Article

Seasonal and yearly variation in photosynthetic capacity of short- and long-shoot needles of the canopy of Japanese larch trees as a basis for determining for O3 sensitivity

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Abstract: Ground-level ozone (O_3) increases in the northeastern part of Eurasia, where larch species are dominant trees and have been planted intensively. The Japanese larch (*Larix kaempferi*; hereafter larch) is classified as the tree species most of its sensitive to O_3 based on data from long-shoot needles of seedlings. This criterion should be reconsidered based on O_3 uptake, which is strongly depended on stomatal conductance (Gs) of adult trees through the difference in relevant needle traits. Because Gs is closely correlated with photosynthetic activity, we measured the *in situ* seasonal and yearly change in photosynthetic function, needle mass per area (LMA) and nitrogen (N) content of both short- and long-shoot needles of the canopy of larch trees over successive 3 years. No difference was observed in the in light saturated photosynthetic rate at ambient CO_2 (P_{sat}) between needles of hetero types of shoot in the latter part of the growing season, but clear differences were found within a specific year, indicating that seasonal changes in climatic factors determine P_{sat} in the long term. The P_{sat}-N relations differed significantly between the needle types. However, P_{sat} at saturated CO_2 (P_{max})-N was quite similar, implying that the CO_2 diffusion difference between short- and long-shoots is responsible for the differing P_{sat} -N. In conclusion, seasonal and yearly variations in photosynthetic capacity are mainly determined mainly by climatic variations, whereas shoot type determined the traits of photosynthetic N utilization as well as Gs regulation.

Keywords: Japanese larch (*Larix kaempferi*), heterophyllous shoots, year-to-year variation, electron transport rate, nitrogen remobilization rate

1. Introduction

In the northeastern part of Eurasia including Japan, larch is the dominant tree species and has been planted intensively [1–3]. Unfortunately, recent increase in ground-level ozone (O_3) and nitrogen (N) deposition in northeast Asia (e.g.[4–6]) is threatens to suppress the growth of larch species and also their hybrid, F_1 [7]. According to a screening experiment with open top chambers, the larch seedling is one of the most O_3 sensitive species of 18 trees native to Japan[8]due to high stomatal conductance (Gs) in long-shoot needles. Most larch species are characterized by high growth rate with heterophyllous shoots (e.g. [1,9]) having deciduous leaf habit in a conifer species[10]. With increasing in tree size, the proportion of long-shoot needles decreases and consequently many physiological traits are evaluated using short-shoot needle.

Much research has analyzed the high growth rate of larch species in relation to photosynthetic function in heterophyllous shoots, i.e. short- and long-shoot needles. Although they concluded that no special metabolic pathway was involved [11–14], a high photosynthetic rate may be realized with the unique arrangement of different shoot types in larch canopies [15–17]. In general, heterophyllous leaves are such that the growth of long-shoots i.e. late leaves is substantially suppressed following shading by early leaves and short-shoot leaves of birch [18]. However, contradictory results corresponding the photosynthetic rate at light saturation (P_{sat}) have been reported for larch trees such that short-shoot needles have lower P_{sat} than long-shoot needles [16]. In contrast, no difference in P_{sat} was found for the two types of shoots [19].

Sensitivity to O_3 in larch depends strongly on N conditions[20,21] as well as Gs (i.e. gas diffusion in both water and CO_2, e.g. [17,22,23]). Is there any significant difference in photosynthetic capacity of short- and long-shoot needles of larch canopy when O_3 attacks them? We have insufficient photosynthetic and Gs data on heterophyllous shoots *in situ* of mature larch canopy trees [20,24]. Only a few studies have been made of photosynthetic difference in heterophyllous shoots of larch [16,19] and the data were obtained for a single season. There is a large yearly variation in P_{sat} of saplings of four deciduous tree species in larch forests in northern Japan, due to the difference in air temperature and precipitation during the leaf development period [25]. To evaluate the yearly variation, at least 2-years of measurement will be needed to determine the roles and functional differences in heterophyllous shoots of larch trees under field conditions.

In generally, Gs substantially affects photosynthetic capacity (e.g. [22,26,27]. Leaf functional structure, including leaf mass per area (LMA) and leaf water availability parameters, influence gas diffusion from air to chloroplasts and vice versa [28]. Are there any variations in the relation of needle N contents and photosynthetic function between short- and long-shoot needles with increasing N deposition in East Asia[7]?

