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Title

Effect of nitrogen load on growth and photosynthesis of seedlings of the hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) grown on serpentine soil

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Abstract

We studied the growth and photosynthesis of the hybrid larch F_1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) grown on serpentine soil, and the effects of soil N load, to determine the performance of this species as reforestation material in serpentine regions. We prepared 16 experimental plots (2 m × 4 m in each), eight on serpentine and eight on brown forest soil, and planted one-year-old cutting seedlings of the hybrid larch F_1 in each plot, in May 2007. Ammonium sulfate was supplied to half of the plots of each soil type in 2008 and 2009, at a load of 47 kg N ha^{-1} $year^{-1}$. Although the growth and photosynthetic capacity of hybrid larch F_1 seedlings in the serpentine soil were limited, the rate of growth in serpentine soil was greater than that of Sakhalin spruce (*Picea glehnii*) that is dominant species in serpentine regions. There was significant interaction between soil type and N load for the growth and photosynthetic parameters. The N load adversely affected growth and photosynthetic parameters in the serpentine soil, while improved them in brown forest soil. Although the growth rate of hybrid larch F_1 without N loading showed high potential as an afforestation species in serpentine region, increasing deposition of N might be a threat to the growth and photosynthesis of the hybrid larch F_1 in serpentine soil.

Key words

gas exchange; infertile soil; nitrogen deposition; nutrient; reforestation material

1. Introduction

Larix species are distributed broadly in cool temperate regions of the northern hemisphere regions (Gower and Richards, 1990). The Japanese larch (*Larix kaempferi*) was introduced from the central subalpine region of Japan to northern Japan as a trial

plantation species in the 1870s, because it grows faster and has better tolerance to cold than other traditional silvicultural species. Unfortunately it has serious disadvantages as a plantation species there, including susceptibility to shoot blight diseases and grazing by voles (Ito, 1963; Koike et al., 2000, 2004). To overcome these problems, a hybrid larch was developed by crossing *Larix gmelinii* var. *japonica* and *L. kaempferi*. The resulting hybrid larch F_1 grew faster than the Japanese larch and had a better capacity for carbon accumulation (Miyaki, 1990; Kita et al., 2009).

Serpentine soils are characterized by an excess of elements such as nickel (Ni), chrome (Cr) and magnesium (Mg), and a low ratio of Calcium (Ca) to Mg; these soils are found worldwide (Proctor, 1971; Brooks, 1987; Roberts and Proctor, 1992; Kayama et al., 2009). Many plant species that are intolerant to serpentine soil suffer from toxicity in such regions. Also, low availability of several essential plant nutrients such as phosphorus (P) is a typical trait of serpentine soil (e.g. Kayama et al. 2009). Accordingly, specialized flora grows there, with endemic species distribution (Brooks, 1987; Roberts and Proctor, 1992). If the vegetation of a serpentine region is disturbed, its recovery is very slow (Curtis and Claassen, 2005). Moreover, serpentine regions are often disturbed by erosion and landslides (Lee et al., 2004; O'Dell and Claassen, 2006; Kayama et al., 2007). It is therefore important to identify tree species suited to reforestation of such regions, to prevent disasters and conserve the environment. As larch species can survive in infertile soil, they have been recognized as useful for timber production and absorption of atmospheric CO_2 as a carbon sink, and also afforestation in infertile soil (Zhang et al., 2000; Ryu el al., 2009; Ryu et al., 2010; Watanabe et al., 2011). Kayama et al. (2009) reported that Japanese larch seedlings grew better on serpentine soil than the Sakhalin spruce (*Picea glehnii*), which is a dominant species in serpentine regions of northern Japan.

The atmospheric deposition of nitrogen (N) into terrestrial ecosystems has recently been increasing due to increased anthropogenic emission of N (Galloway et al., 2008; Ohara et al., 2007). Several researchers have reported high N deposition, of 10 to 20 kg N ha^{-1} $year^{-1}$ by wet N deposition (bulk precipitation) and 10 to 50 kg N ha^{-1} $year^{-1}$ by throughfall and stemflow, in forested areas of Japan (Baba and Okazaki, 1998; Baba et al., 2001; Okochi and Igawa, 2001; Sase et al., 2008; Kimura et al., 2009). In general, N is a limiting resource for tree growth in forest ecosystems, and atmospheric N deposition acts as a fertilizer (e.g. Bobbink et al., 2003; Magnani et al., 2007). However, there are several reports that experimental N supply to soil did not enhance tree growth (Nakaji et al., 2001; Watanabe et al., 2006). The main factor limiting plant growth in serpentine region may be excess Ni and Mg rather than N deficiency, and/or low availability of other nutrients. On this basis, we suggest that an increase in N deposition would not enhance the growth of hybrid larch F_1 in serpentine soil. We tested this hypothesis by studying the effects of N load on the growth and photosynthesis of hybrid larch F_1 seedlings grown on serpentine soil.

2. Materials and methods

2.1. Study site

This study was conducted in the Teshio Experimental Forest of Hokkaido University (45˚06’N, 142˚12’E, 110 m a.s.l.). Serpentine soils are distributed in the eastern part of the Teshio Experimental Forest, and Sakhalin spruce is dominant in this region (Tatewaki and Igarashi, 1971, Nakata and Kojima, 1987). The mean annual precipitation was approximately 1200 mm. The annual mean, maximum and minimum temperatures were 5.2, 32.0 and –33.5˚C, respectively, for the recent 30 years as measured by a thermo-recorder at the Kami-toikan Meteorological Station, about 7 km far from study

site.

