4.5 × 7.5 m) contained 15 seedlings. The plots with and without N application were distributed alternately. In each growing season, ammonium nitrate solution (14 mM) was supplied by watering pot to the soil surface of the N50 plots. In 2008, N was supplied on 25 June, 11 July, 1 August, and 22 August. In 2009, N was supplied on 18 May, 27 June, 6 August, and 18 September. In 2010, N was supplied on 31 May, 7 July, 11 August and 13 September. The same amount of tap water without ammonium nitrate was supplied to the N0 plots. Kimura *et al*. (2009) reported the amount of

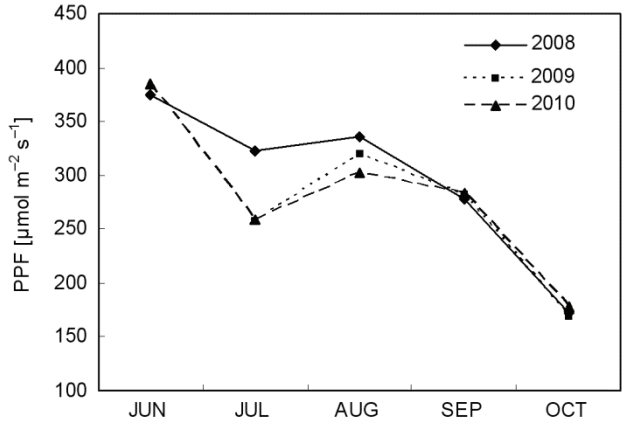

Fig. 1. Monthly average photosynthetic photon flux (PPF) during the experiment period. $n = 3$.

atmospheric nitrogen deposition in the forest in the Kanto region of Japan to be 30–50 kg(N) ha^{-1} year^{-1}. Moreover, Galloway *et al*. (2004) assert that N deposition to the ground will increase; that is why we chose to administer 50 kg(N) ha^{-1} year^{-1}.

Soil solution was collected from 2008 to 2010 during the growing season (10 days before and after N application) using a tension lysimeter (*Dik-8390, Daiki Rika Kogyo Co., Ltd.*, Saitama, Japan). The pH of the soil solution was measured using a pH meter (*M-12, Horiba*, Kyoto, Japan). The contents of inorganic nitrogen (*i.e*. NH_4^+ and NO_3^-) were measured with a continuous flow type analyzer (Auto analyzer, *BL-TEC, AACS-4*, Osaka, Japan). Contents of potassium (K), calcium (Ca), and magnesium (Mg) were measured by inductively coupled plasma-atomic emission spectrometry (*ICP-AES, IRIS, Jarrel Ash*, Franklin, MA, USA).

Light environment of needles: The shade crown was defined as within 50 cm from the ground and 50 cm horizontally away from the stem, where mutual shading of lower shoots had begun in early July, 2010. The sun crown was defined as 150 cm high off the ground and 50 cm horizontally away from the stem. Light intensity at the shade- and sun-crown positions were measured (using a *HOBO*, the same with above) in September after long-shoot development had ceased (Kitaoka *et al*. 2000).

Needle gas exchange was measured for long-shoot needles from both the shade and sun crowns from mid-September in 2010, when the needles had matured. The needles studied in both upper and lower crowns were the long-shoot needles which had emerged in early June and matured by late August (Kitaoka *et al.* 2000). These measurements were made in September 2010 using an open gas-exchange system (*LI-6400, Li-Cor Inc*., Lincoln, NE, USA) with a light-emitting diode light source (*LI-6400-40*). Using two or three seedlings per plot, we established the intercellular CO_2 concentration (C_i)-response curve for the P_N with 11 steps of external CO_2 concentration (C_a). The order of the C_a values during the measurement was 380, 300, 220, 140, 60, 380, 500, 800; 1,100; 1,400; and 1,700 ppm (Long and Bernacchi 2003). During the measurements, the needle temperature and light PPF from light-emitting diode light source were maintained at 25.0 ± 1.0ºC and 1,500 μmol m^{-2} s^{-1}, respectively, which had been determined as the conditions maximizing the photosynthetic capacity of this species (Ryu *et al*. 2009). The leaf-to-air vapor pressure deficit was approximately 1.5 kPa, reflecting ambient water vapor conditions in the experimental field.

The P_N/C_i curve was used to estimate P_{Nmax} and stomatal conductance (g_s) at 380 μmol(CO_2) mol^{-1}, the net assimilation rate at 1,700 μmol(CO_2) mol^{-1} (P_{max}), the maximum rate of carboxylation (V_{cmax}), and the maximum rate of electron transport (J_{max}) (Farquhar *et al*. 1980, Long and Bernacchi 2003). The values of the Rubisco Michaelis constants for CO_2 (K_c) and O_2 (K_o), and the CO_2 compensation point in the absence of dark respiration (Γ*), were calculated according to Bernacchi *et al.* (2001) based on analysis of the P_N/C_i curve. All gas-exchange parameters were expressed on the basis of the projected needle area as measured with an image scanner (*CanoScan LiDE 600F, Canon*, Tokyo, Japan).