To shed light on these questions, we monitored seasonal and yearly changes in the *in situ* canopy photosynthetic capacity (P_{sat} and P_{max}=P_{sat} at CO_2 saturation, Gs) in short- and long-shoot needles, together with environmental factors, using a canopy tower. The photosynthetic-N relations (P_{sat}-N and P_{max}-N, V_{cmax}-N and J_{max}-N) and the needle nitrogen remobilization rate (NRMA) were also examined in relation to factors affecting photosynthesis in the larch canopy. The aim of this study is to explain the seasonal and yearly variations in the photosynthetic capacity of both types of needles as a basis for studying stomatal O_3 absorption in adult larch trees, and to compare Gs between heterophyllous needles of seedlings and adult trees.

2. Materials and Methods

2.1.Study site

The study was conducted in a plantation of Japanese larch (*Larix kaempferi* (Lamb.) Carrière; here after larch) plantation beginning in 1953 at Tomakomai National Forest, northern Japan (42° 40′N, 141°36′E, 200-300 m a.s.l.) from 2001 to 2003. The soil material comprises immature volcanic ash (=Vitric Andosols) with shallow soil depth (about 20cm), due to the eruption of Mt. Tarumae, a volcano, in 1739. The mean annual precipitation is about 1250 mm, and the mean monthly air temperature ranges from -3.2 °C to 19.1 °C. The snow-free period is from late March to early December and snow is rarely deeper than 50cm. The soil usually freezes from January to mid-April. Further details erlated to flux monitoring are given by Hirata et al. [29]. This plantation was destroyed in September 2004 by typhoon No. 18.

2.2.Plant materials

A 20 m tower was built to reach up to the canopy of larches with heterophyllous shoots, i.e. short- and long-shoots. [15,30], separated needles of long-shoots were found to grow directly on current-year branches; these needles are generally thick and have a large connecting interface between leaves and branches. Short-shoots, in contrast, grow on older branches with bundles of needle leaves, and these needles generally have a thin leaf blade and small connecting interface [30,31]**.** The average height of the larch plantation was 12 m. We selected short- and long-shoot needles from the upper canopy of three individual trees for the monthly measurements.

2.3.Air temperature, soil moisture monitoring

Temperatures at the study site were monitored by auto-logged thermo-monitors (HMP45D, VAISALA, Helsinki, Finland) at a height of 8m above ground. The soil moisture was measured by a TDR sensor (CS615, Cambell Scientific Inc, Utah, USA) at depth 0.2 m. Data were recorded twice per hour. (Asia Flux web: http://asiaflux.net/index.php?page_id=113)

2.4.Leaf photosynthesis and leaf nitrogen measurement

Seasonal changes in the net photosynthetic rate (P_{sat}=net photosynthetic rate at light saturation and ambient CO_2) of short- and long-shoot needles were measured by a portable gas analyzer (LCA4, ADC Bioscientific Ltd, Haddeson, U.K.) equipped with a 2050-HB illumination system (Walz, Effeltrich, Germany) suitable for determination of P_{sat}, and a LI-6400 (Li-Cor, Lincoln, NE, USA) for determining the photosynthetic capacity at light and CO_2 saturation (P_{max}). Measurements of P_{sat} were made under steady state conditions at a leaf temperature of 24-28^{o}C and a near ambient CO_2 concentration of about 360 ppm (accurate between 2001 and 2003) and a PPF (Photosynthetic Photon Flux) of about 2000 μmol m^{-2} s^{-1} as determined previously [19]. After determining P_{sat}, we determined P_{max} by increasing the CO_2 concentration to 1800ppm. During the measurement of P_{max}, the stomatal conductance remained above 0.05 mol $m^{-2}s^{-1}$.

Leaf area was measured using a LI-3000 leaf area system (Li-Cor, Lincoln, NE, USA); the result was then used to calculate the photosynthetic rate per unit area. Following thismeasurement, the needle dry mass was measured after drying at 65^{o}C at least 48 hours, and the LMA (leaf mass per area) was calculated. Leaf nitrogen (N) content was determined with a N-C analyzer (NC-900, Shimadzu, Kyoto, Japan). The N content was calibrated and checked against a standard (acetanilide: N=10.36%, C=71.09%; Wako, Osaka, Japan).