We prepared eight experimental plots (each 2 m × 4 m) in each serpentine soil and brown forest soil as controls. According to the FAO-UNESCO systems, serpentine soil and brown forest soil were classified as Podzol and Cambisol, respectively (Nakata and Kojima, 1987). There was a fault between the two soil sites. Climates of the plots were considered to be almost identical because the distances between two soil sites were within 100 m (Kayama et al. 2005). The experimental plots within each soil type were adjoined.

2.2. Plant materials and nitrogen load

In May 2007, cutting seedlings of one-year-old hybrid larch F_1 were planted in experimental plot, four seedlings to each plot and thereby 64 seedlings in total. Each plot was weeded periodically thereafter. After planting, the seedlings were grown for one year without any treatment, to acclimatise to the conditions at the study site.

In May 2008, pellets of ammonium sulfate (commercial fertilizer, Akagi horticulture Co., Ltd., Gunma, Japan) were homogeneously supplied to the soil of four plots in each soil type by handwork. The pellet is usually dissolved with one event of precipitation because of their high solubility. The rate of N load was 47 kg N ha^{-1} $year^{-1}$ and this amount is matched with the high N deposition in central Japan (Kimura et al., 2009). We supplied half amount of N (23.5 kg N ha^{-1}) in May 2008 and 2009 for stimulating a seasonal variation of N deposition. Because the Teshio Experimental forest has relatively high amount of snow, which depth in mid winter reaches 100-150 cm, large portion of N is supplied into soil with snow melting in the spring (Nomura et al., 1999; Satoh et al. 1999). The rest N (23.5 kg N ha^{-1}) was supplied in August and November in 2008 and August in 2009

2.3. Soil properties

In July 2009 we collected a soil core (100 ml) from each plot (four cores per treatment), to evaluate the physical properties of the soil: the dry bulk density, soil water content and maximum water holding capacity. After collection we measured the weight of the soil core (M_1). It was then placed on a tray holding shallow water for 24 hours at room temperature. The resulting saturated soil core was weighed (M_2). It was then dried in an oven for seven days at 105˚C, and weighed (M_3). The dry bulk density was calculated as M_3 divided by 100 ml volume of soil cores (g cm^{-3}). The soil water content and maximum water holding capacity were calculated as follows:

Soil water content (g g^{-1}) = $(M_1 - M_3) / M_3$

Maximum water holding capacity (g g^{-1}) = $(M_2 - M_3) / M_3$

To determine the chemical properties of the soil, including pH, total carbon (C) and N concentrations, inorganic N (i.e. NO_3^- and NH_4^+), available P, exchangeable cation, and acid soluble metals, we collected soil samples at the end of the experiment in September 2009. Soil samples were collected from each plot (four samples per treatment), and were air-dried for one month. The dried soil samples were crushed with a mortar and passed through a sieve of mesh 2 × 2 mm. This soil sample was used for the following measurements.

The soil pH was measured with a pH meter (HM-30V, DKK-TOA Co. Ltd., Tokyo, Japan) after 10 g soil sample had been shaken with 50 ml of ion-exchanged water for 1 h (van Reeuwijk, 1993). The total C and N concentrations in the soil was measured

by gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) after combustion with circulating O_2 by an NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Osaka, Japan). Available P was extracted by the Bray-2 method (Bray and Kurtz, 1945), as follows: 1 g soil sample was shaken with extracting solution containing 0.03 M ammonium fluoride and 0.1 M hydrochloric acid. The P extracted was determined by the molybdenum blue method with ascorbic acid, using a spectrophotometer (Gene spec III, Hitachi, Tokyo, Japan) (Murphy and Riley, 1962). To extract exchangeable cations from the soil, 5 g soil sample was mixed with 50 ml of 1 M ammonium acetate solution and was shaken for 1 h (Thomas, 1982). Solutions for analyzing acid soluble metals were obtained by adding 50 ml 0.1 M hydrochloric acid to 5 g soil sample with shaking for 1 h (Baker and Amacher, 1982). The concentrations of elements in the sample solutions were determined using a inductively coupled plasma optical emission spectrometer (ICP-OES, IRIS, Jarrel Ash, Franklin, MA, USA). We determined the concentrations of Ca, Mg, potassium (K), Cr and Ni as exchangeable cations, and Cr and Ni as an acid-soluble metal.

2.4. Growth measurement

In May and October 2008 and in May, July and September 2009, we measured the height and stem diameter of all seedlings. In September 2009, all seedlings were harvested in order to determine the dry mass of the plant organs. The seedlings were separated into needles, stems and roots. The plant organs were dried at 70˚C for 1 week and weighed.