Foliar nutrients and Chl: After the gas-exchange measurements, the needles were collected and the leaf mass per area (LMA) was determined. This was calculated as the ratio of dry mass (measured after oven-drying at 60ºC for 5 days) to the needle surface area. The leaf area was determined by a leaf image scanner as described above. The dried needles were then ground into fine powder and their N content was determined by the combustion method, using an NC analyzer (*NC-900, Sumica-Shimadzu*, Kyoto, Japan). The photosynthetic N-use efficiency (PNUE) was calculated as P_{Nmax} divided by the needle N content.

The needle powder samples were digested by HNO_3, HCl, and H_2O_2. The contents of phosphorus (P), magnesium (Mg), and potassium (K) in these sample solutions were determined using the *ICP-AES* referred to above. The fresh needle samples for Chl measurement were stored at –80°C; Chl was extracted with dimethyl sulfoxide according to the method of Barnes *et al.* (1992) and Shinano *et al.* (1996) and was quantified using a spectrophotometer (*Gene spec III, Hitachi*, Tokyo, Japan).

Nitrogen allocation to the photosynthetic system: The photosynthetic apparatus was divided into four parts: (*1*) light-harvesting Chl complex protein (LHCP) and photosystems; (*2*) bioenergetics (electron carriers except for photosystems, coupling factor and Calvin-cycle enzymes except for Rubisco); (*3*) Rubisco and (*4*) others; these are denoted by N_1, N_2, N_3 and N_4, respectively. We estimated N_1, assuming a N content per unit Chl content of 37.1 mol mol^{-1} (Barnes *et al*. 1992), as:

N_1 (%) = (Chl × 0.5194)/LMA N_m.

We estimated N_2 from gas-exchange characteristics according to the following equation (Field 1983, Takashima *et al.* 2004):

N_2 (%) = J_{max}/(156 × 9.53 LMA N_m),

where N_m denotes N per unit leaf mass. It is assumed here that nitrogen in bioenergetics is proportional to J_{max}, where the ratio of J_{max} to the cytochrome *f* content is 156 mmol mol^{-1} s^{-1} (Niinemets *et al.* 1997), and nitrogen in bioenergetics per unit cytochrome *f* has value 9.53 mol mmol^{-1}]. We estimated N_3 using the following equation (Niinemets *et al.* 1999, Tissue and Lewis 2010):

N_3 (%) = V_{cmax}/(6.25 V_{cr} LMA N_m),

where V_{cr} is the specific activity of Rubisco (the maximum rate of RuBP carboxylation per unit Rubisco protein), and the coefficient of 6.25 [g(Rubisco) g^{-1}(N in Rubisco)] converts N content to protein content. The value of V_{cr} is 20.5 μmol(CO_2) g^{-1}(Rubisco) s^{-1} at 25°C for purified Rubisco enzyme from *Spinacia oleracea* (Jordan and Ogren 1984). This method is unable to determine the amount of inactivated Rubisco; the calculated value of N_3 is therefore an underestimate (Warren and Adams 2004). The remaining N corresponds to N_4.

Statistical analysis: We used analysis of variance (*ANOVA*) to estimate the effects of N and of crown position, and of their interaction, on the physiological and growth properties of hybrid larch F_1 seedlings. The tests were performed using *SPSS 16.0* (*SPSS Inc.*, USA) statistical software.

Results

Growth environment: The average height ± SD in N0 plots was 341.96 ± 86.96 cm and 353.82 ± 64.63 cm in N50 plots, respectively. During the measuring period in mid-September 2010, the average values ± SD of the PPF (6:00–18:00 h) in the N0 and N50 plots were 144.0 ± 60.8 and 112.9 ± 43.8 μmol m^{-2} s^{-1} in the shade crown; and 324.5 ± 106.8 and 320.3 ± 121.0 μmol m^{-2} s^{-1} in the sun crown, respectively. Light intensity in the shade crowns of the N0 plots and N50 plots was significantly lower than in the sun crowns (P=0.05). No significant difference in the PPF was observed between the N0 and N50 plots (P=0.159).