2.5.Analysis of A-Ci curves

A-Ci curves can be used to estimate V_{cmax} (maximum Rubisco activity) and J_{max} (maximum electron transport rates) via the model [32–35] as follows:

$$P_n = V_{c\,max}\frac{C_i-\Gamma^*}{C_i+K_c(1+\frac{O}{K_o})}-R_d, \quad \text{if Wc <Wj} \qquad (1)$$

$$P_n = J\frac{C_i-\Gamma^*}{4(C_i+2\Gamma^*)}-R_d, \quad \text{if } W_c>W_j \qquad (2),$$

in equation (2) at light saturation, J is equal to J_{max} [36], and therefore,

$$P_n = J_{\max} \frac{C_i - \Gamma^*}{4(C_i + 2\Gamma^*)} - R_d, \tag{3}$$

(3),

Here, P_n is the net photosynthetic rate, V_{cmax} is the maximum rate of calboxylation allowed by Rubisco, J is the potential electron transport rate, Ci is the intercellular concentration of CO_2 (μmol mol^{-1}) and O signifies that of O_2, K_c is the Michaelis-Menten constant for carboxylation and K_o that for oxygenation, Γ^* is the CO_2 compensation point (μmol m^{-2} s^{-1}), R_d denotes day respiration (μmol m^{-2}s^{-1}), W_c is Rubisco-limited photosynthetic rate (potential rate limited by the activity of Rubisco and the concentration of CO_2 and O_2), W_j denote RUBP limited photosynthetic rates (the rate of RUBP carboxylation in photosynthesis is either equal to the potential rate allowed by the concentration of RUBP), and Γ^*=3.69 (Kpa), Kc = 40.4 (Kpa), Ko = 24.8 (Kpa) [37].

2.6.Calculation of nitrogen remobilization during autumn senescence

Several studies have examined N remobilization from old leaves [25,38,39]. We express the N remobilization rate (NRMR) as

$$NRMR(\%) = 100\left(\frac{N_{LSP} - N_{died}}{N_{LSP}}\right) \tag{4}$$

(4),

where N_{LSP} is the mean value of N (g m^{-2}) during the LSP (Leaf Stable Period; [40]), and N_{died} is the N content of leaves (g m^{-2}) collected in late October the needle color was pale green to yellow), just before leaf shedding [25].

2.7.Statistical analysis

All of the statistical tests were carried out with using the R language [41]. To compare the P_{sat}-N linear relation, the V_{cmax}-N linear relation and J_{max}-N linear relation, we used analysis of covariance (ANCOVA). To compare the P_{sat} for long and short shoots, we use t-tests. To compare the seasonal and yearly fluctuations in photosynthesis and leaf characteristics, and to compare the difference in photosynthetic capacity and the nitrogen remobilization rate between short- and long-shoots, we used component analysis of variance (ANOVA).

3. Results

3.1.Seasonal changes of air temperature and soil moisture

The peak air temperature was observed during July and August through the 3 years. Soil moisture data did not reveal any obvious dry season or moist season. Over the 3 years, the air temperature raised from -10 °C to 21°C. The soil moisture in the vegetation season raised from 22% to 38% (v/v), and it was as low as 10% in the winter (Figure 1). The air temperature in July and August 2001 was much lower than in 2002 or 2003, and the soil moisture in 2003 was much lower than in 2002.

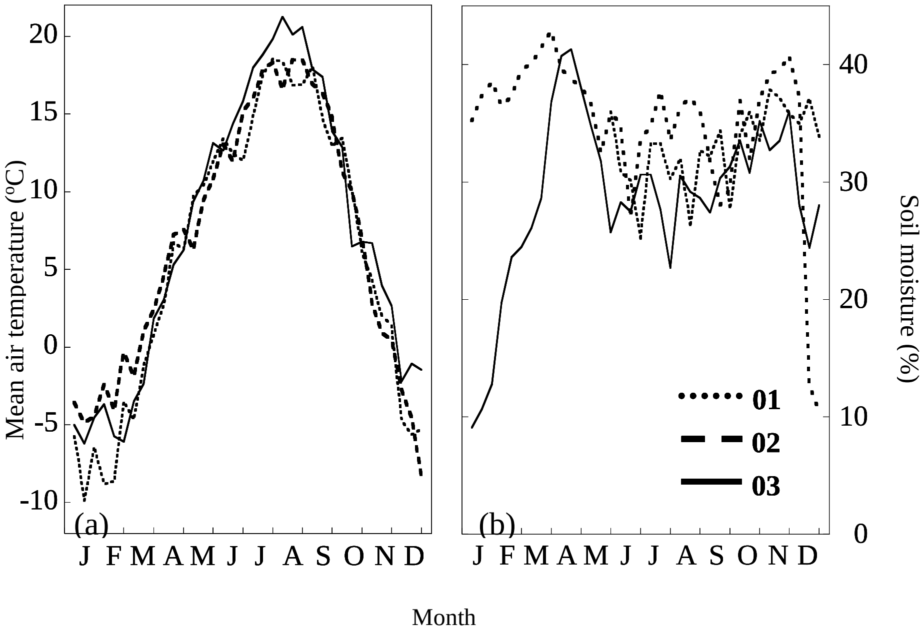

Figure 1. Seasonal and annual changes in mean daily air temperature (a) and soil moisture (b)