2.5. Measurement of gas exchange rates in needles

The gas exchange rates of fully expanded young needles were measured in July 2009 using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Two

seedlings per treatment-plot combination were selected randomly for measurement, to a total of eight seedlings per treatment. The gas exchange rates were measured between 0800 and 1500 hours. The needle temperature during the measurement was maintained at 25 ± 0.5˚C and the photosynthetic photon flux density at 1500 μmol m^{-2} s^{-1}. The vapor pressure deficit was 1.2 ± 0.2 kPa. To obtain the intercellular CO_2 concentration (C_i)-response curve of the net photosynthetic rate (A), i.e., the A/C_i curve, A was determined at ten CO_2 concentration levels in the chamber (C_a, 60-1700 μmol mol^{-1}). We determined A, the transpiration rate and stomatal conductance at 380 μmol mol^{-1} CO_2 (A_{380}, E and G_s, respectively), and A at 1700 μmol mol^{-1} CO_2 (A_{max}). The stomatal limitation of photosynthesis, maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were calculated from the A/C_i curve (Farquhar et al., 1980; Long and Bernacchi, 2003). Values of the Rubisco Michaelis constants for CO_2 (K_c) and O_2 (K_o) and CO_2 compensation point in the absence of dark respiration (Γ*), for analysis of the A/C_i curve, were as according to Bernacchi et al. (2001) All gas exchange parameters were expressed on the basis of the projected needle area, which was measured with an image scanner (CanoScan LiDE 600F, Cannon, Tokyo, Japan).

2.6. Measurement of needle traits

After the gas exchange rate had been measured, the needles were collected in order to determine the leaf mass per area (LMA) and the chlorophyll content and amount of nutrient elements. After the needle projected area was measured using an image scanner, the samples for chlorophyll quantification were frozen and stored in a freezer at -80°C. Chlorophyll in needles was extracted with dimethyl sulfoxide as according to Barnes et al. (1992) and Shinano et al. (1996), and was determined using a spectrophotometer (Gene spec III, Hitachi, Tokyo, Japan). The other samples, for determination of LMA and

nutrient elements, were dried in an oven for five days at 70˚C. The LMA was calculated as the ratio of dry mass to the area of the needles. The dried needles were ground to a fine powder with a sample mill. The N content of the needle sample was determined with a gas chromatography-NC analyzer system, as mentioned above. We calculated the ratio of A_{380} to the N content as photosynthetic N use efficiency (PNUE). To measure the concentrations of P, Ca, Mg, K, and Ni in the needles, the powdered sample was digested with nitric acid, hydrochloric acid, and hydrogen peroxide. The concentrations of elements in the sample solutions were determined using an ICP-OES (ICPS-7000, Shimadzu, Kyoto, Japan).

2.7. Estimation of nitrogen allocation to photosynthetic functions

The photosynthetic apparatus was divided into three parts: Rubisco, bioenergetics (electron carriers except for photosystems, coupling factor and Calvin cycle enzymes except Rubisco), and light-harvesting complex and photosystems; the fraction of needle N deployed in each function is denoted by N1, N2 and N3. We estimated N1 according to the following equation (Niinemets et al., 1999; Tissue and Lewis, 2010):

$$N1 = V_{cmax} / (6.25\, V_{cr}\, LMA\, N_M)$$

where V_{cr} is the specific activity of Rubisco (the maximum rate of RuBP carboxylation per unit Rubisco protein), N_M denotes N per unit leaf mass, and the factor of 6.25 (g Rubisco [g N in Rubisco]$^{-1}$) converts N content to protein content. Here, V_{cr} is equal to 20.5 μmol CO_2 (g Rubisco)$^{-1}$ s^{-1} at 25 °C for purified Rubisco enzyme from *Spinacia oleracea* (Jordan and Ogren, 1984). Because this method cannot determine the amount of inactivated Rubisco, the calculated value of N1 is an underestimate (Warren and Adams,

2004). The N2 was estimated from gas exchange characteristics according to the equation (Kitaoka and Koike, 2004; Takashima et al., 2004):

$$N2 = J_{max} / (156 \times 9.53\ LMA\ N_m)$$

We assume that N 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 and Tenhunen, 1997), and N in bioenergetics per unit cytochrome f is 9.53 mol $mmol^{-1}$ (Hikosaka and Terashima, 1995). N3 was estimated according to the following equation.

$$N3 = 37.1\ Chl / (LMA\ Nm)$$

where Chl is chlorophyll content (mol m^{-2}) and the N content per unit chlorophyll is 37.1 mol mol^{-1} (Evans and Seemann, 1989).

2.8. Statistical analysis

Statistical analyses were undertaken using R software, version 2.8.1 (R Development Core Team, 2009). Student's t-test was applied to the difference in physical properties between the two soil types. Two–way analysis of variance (ANOVA) was used to test the effects of soil type and N treatment. In the analysis, a plot was nested within each soil and N treatment and was added to the model as a random effect (Underwood, 1981). When a significant interaction between soil type and N treatment was detected, Fischer's least significant difference test was performed to identify significant differences between the four treatments.

3. Results

3.1. Soil properties

The soil water content and maximum water holding capacity of serpentine soil were significantly higher than values in brown forest soil, but there was no significant difference in dry bulk density between the soil types (Table 1).

The content of Ca, Mg, acid-extracted Cr and Ni (in both extractions) in serpentine soil was each significantly higher than in brown forest soil, whereas the P content was significantly lower in serpentine soil (Table 2). The N load caused a significant increase in the total N and NO_3^- content, and a fall in the K content. Significant interactions between soil type and N load were observed in the values of the pH, the NH_4^+ content and the ammonium acetate-extracted Ni content. The pH was higher in serpentine soil than in brown forest soil, and decreased with the N load. This tendency in pH did not change despite the significant interaction. An increase in the content of NH_4^+ and Ni induced by N loading was found in serpentine soil, although N loading did not affect the Ni content of brown forest soil.