No significant difference was found in any soil parameters between the plots used for N0 and N50 before the N treatment in May 2008. Soil pH value was 4.17–4.21. The contents of total N, ammonium-N (NH_4^+), nitrate-N (NO_3^-) in the soil solution were respectively 3.05–3.08 g kg^{-1}, 3.44–4.11 mg kg^{-1}, and 9.37–10.0 mg kg^{-1}. The average value of each parameter was calculated during the three years of the study from 2008 to 2010. The content of NO_3^- was significantly increased in N50 (34.76% higher than N0), but the content of NH_4^+ did not change significantly. The contents of Ca and Mg in the soil solution were significantly greater in the N50 plot (Ca: 547.67 μM; Mg: 83.21 μM) than in the N0 plot (Ca: 377.79 μM; Mg: 55.09 μM). The content of K did not change significantly with N50 (N0, 113.01 μM; N50, 116.08 μM).

Needle gas exchange: Values of P_{Nmax} and g_s did not change significantly in response to N application, or differ with canopy position. Values of V_{cmax} and J_{max} were significantly lower in the shade crown, and were unaffected by N application. PNUE in the shade crown was significantly higher than in the sun crown. C_i was significantly higher in the shade crown than in the sun crown. No significant interaction was found between N application and crown position for any parameter (Table 1).

Needle nutrients, Chl content and LMA: N application did not significantly affect any parameters. The content of N, P, K, chlorophyll in needles and LMA in the shade crown, were significantly lower than in the sun crown. No significant interaction was found between N application and crown position (*i.e.* shade- *vs.* sun crown) for any parameter (Table 2).

Table 1. Photosynthetic capacity (± SD) in needles located at upper crown (U) and lower crown (L) of hybrid larch F_1 seedlings grown at two levels of N: N0 – 0 kg(N) ha^{-1}; N50 – 50 kg(N) ha^{-1}. V_{cmax} – maximum rate of carboxylation, J_{max} – maximum rate of electron transport, P_{Nmax} – light-saturated net photosynthetic rate, g_s – stomatal conductance, C_i – intercellular CO_2 concentration, PNUE – photosynthetic nitrogen-use efficiency. n = 8. *ANOVA*: * – P<0.05; ** – P<0.001; n.s. – not significant.

	N level	V_{cmax} [μmol m^{-2} s^{-1}]	J_{max} [μmol m^{-2} s^{-1}]	P_{Nmax} [μmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]	PNUE [μmol mol^{-1} s^{-1}]
U	N0	110.87 ± 14.84	176.72 ± 31.51	9.28 ± 2.08	0.09 ± 0.04	188.20 ± 28.90	55.84 ± 14.21
	N50	112.91 ± 41.18	188.57 ± 48.12	9.36 ± 2.54	0.09 ± 0.02	201.48 ± 63.20	53.75 ± 13.79
L	N0	90.14 ± 18.23	158.98 ± 27.00	9.94 ± 1.91	0.11 ± 0.04	220.55 ± 50.03	89.39 ± 14.18
	N50	64.88 ± 9.86	144.96 ± 32.00	7.88 ± 2.23	0.10 ± 0.05	233.94 ± 34.36	70.82 ± 14.97
N		n.s.	n.s.	n.s.	n.s.	n.s.	*
Crown		**	*	n.s.	n.s.	*	**
N × Crown		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 2. Nutrient contents, chlorophyll (Chl) and leaf mass per area (LMA) ± SD of needles located at upper crown (U) and lower crown (L) of hybrid larch seedlings grown at two levels of N: N0 – 0 kg(N) ha^{-1}; N50 – 50 kg(N) ha^{-1}. Ca – calcium; Chl – chlorophyll; K – potassium; LMA – leaf mass per area; N – nitrogen; Mg – magnesium; P – phosphorus. n = 8. *ANOVA*: * – $P<0.05$; ** – $P<0.001$; n.s. – not significant.

	N level	N [g m^{-2}]	P [g cm^{-2}]	K [g m^{-2}]	Ca [g m^{-2}]	Mg [g m^{-2}]	Chl [g m^{-2}]	LMA [g m^{-2}]
U	N0	2.37± 0.31	0.22 ± 0.09	0.81 ± 0.14	0.43 ± 0.10	0.11 ± 0.04	0.54 ± 0.13	104.38 ± 9.70
	N50	2.49 ± 0.53	0.19 ± 0.06	0.72 ± 0.23	0.41 ± 0.09	0.10 ± 0.04	0.60 ± 0.24	103.89 ± 19.41
L	N0	1.56 ± 0.19	0.18 ± 0.06	0.45 ± 0.12	0.35 ± 0.10	0.13 ± 0.03	0.41 ± 0.05	75.50 ± 8.87
	N50	1.56 ± 0.26	0.15 ± 0.06	0.40 ± 0.13	0.41 ± 0.10	0.12 ± 0.02	0.40 ± 0.08	76.59 ± 12.37
N		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Crown		**	*	**	n.s.	n.s.	*	**
N × Crown		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3. N allocation ± SD to photosynthetic functions in needles located at upper crown (U) and lower crown (L) of the hybrid larch F_1 grown under two levels of N: N0 – 0 kg(N) ha^{-1}; N50 – 50 kg(N) ha^{-1}. Photosynthetic functions are categorized as light-harvesting complex protein (LHCP) and photosystems (N_1), bioenergetics (electron carriers except for photosystems, coupling factor and Calvin cycle enzymes except for Rubisco (N_2), Rubisco (N_3) and other (N_4). n = 8. *ANOVA*: * – $P<0.05$; n.s. – not significant.