3.2. Seasonal and annual changes in P_{sat}

Short-shoot needles flushed in mid-May and, about 20 days later, long-shoot needles began to develop. In short-shoot needles, P_{sat} increased gradually from mid May to August, then decreased toward late October; values ranged from about 2.0 to 8.0 μmol $m^{-2}s^{-1}$. Long-shoot needles displayed a similar tendency, although P_{sat} increased from June to August and then decreased toward late October (Figure 2). Seasonal changes in P_{sat} were similar in 2001 and 2002. There is clear variation of P_{sat} between the 3rd years; P_{sat} of short-shoot needles was generally higher than for long-shoot ones in 2001 and 2002, but the reverse was true in 2003. Moreover, the difference between long- and short-shoot needles was significantly greater in the early growing season than in the late growing season (Figure 2). In mid summer, P_{sat} was 30 % higher in 2003 than in 2001 for short-shoot needles, and 40 % higher for long-shoot needles (Figure 2). However, the difference in P_{sat} between short- and long-shoot needles was not significant ($p > 0.05$).

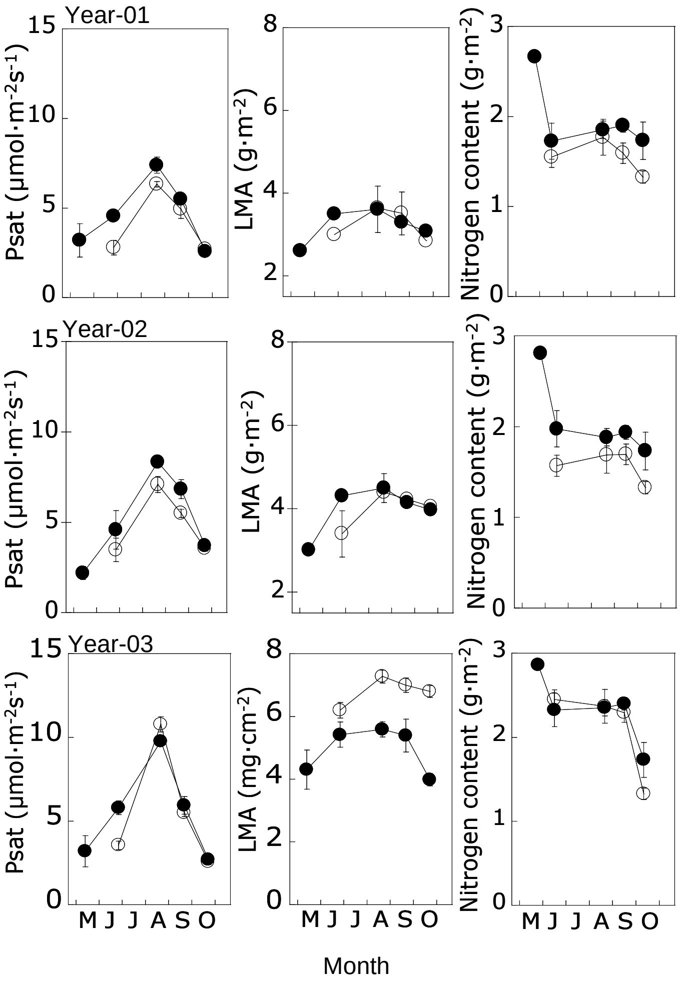

Figure 2. Seasonal and annual changes in P_{sat}, LMA and nitrogen content from year-01 to -03. Closed circles are short-shoots, and open ones are long-shoots.

3.3.Photosynthesis-nitrogen relation

Photosynthetic nitrogen use efficiency (PNUE) is expressed via the gradient of the linear part of the P_{sat}-N relation. A steeper slope means a more efficient PNUE. For both short- and long- shoot needles, closely linear relations between needle N and P_{sat} were observed, although, the difference between their gradients was significant ($p < 0.05$). The gradient was steeper for short- shoot needles (gradient of the regression line = 11.1) than for long-shoot needles (5.6) (Figure3 a). The value of P_{max} for short- and long-shoot needles was equally strongly correlated with their needle N concentration (that =9.5) (Figure 3 b).