3.2. Growth response

Figure 1 shows trends in the increments of height and stem diameter of the seedlings. For serpentine soil, all parameters were lower than those in brown forest soil. N loading tended to increase growth parameters in brown forest soil but reduce them in serpentine soil. The dry mass of all organs and the whole-plant dry mass of the seedlings in serpentine soil were significantly lower than those in brown forest soil (Table 3). There was significant interaction between soil type and N load for the dry mass of the stem and roots, and also the whole-plant dry mass. N loading did not significantly affect (rather decrease) the dry mass of seedlings grown on serpentine soil, whereas in brown forest

soil N it led to a significant increase in the root, and a marginally significant increase in stem and whole-plant dry mass. (The *P* values were 0.074 for root and 0.100 for whole-plant dry mass.)

3.3. Needle gas exchange parameters and traits

All parameters except for stomatal limitation and the LMA of the needles in serpentine soil were significantly lower than the corresponding values in brown forest soil (Table 4). Stomatal limitation and LMA responded oppositely from the other parameters to soil type. The N load significantly increased the value of N_{area}. For A_{max}, E, V_{cmax}, J_{max} and PNUE there was significant interaction between soil type and N loading. The N load significantly reduced the values of these parameters in serpentine soil, but no significant effect of N loading was found in brown forest soil. The interaction of soil type and N loading for A_{380} was marginally significant (P = 0.058) and showed a similar trend. N allocation to photosynthetic functions in needles is shown in Figure 2. N loading significantly decreases N allocation to photosynthetic apparatus in serpentine soil but not in brown forest soil. A similar trend in N allocation was observed in each component of photosynthetic function (i.e. N1, N2 and N3).

3.4. Concentrations of elements in needles

Mass-based N, P, K and Ni concentrations in needles of seedlings grown in serpentine soil were significantly lower than those in brown forest soil, but the Mg concentration was higher for serpentine soil (Fig. 3). N loading significantly increased the mass-based N concentration. There was no significant interaction between soil type and N loading for the concentration of any element in needles. The concentration of Cr in needles was quite low and we could not detect Cr in about half of the samples. Figure 4 shows the relations

between A_{380} and amounts of elements in needles. Significant positive correlations were observed between A_{380} and the N, P, K and Ni content in needles, but A_{380} was negatively correlated with Mg content in the needles.

4. Discussion

4.1. Growth and photosynthesis of hybrid larch F_1 seedlings grown in serpentine soil

In serpentine soil, the growth and photosynthetic capacity of hybrid larch F_1 seedlings were severely limited compared to those in brown forest soil (Fig. 1 and Table 3). High soil water content, high concentrations of Ni and Mg, and a low concentration of P was found for the serpentine soil in the present study site (Table 1, Table 2). Although the decrease in the T/R ratio is considered to be an accommodation to the low P availability (Lambers et al., 2008), the P concentration in needles on serpentine soil was lower than on brown forest soil (Table 3, Fig. 3). Needle N and K concentrations were also lower in serpentine soil, even there was no significant difference between the two soil types in the availability of those elements. Furthermore, although Ca availability was higher in serpentine soil, there was no difference in the concentration of Ca in needles between two soil types. Excess Mg availability in serpentine soil might inhibit the uptake of the other nutrient by root or transport from root to above ground as indicated by Kobayashi et al. (2005). Accommodation to serpentine soil is therefore insufficient to compensate for its infertile condition.

Photosynthetic activity is generally regulated by the foliar N content (Lambers et al., 2008). Lower N content was observed in the needles of seedlings grown on serpentine soil, and this is believed to be the factor responsible for decreasing the photosynthetic activity (Table 4, Fig. 4). The PNUE also decreased in serpentine soil. The decrease in the CO_2 uptake owing to stomatal closure led to the decrease in PNUE (Table

4). The content of other elements than N in needles may also affect PNUE (Watanabe et al., 2011). Pâques (1994) reports that the optimal concentrations of elements for growth in needles of the hybrid larch (*Larix* × *eurolepis* Henry) growth are 19.5-27.3 mg g^{-1} for N, 1.4-2.4 mg g^{-1} for P, 5.2-8.6 mg g^{-1} for K, 3.3-6.2 mg g^{-1} for Ca and 1.1-2.0 mg g^{-1} for Mg. We found that the concentrations of N, P and Ca in needles grown on serpentine soil were less than the optimum (Fig. 3). The value of A_{380} was significantly positively correlated with the N and P concentrations (Fig. 4). The concentration of P in needles therefore appears to be a further factor that decreases photosynthetic activity in serpentine soil, consistent with Kayama et al. (2009). This possibility can be verified by an experiment of P fertilization in the future. The concentration of Mg in needles was significantly negatively correlated with A_{380} (Fig. 4). However, the needle Mg concentrations on serpentine soil were not high enough to decrease photosynthetic activity and growth (Rao et al., 1987; Kobayashi et al., 2005).