	N level	N_1 [%]	N_2 [%]	N_3 [%]	N_4 [%]
U	N0	25.00 ± 2.74	5.16 ± 1.41	36.74 ± 6.60	43.40 ± 8.20
	N50	16.01 ± 5.80	5.28 ± 1.58	36.43 ± 14.65	42.28 ± 20.00
L	N0	16.55 ± 4.77	6.89 ± 1.01	43.92 ± 14.26	30.34 ± 13.70
	N50	16.72 ± 3.22	6.35 ± 1.29	33.00 ± 7.78	43.93 ± 10.03
N		n.s.	n.s.	n.s.	n.s.
Crown		n.s.	*	n.s.	n.s.
N × Crown		n.s.	n.s.	n.s.	n.s.

N allocation in the photosynthetic system: There was no significant effect of N application or of canopy position on N allocation to N_1 and N_3 (Table 3). N allocation to N_2 was significantly greater in the shade- than in the sun crown. There was no significant interaction between N application and crown position for any parameter.

Discussion

Low values of P_{Nmax} in shade crown were correlated with low foliar N concentration, and high P_{Nmax} was correlated with high foliar N concentration (Table 2). It is possible that there was no photoinhibition of photosynthesis in the shade crown in our study (Baker and Bowyer 1994). Field (1983) proposed that canopy photosynthesis is maximized when sunlit leaves have greater N content than shaded leaves. Our data are therefore consistent with previous observations of herbaceous and woody plants (Fife *et al.* 1997, Wilson *et al.* 2000, Warren *et al*. 2003, Posch *et al.* 2008, Johnson *et al.* 2010). Our results did not, however, confirm that P_{Nmax} is greater in the sun crown irrespective of foliar N. In fact a higher V_{cmax} and J_{max}, as well as higher N content, were observed in the sun crown.

The value of C_i was higher in the shade crown than in the sun crown (Table 1). Greater C_i does not necessarily induce a difference in P_{Nmax} between the shade and sun crowns, although V_{cmax} and J_{max} were lower in the shade crown than in the sun crown, and PNUE was higher in the shade crown. The value of C_i is the result of a balance between the supply of CO_2 from the atmosphere, through the stomata, and CO_2 demand in chloroplasts (Lambers *et al.* 2008). The reductions in V_{cmax} and J_{max} induce an increase in C_i. The plant then usually closes stomata to prevent loss of water by transpiration. It might not be necessary for needles in the shade canopy to close their stomata completely, however, because of the lower temperature in the shade crown than in the sun crown; also, there is only a small hydraulic limitation (Kupper *et al.* 2006), and the needles may be young (Escudero and Mediavilla 2003). In fact we found that g_s in the shade crown was higher in the shade crown than in the sun crown, not lower.

The change in N allocation to photosynthesis may partly compensate for the decrease in photosynthetic activity due to the increase in PNUE of the needles in the shade crown. The N allocation to N_2, which is related mainly to the function of RuBP regeneration, was significantly higher in the shade crown (Table 3). This implies in turn that shade crown needles increased their bioenergetic transport efficiency. An increase in N allocation to N_1 might also increase photosynthetic activity by exploiting more light energy for photosynthesis (Kitaoka

and Koike 2004, Kitaoka *et al.* 2009). Our results showed, however, that N allocation to N_1 was not significantly enhanced in the shade crown (Table 3). Based on this study, we suggest that 14.69–16.72% N was allocated to N_1. This is an optimal ratio that allows for adequate light energy for photosynthesis, even in the shade crown.

Foliar nutrients other than N may also be important in the assimilation of carbon by plants. Foliar N, P and K were significantly less in the shade crown than in the sun crown, and foliar Ca and Mg did not differ significantly between the sun and shade crowns (Table 2). These reductions in N, P, and K contents were due mainly to the decrease in LMA, rather than the decrease in mass-based contents (data not shown). The mass-based needle Ca and Mg contents were higher in the shade crown than the sun crown. This enhancement of the Ca and Mg contents may be important for acclimation to low light conditions.

In conclusion, allocation of N within the canopy of the hybrid larch F_1 was significantly influenced by the irradiation conditions. Indeed, N allocation within needles of the hybrid larch changed with the light conditions; more foliar N was reallocated to bioenergetics transport apparatus in photosynthetic systems. However, N application did not increase net photosynthesis.

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