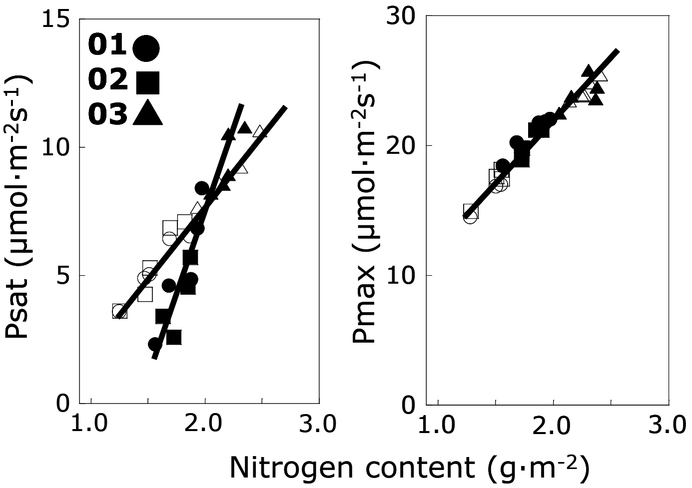

Figure 3. P_{sat}-nitrogen relation (a) and P_{max}-nitrogen relation (b). Closed symbols are short- shoots and open symbols are long-shoots. Data is from after leaf maturation till leaf senescence (June to October for short-shoots, July to October for long -shoots). Best-fitting equations: (a), P_{sat}=-3.90+5.62N, r^2=097, $p<0.001$ (long-shoots); P_{sat}=-15.1+11.1N, r^2=091, $p<0.001$ (short-shoots); (b), P_{max}=2.22+9.47N, r^2=0.90, $p<0.001$ (short- and long-shoots)

3.4.Variation of V_{cmax}, J_{max} and needle N

No significant difference was found for V_{cmax} in both short- and long-shoot needles in 2001 and 2002 ($p > 0.05$, Figure 4). However, V_{cmax} for short-shoot needles in 2003 was significantly higher than in the other two years ($p < 0.01$). In long-shoot needles, V_{cmax} tended to be higher in 2003 in 2001 and 2002 ($p < 0.13$).

For J_{max}, there were no significant differences between short- and long-shoot needles in 2001 and 2002. As with V_{cmax} however, J_{max} for short-shoot needles in 2003 was significantly lower than for long-shoot needles. In long-shoot needles, J_{max} was significantly higher in 2001 or 2002 ($p < 0.01$).

There was no marked difference, between short-shoot and long shoot needles (Figure 5 a). A significant difference was found between short-shoot and long-shoot needles in the J_{max}-N relation: as needle N increases, J_{max} increased more slowly for short shoot than for long-shoot needles (Figure 5 b).

3.5.Nitrogen remobilization rate (MRMR)

Large yearly variations in NRMR were observed for both short-shoot and long-shoot needles (Figure 6). For short-shoot needles the minimum NRMR was 17%, observed in 2002, and the maximum was 25% in 2003. For long-shoot needles, the minimum NRMR was 26% and the maximum was 43% (Figure 6). In each year, long-shoot needles were able to remobilize more nitrogen during leaf senescence than short-shoot needles ($p<0.01$) (Figure 6). On average, long-shoot remobilized 33% before needle shedding whereas short-shoots remobilize only 20%.

4. Discussion

4.1.Differences in photosynthetic characteristics between short- and long-shoot needles

Ozone (O_3) sensitivity of larch seedlings is strongly related to the nitrogen condition of the soil (e.g. [21,24]) as well as the stomatal conductance (Gs) [6]. O_3 sensitivity appears to increase with the foliar N content in many plant species, since both P_{sat} and Gs increase (e. g. [4,6]).

Based on the AOT40 and biomass relation in larch seedlings, O_3 sensitivity decreased with greater N loading [42]. Here, we compared P_{sat} and Gs in short- and long-shoot needles of adult trees and seedlings [20], in Figure 7. Values of P_{sat} and Gs for short-shoot needles in seedlings were lower

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than those in mature trees during August to September. For long-shoot needles (present study) during late summer, no difference in Gs was found between seedlings and mature trees.