Surprisingly, the Ni concentration in the needles of seedlings grown on serpentine soil was lower than that for brown forest soil (Fig. 3). Ectomycorrhizal symbiosis plays an important role in the exclusion of Ni (Hartley et al., 1997; Leyval et al., 1997; Kayama et al., 2005, 2006) and we observed the formation of ectomycorrhizal roots in all seedlings in the present study (data not shown). In general, infection level to ectomycorrhiza is higher in infertile soil than that in fertile soil (van der Heijden and Sanders, 2002). Although we did not quantitatively evaluate the infection level to ectomycorrhiza in root, the extent of the infection level in serpentine soil may be higher than that in brown forest soil. Actually, the relative C allocation to roots was estimated to be higher in serpentine soil, because of the lower T/R ratio (Table 3), and that to ectomycorrhiza may therefore be higher, giving rise to well-developed ectomycorrhiza which robustly exclude Ni in serpentine soil. On the other hand, the species composition

of ectomycorrhiza is quite dependent on the soil type (van der Heijden and Sanders, 2002; Qu et al., 2004). The difference in species composition of ectomycorrhiza between two soil types might also explain the lower absorption of Ni in serpentine soil as compared to that in brown forest soil. As another possibility, greater accumulation of Ni in the needles in brown forest soil may be responsible for the higher transpiration rate (Table 4).

According to Kayama et al. (2005; 2006; 2009), Ni concentration in the matured needle of the seedlings of conifer species (Sakhalin spruce, Yezo spruce (*Picea jezoensis* Carr.) and Norway spruce (*Picea abies* Karst) and Japanese larch) grown on serpentine soil were ranged from 15 to 70 μg g^{-1}. Kayama et al. (2009) demonstrated no decline of net photosynthetic rate in the range below 30 μg g^{-1} of Ni concentration in needles of Japanese larch seedlings grown in the same experimental forest with the present study. The Ni concentration in the needle of hybrid larch F_1 in the present study was rather low (15-25 μg g^{-1}, Fig. 3). Therefore, we consider the Ni concentaration in needles was within a range that does not exert a negative effect on photosynthesis, and the correlation between A_{380} and Ni concentration simply was a result with the other mechanism.

4.2. Effects of N load on the growth and photosynthesis of hybrid larch F_1 seedlings

The N loading increased the growth of the hybrid larch F_1 seedlings grown on brown forest soil, although the increse was only just statistically significant (Table 3). In serpentine soil, we found the opposite: N loading reduced growth. Similar impairment was observed in A_{380}, A_{max}, E, V_{cmax}, J_{max} and PNUE in the needles (Table 4). The different responses of growth in serpentine soil and brown forest soil to N loading are due to the differing responses in photosynthetic activity. The negative effects of N loading on photosynthetic activity in serpentine soil was mainly due to the reduction in PNUE of the

needles as a result of the decreased nitrogen allocation to photosynthetic apparatus (Table 4, Fig. 2). The concentrations of elements in needles were not the causal factor responsible (Fig. 3). Stomatal limitation may contribute to the response of PNUE to N loading in serpentine soil, although the interaction between soil type and N loading for PNUE was not significant because of the relatively large variations (Table 4). Further study is needed to clarify the effects of N deposition on photosynthesis of the hybrid larch F_1 grown on serpentine soil.

Soil N loading induced a significant increase in the available Ni concentration in soil (Table 2). Ni availability increases with decreasing soil pH (Mizuno, 1967; Seregin and Kozhevnikova, 2006). For N loading of soil we applied ammonium sulfate, so that some of the NH_4^+ should change to NO_3^- through nitrification processes and release H^+ to the soil (i.e. decrease the pH) (Fisher and Binkley, 2000). Although the available Ni concentration in serpentine soil was significantly increased by N loading, the concentration of Ni in needles was not increased (Table 2, Fig. 3). This might be because ectomycorrhizal fungi act to exclude Ni, as stated above. However, it is possible that the cost of C needed to maintain the ectomycorrhizal symbiosis that prevents seedlings from reflecting the higher Ni concentration is greater in the seedlings grown on serpentine soil with N loading, causing the reduction in growth.

Growth of the hybrid larch F_1 seedlings without N loading is higher than that of the Sakhalin spruce, native species of serpentine regions, and similar to that of seedlings of the Japanese larch (Kayama et al., 2006; 2009). We therefore assert that the hybrid larch F_1 is useful for reforestation in serpentine regions, as well as the Japanese larch. However, the N load-induced reduction in growth and photosynthetic activity on serpentine soil is of concern for reforestation with the hybrid larch F_1 given increasing N deposition.

5. Conclusion

The results of the present study clearly support our hypothesis. N loading adversely affected growth and gas exchange parameters of seedlings grown in serpentine soil, whereas the opposite trends were observed in brown forest soil. The growth rate of hybrid larch F_1 without N loading showed high potential as an afforestation species in serpentine region. However, increasing deposition of N might be a threat to the growth and photosynthesis of the hybrid larch F_1. The emission control for anthropogenic N compound especially in East Asia region is highly important for the afforestation with hybrid larch F_1 in serpentine regions of Japan. In the next step, we should develop more long-term study from seedlings to mature tree to confirm the ability of hybrid larch F_1 as afforestation species with special attention to the increase of N deposition.

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Reference

Baba, M., Okazaki, M., 1998. Acidification in nitrogen-saturated forested catchment. Soil Sci. Plant Nutr. 44, 513–525.

Baba, M., Suzuki, Y., Sasaki, H., Matano, K., Sugiura, T., Kobayashi., H., 2001. Nitrogen retention in Japanese cedar stands in northern Honshu, with high nitrogen deposition. Water Air Soil Pollut. 130, 1103–1108.

Baker, D.E., Amacher, M.C., 1982. Nickel, copper, zinc, and cadmium, in: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of soil analysis, Part 2. Chemical and microbiological properties, 2nd edn. Soil Science Society of America Inc., Madison, pp. 323–336.

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison., A.W., 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. Environ. Exp. Bot. 32, 85–100.

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis, A.R., Long., S.P., 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant. Cell Environ. 24, 253‑259.

Bobbink, R., Ashmore, M., Braun, S., Flückiger, W., Van den Wyngaert., I.J.J., 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update, in: Achermann, B., Bobbink, R. (Eds.), Empirical Critical Loads for Nitrogen, Environmental Documentation No. 164, Swiss Agency for the Environment, Forests and Landscape, Berne, Switzerland, pp. 43-170.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59, 39–45.

Brooks, R.R., 1987. Serpentine and its vegetation. Dioscorides Press, Portland

Curtis, M.J., Claassen, V.P., 2005. Compost incorporation increases plant available water in a drastically disturbed serpentine soil. Soil Sci. 170, 939–953.

Evans, J.R., Seemann, J.R., 1989. The allocation of protein nitrogen in the photosynthetic apparatus: cost, consequences and control. In: Brigs, W.R. (Ed.), Photosynthesis, Alan R. Liss, New York, USA, pp. 183–205.

Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149, 78–90.

Fisher, R.F., Binkley, D., 2000. Ecology and management of forest soil. Third edition. John Wiley & Sons, Inc., New York, USA.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320, 889–892.

Gower, S.T., Richards, J.H., 1990. Larches: deciduous conifers in an evergreen world. Bioscience 40, 818–826.

Hartley, J., Cairney, J.W.G., Meharg, A.A., 1997. Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment? Plant Soil 189, 303–319.

van der Heijden, M.G.A., Sanders, I.R., 2002. Mycorrhizal ecology. Springer-Verlag, Berlin-Heidelberg.

Hikosaka, K., Terashima, I., 1995. A model of the acclimation of photosynthesis in the leaves of C3 plants to sun and shade with respect to nitrogen use. Plant, Cell Environ. 18, 605–618.

Ito, K., 1963. Shoot blight dieses larches – A destructive diseases in larch plantations in Japan. Bull. Gov. For. Exp. St. 159, 89‑110.

Jordan, D.B. and Ogren, W.L., 1984. The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. Dependence on ribulosebisphosphate concentration, pH and temperature. Planta 161, 308–313.

Kayama, M., Quoreshi, A.M., Uemura, S., Koike, T., 2005. Differences in growth characteristics and dynamics of elements absorbed in seedlings of three spruce species raised on serpentine soil in northern Japan. Ann. Bot. 95, 661–672

Kayama, M., Choi, D.S., Tobita, H., Utsugi, H., Kitao, M., Maruyama, Y., Nomura, M., Koike, T., 2006. Comparison of growth characteristics and tolerance to serpentine soil of three ectomycorrhizal spruce seedlings in northern Japan. Trees 20, 430–440.

Kayama, M., Choi, D.S., Sasa, K., Satoh, F., Nomura, M., Koike, T., 2007. A trial for reforestation after forest fires with Sakhalin spruce in the northern most Japan. Eurasian J. For. Res. 10, 31–39.

Kayama, M., Makoto, K., Nomura, M., Satoh, F., Koike. T., 2009. Nutrient dynamics and carbon partitioning in larch seedlings (*Larix kaempferi*) regenerated on serpentine soil in northern Japan. Landscape Ecol. Eng. 5, 125–135.

Kimura, S.D., Saito, M., Hara, H., Xu, Y.H., Okazaki, M., 2009. Comparison of nitrogen dry deposition on cedar and oak leaves in the Tama Hills using foliar rinsing method. Water Air Soil Pollut. 202, 369–377.

Kita, K., Fujimoto, T., Uchiyama, K., Kuromaru, M., Akutsu., H., 2009. Estimated amount of carbon accumulation of hybrid larch in three 31-year-old progeny test plantations. J. Wood Sci. 55, 425–434.

Kitaoka, S., Koike, T., 2004. Invasion of broad-leaf tree species into a larch plantation: seasonal light environment, photosynthesis and nitrogen allocation. Physiol. Plant. 121, 604‑611.

Kobayashi, H., Masaoka, Y., Sato, S., 2005. Effects of excess magnesium on the growth and mineral content of rice and Echinochloa. Plant Prod. Sci. 8, 38–43.

Koike, T., Yazaki, K., Funada, R., Maruyama, Y., Mori, S., Sasa, K., 2000. Forest heath and vitality in northern Japan -A case study on larch plantation-. Res. Note Fac. For. Univ. Joensuu 92, 49–60.

Koike, T., Kuromaru, M., Qu, L.Y., Choi, D.S., Takagi, K., Masyagina, O.V., Wang, W.J., Sasa, K., 2004. Process of larch plantation in northern Japan and development of hybrid larch (F_1) for overcoming biological stresses. Proceed. Int. Cong. Forest bioactive resources, Far East Forest Research Institute, Khabarovsk, 57–63.