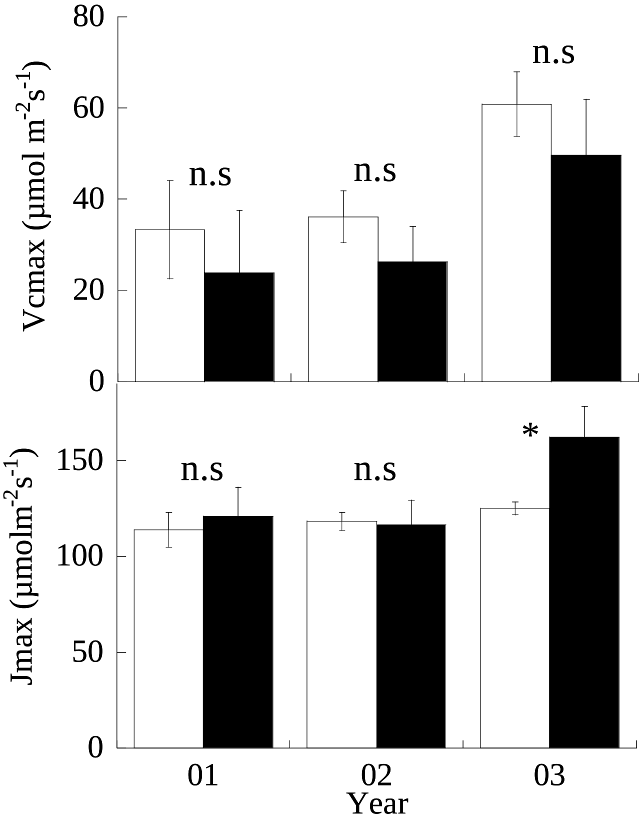

Figure 4. Maximum rates of RuBP carboxylation (V_{cmax}) and the maximum rate of electron transport driving RuBP regeneration (J_{max}) of both short- and long-shoot needles in August of the three years. Open: short-shoot needles, Closed: long-shoot needles. * indicates statistical difference between long and short-shoot needles.

Upon comparing P_{sat} and Gs in the same season, a positive relationship between Gs and P_{sat} was found in early and late summer except long-shoot of seedlings. This relation is well known in many plants (e.g. [26]). In long-shoots of seedlings, P_{sat} was highest at lower Gs which usually corresponds to dry conditions but nitrogen-rich leaves were formed(e.g. [25]).

A positive correlation between leaf nitrogen and photosynthesis has been observed in many species [43], although with large variations [44], and even seasonal variations within a single species [35]. Weak linear relations have also been observed in some canopy species [45]. This disparity may occur because photosynthetic capacity rises linearly with increasing nitrogen content (per unit leaf surface) until other factors cause saturation [46].

Although accurate linear relations between P_{sat} and N were found in short-shoots and long-shoots. Here was a significant difference between the two shoot types. This difference became negligible when P_{max} took the place of P_{sat}, suggested that CO_2 diffusion is a reason for this disparity. It is still remains to determine which photosynthetic processes may mostly affect these CO_2 diffusion differences, such as mesophyll resistance or cell-wall resistance (e.g. [27,47]).

The value of V_{cmax} (the maximum rate of carboxylation) increased linearly with leaf nitrogen, and no differences were found between short-shoots and long-shoots. On the other hand, J_{max} (the maximum electron transport rates) of short-shoot, increased more steeply with increasing leaf nitrogen than in long-shoots (Figure 5b). Thus, although the efficiency of leaf nitrogen in carboxylation enzymes was finally similar in both short- and long-shoots the lower efficiency in RuBP regeneration in long-shoots reduced the electron transportation rate, giving rise to the differences observed in the P_{sat}-N relation (Figure 3).

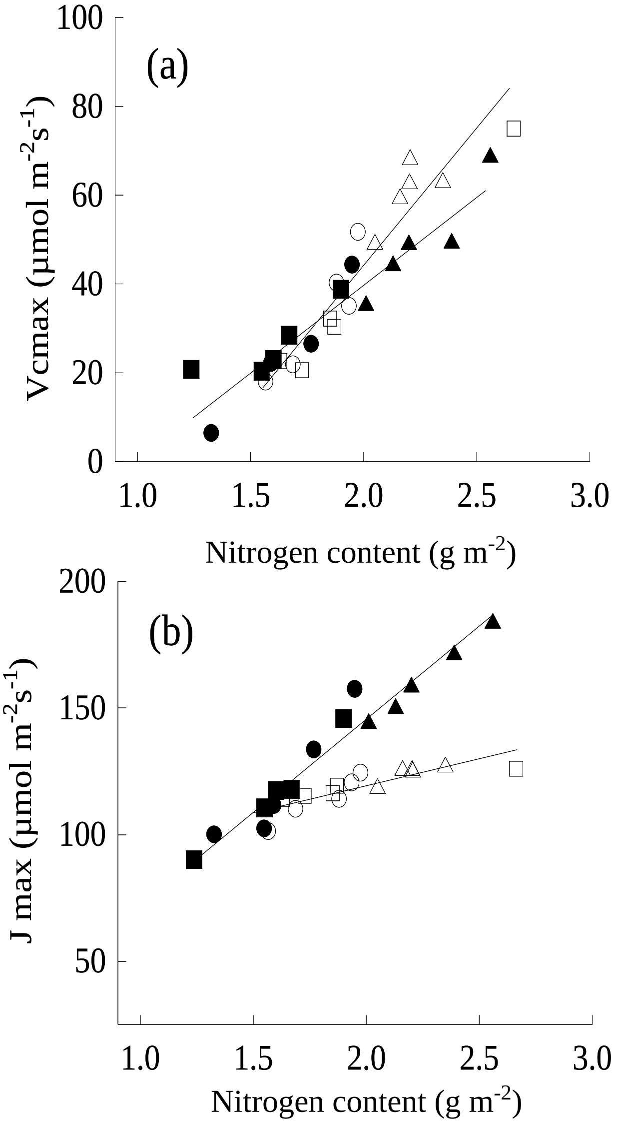

Figure 5. (a) Relationship between the maximum rates of RuBP carboxylation (V_{cmax}) and leaf nitrogen content (b) the maximum rate of electron transport driving RuBP regeneration (J_{max}) and leaf nitrogen content of both short and long shoot needles. ○ : 2001, △: 2002,□ :2003. Open: short-shoot needles, Closed: long-shoot needles. Short-shoot needles, V_{cmax} = -79.557 + 61.998 N, J_{max} = 77.161 + 21.119 N; Long-shoot needles, V_{cmax} = -40.071 + 40.113 N, J_{max} = -0.899 + 73.302 N. Short shoot needles: r^2 = 0.89 in V_{cmax}, 0,700 in $J_{max,}$ Long shoot needles: r^2 = 0.90 in V_{cmax}, 0.95 in J_{max}

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The differences in CO_2 diffusion between short- and long-shoots strongly affects the use of N in RuBP regeneration, but not the carboxylation processes. A wide ranges of species (107 species) have been found to cooperate with carboxylation and electron transportation through equal N allocation to both processes [33]. Since N may be equally portioned between carboxylation and RuBP regeneration, one possible reason for the lower nitrogen efficiency in RuBP regeneration is that nitrogen cannot be used efficiently where these is a shortage of other resources, such as CO_2. This is verified by the unchanging linear relation between P_{max} and N when both CO_2 and light are saturated (Figure 3b).

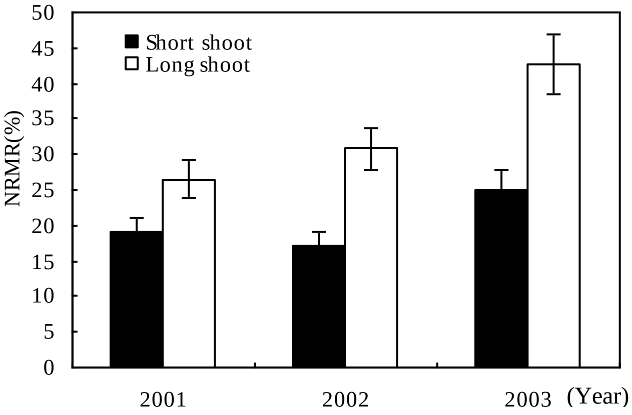

Figure 6. Nitrogen remobilization rates (NRMR) of short- and long-shoots from year-2001 to -03. The vertical bars show a 10% error.

We found that nitrogen was remobilized at different rates in short- and long-shoots; short-shoots tended to remobilize less N during leaf senescence than long-shoots. Moreover, needles of long shoots are positioned at the shoot top, where new shoot elongation will take place in the next year. This process will ultimately recycle more nutrients for next year's growth at the shoot top.

An increase in LMA should lead to an increase in photosynthetic organ per unit area, and it may also change the nitrogen allocation to photosynthetic protein [48,49]. Yearly variation in LMA would therefore, affect the yearly variation of PNUE and P_{sat}. Yearly variation of PNUE and P_{sat} may be attributed to LMA, influenced in turn by year-to-year variations in temperature and humidity as observed in deciduous broadleaved trees [25].

The photosynthesis-N relationship of larch needles with different shoot types may be affected by many factors: CO_2 diffusion, leaf nitrogen content, stomatal conductance and the kinetics of Rubisco (Figure 3), as found in many plant species[50]. Their conclusion agrees with our findings, i.e. yearly variation of P_{sat} and the P_{sat}-N relationship is due to a combination of changes in LMA, leaf nitrogen and photosynthetic functions in the needles.

4.2.Seasonal and yearly variation in photosynthetic rate: climatic limitation

In this study, there were large seasonal and yearly variations in the photosynthetic capacity of the differing shoot types. The difference is not intrinsic; for example, short-shoot needles had a lower photosynthetic rate (P_{sat}) in 2001 and 2002, but a higher rate in 2003 (Figure 2). Photosynthesis in needles is determined by the climatic conditions surrounding leaves and also by leaf anatomical structure; i.e. by CO_2 diffusion, N allocation in the leaf, and the kinetics of Rubisco [25,51,52].