Lambers, H., Chapin III, F.S., Pons, T.L., 2008. Plant physiological ecology, second edition. Springer, New York.

Lee, B.D., Graham, R.C., Laurent, T.E., Amrhein, C., 2004. Pedogenesis in a wetland meadow and surrounding serpentinitic landslide terrain, northern California, USA. Geoderma 118, 303–320.

Leyval, C., Turnau, K., Haselwandter, K., 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7, 139–153.

Long, S.P., Bernacchi, C.J., 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. Exp. Bot. 54, 2393‑2401.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P.G., Kolari, P., Kowalski, A.S., Lankreijer, H., Law, B.E., Lindroth, A., Loustau, D., Manca, G., Moncrieff, J.B., Rayment, M., Tedeschi, V., Valentini, R., Grace, J., 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature, 447, 848-852.

Miyaki, Y., 1990. Superior characters of the F_1 hybrid *Larix gmelinii* × *L. kaempferi* and the growth gain in full-sib family selection. For. Tree Breed. Hokkaido 33, 7–12. (in Japanese)

Mizuno, N., 1967. Studies on chemical characteristics of serpentine soil in Hokkaido: 1. The difference of contents of Nickel and Molybdenum in plants and soils. Res. Bull. Hokkaido Agr. Exp. Station, 15, 48–55.

Murphy, J., Riley, J.P., 1962. A modified single solution method for determination of phosphate in natural waters. Anal. chem. Acta 27, 31–36.

Nakaji, T., Fukami, M., Dokiya, Y., Izuta, T., 2001, Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. Trees 15, 453–461.

Nakata, M., Kojima, S., 1987. Effects of serpentine substrate on vegetation and soil development with special reference to *Picea glehnii* in Teshio district, Hokkaido, Japan. For. Ecol. Manage. 20, 265–290.

Niinemets, Ü., Tenhunen, J.D., 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. Plant Cell Environ. 20, 845–866.

Niinemets, Ü., Tenhunen, J.D., Canta, N.R., Chavis, M.M., Faria, T., Pereira J.S., Reynolds, J.F., 1999. Interactive effects of nitrogen and phosphorus on the acclimation potential of foliage photosynthetic properties of cork oak, *Quercus suber*, to elevated atmospheric CO_2 concentrations. Glob. Chang. Biol. 5, 455–470.

Nomura, M., Satoh, F., Ashiya, D., Masumoto, H., 1999. Observation and simulation of snowcover in a mountain region. Res. Bull. Hokkaido Univ. For. 56(2), 11–19.

Okochi, H., Igawa, M., 2001. Elevational patterns of acid deposition into a forest and nitrogen saturation on Mt. Oyama, Japan. Water Air Soil Pollut., 130, 1091–1096.

O’Dell, R.E., Claassen, V.P., 2006. Relative performance of native and exotic grass species in response to amendment of drastically disturbed serpentine substrates. J. Appl. Ecol. 43, 898–908.

Ohara, T., Akimoto, H., Kurokawa, J., Horii, N., Yamaji, K., Yan, X., Hatasaka, T., 2007. An Asian emission inventory of anthropogenic emission sources for the period 1980-2020. Atmos. Chem. Phys. 7, 4419–4444.

Pâques, L.E., 1994. Relationship between foliar nutrient concentrations and growth of hybrid larch (*Larix* × *eurolepis* Henry). For. Ecol. Manage. 63, 153-167.

Proctor, J., 1971. The plant ecology of serpentine III. The influence of a high calcium/magnesium ratio and high nickel and chromium levels in some British and Swedish serpentine soil. J. Ecol. 59, 827–842.

Qu, L.Y., Shinano, T., Quoreshi, A.M., Tamai, Y., Osaki, M., Koike, T., 2004. Allocation of ^{14}C-Carbon in two species of larch seedlings infected with ectomycorrhizal fungi. Tree Physiol. 24, 1369–1376.

R Development Core Team., 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available via DIALOG. http://www.R-project.org.

Rao, M., Sharp, R.E., Boyer, J., 1987. Leaf magnesium alters photosynthetic response to low water potentials in sunflower. Plant Physiol. 84, 1214–1219.

Roberts, B.A., Proctor, J., 1992. The ecology of areas with serpentinized rocks. Kluwer, Dordrecht

Ryu, K., Watanabe, M., Shibata, H., Takagi, K., Nomura, M., Koike, T., 2009. Ecophysiological responses of the larch species in northern Japan to environmental changes as a basis for afforestation. Landscape Ecol. Eng. 5, 99–106.

Ryu, K., Watanabe, M., Takagi, K., Nomura, M., Koike, T., 2010. Effects of high nitrogen load on the photosynthesis and growth of the hybrid larch (*Larix gmelinii* × *L. kaempferi*) seedlings: the responses for the nitrogen treatment during two growing seasons. Transactions of the meeting in Hokkaido Branch of the Japanese Forest Society 58, 17–20. (in Japanese)

Sase, H., Takahashi, A., Sato, M., Kobayashi, H., Nakata, M., 2008. Seasonal variation in the atmospheric deposition of inorganic constituents and canopy interactions in a Japanese cedar forest. Environ. Pollut. 152, 1–10.