In a *Pinus taeda* canopy, year-to-year variation in the microenvironment led to large variations in the net photosynthetic rate at light saturation and ambient CO_2 (P_{sat}) [53]; this shows the results of stomatal limitation. In a mixed deciduous forest, P_{sat} differs significantly between species, and also among individuals of a given species [54]. Year-to-year variation, as well as differences between species, were found in the P_{sat}-N relation, and were attributed to variations in climate and species-specific features in 3 species of deciduous broadleaved tree seedlings [25].

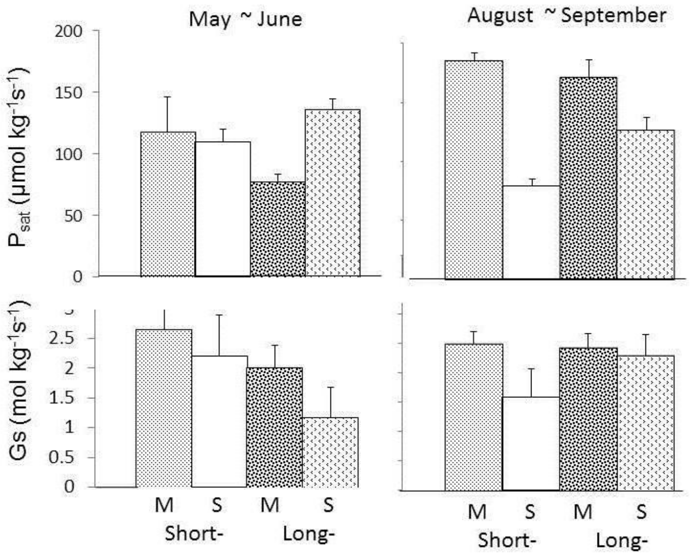

Figure 7. Comparison of P_{sat} and Gs between heterophyllous shoots of mature trees (M: present study) and seedlings (S: after Watanabe et al. [42]). Short = short shoot needles, Long= long shoot needles.

Temperature significantly influences the yearly value of P_{sat} through the vapor pressure deficit [25,46,53]. A recent simulation study also suggests significant impacts of VPD on canopy photosynthesis in *Larix olgensis* Henry [3]. In 2001, the air temperature in July and August was 3 °C lower than in 2002 and 2003, and in 2002 it was about 1°C lower than in 2003 (Figure 1). This correlates with the changes in P_{sat} at mid-summer (Figure 2), and with nutrient absorption and remobilization (Figure 2 and 6). With increasing temperature, the needles of larch trees developed adequately, with higher photosynthetic capacity. More energy from the photosynthates could consequently be used in absorbing nitrogen from soil; more nitrogen was detected in needles (Figure 2). This in turn enhanced the photosynthetic rate (e.g.[35,51]).

There was no significant difference in LMA between short- and long-shoots during the growing season [40], although short-shoots had much higher LMA values in June, due to the needle development process. High LMA values were observed in 2003 when the soil was dryer than in 2001 and 2002, however (Figure 1). Species inhabiting dry regions often have leathery foliar organs with high-LMA value [51,55–57], indicating greater investment in leaf construction to endure desiccation in a water-limited environment. This applies to our finding of higher LMA in the dry year, in which needle structure was changed by the dry conditions. In dry years, larch requires more carbon investment in needle formation, which is rewarded by higher photosynthetic rates per unit leaf area in both short- and long-shoots (Figure 2), and also higher NRMR for storage for the next year's growth (Figure 6), consistent with the process of leaf economy (e.g. [58]).

In conclusion, larch trees, being typical heterophyllous conifers, do not exhibit any permanent difference in photosynthetic capacity (P_{sat}) between short- and long-shoots, indicating that climatic factors rather than shoot types affect P_{sat}. There is a clear age-related functional decline of short-shoot needles in relation to absorption of O_3 though stomata. Basic photosynthetic capacity in short- and long-shoot needles was not found at saturated CO_2 and light (Fig 3b) in mature trees, except in the N and J_{max} relations. From the N- J_{max} relation, J_{max} of long-shoots increased with N much more steeply than for short-shoots, which may suggests that stomatal regulation differences significantly affect efficiency of the electron transportation only via RuBP regeneration. This should be taken into account when we scale up the results of leaf gas exchange traits, including stomatal O_3 absorption capacity of seedlings to mature canopy larch specimens. Much higher N remobilization rates were found in long-shoots, furthermore.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, S.K., M.W. and T.K.; planning of this study, S.K. and L.Q.; field researches, S.K., Q.M., Y.W. and M.W.; original draft preparation, S.K. ; writing—review and editing, S.K., M.W. and T.K.; supervision, T.K.; funding, L.Q. and T.K.

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