Satoh, F., Nomura, M., Masumoto, H., Ashiya, D., Sasa, K., 1999. Ionic elution from the acidic snowpack during spring thaw period in the northern part of Hokkaido. Res. Bull. Hokkaido Univ. For. 56(2), 1–10.

Seregin, I.V., Kozhevnikova, A.D., 2006. Physiological role of nickel and its toxic effects on higher plnats. Russ. J. Plant Physiol. 53, 257-277.

Shinano, T., Lei, T.T., Kawamukai, T., Inoue, M.T., Koike, T., Tadano, T., 1996. Dimethylsulfoxide method for the extraction of chlorophylls a and b from the leaves of wheat, field bean, dwarf bamboo, and oak. Photosynthetica 32, 409-415.

Takashima, T., Hikosaka, K., Hirose, T., 2004. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. Plant Cell Environ. 27, 1047–1054.

Tatewaki, M., Igarashi, T., 1971. Forest vegetation in the Teshio and the Nakagawa district experimental forests of Hokkaido University, Prov. Teshio, N. Hokkaido, Japan. Res. Bull. Hokkaido Univ. For. 28, 1–192. (in Japanese with English summary)

Tissue, D.T., Lewis J.D., 2010. Photosynthetic responses of cottonwood seedlings grown in glacial through future atmospheric [CO_2] vary with phosphorus supply. Tree Physiol. 30, 1361–1372.

Thomas, G.W., 1982. Exchangeable cations. in: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of soil analysis, Part 2. Chemical and microbiological properties, 2nd edn. Soil Science Society of America Inc., Madison, pp. 159–165.

Underwood, A.J., 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Annu. Rev. 19, 513–605.

van Reeuwijk, L.P., 1993. Procedures for soil analysis. International soil reference and information centre, Wagningen

Warren, C.R., Adams, M.A., 2004. Evergreen trees do not maximize instantaneous photosynthesis. Trends in Plant Science, 9, 270-274.

Watanabe, M., Yamaguchi, M., Iwasaki, M., Matsuo, N., Naba, J., Tabe, C., Matsumura, H., Kohno, Y., Izuta, T., 2006. Effects of ozone and/or nitrogen load on the growth of *Larix kaempferi*, *Pinus densiflora* and *Cryptomeria japonica* seedlings. J. Jpn. Soc. Atmos. Environ. 41, 320-334.

Watanabe, M., Watanabe, Y., Kitaoka, S., Utsugi, H., Kita, K., Koike, T., 2011. Growth and photosynthetic traits of hybrid larch F_1 under elevated CO_2 concentration with low nutrient availability. Tree Physiol. 31, 965-975.

Zhang, P., Shao, G., Zhao, G., Le Master, D.C., Parker, G.R., Dunnung Jr., D.B., Li, Q., 2000. China's Forest Policy for the 21th century. Science 288, 2135–2136.

Figure captions

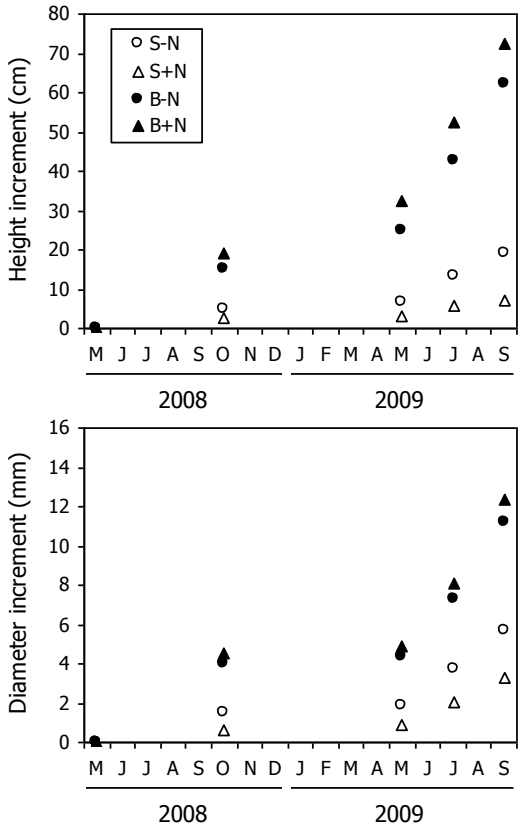
Fig. 1 Increments of height and stem diameter of hybrid larch F_1 seedlings from May 2008 to September 2009. The seedlings were grown on serpentine soil (S) or brown forest soil (B) in combination with N loading at 0 (-N) or 47 kg N ha^{-1} $year^{-1}$ (+N) (n = 16).

Fig. 2 Nitrogen allocation in the needles of hybrid larch F_1 seedlings. The seedlings were grown on serpentine soil (S) or brown forest soil (B) in combination with N loading at 0 (-N) or 47 kg N ha^{-1} $year^{-1}$ (+N) (n = 8). N1, nitrogen allocated to Rubisco; N2, nitrogen allocated to electron carriers except for photosystems, coupling factor and Calvin cycle enzymes apart from Rubisco; N3, nitrogen allocated to light-harvesting complex and photosystems. Values with different letters attached differ significantly for the sum of N1, N2 and N3, with $P < 0.05$

Fig. 3 Concentrations of elements in needles of hybrid larch F_1 seedlings grown on serpentine soil (S) or brown forest soil (B) in combination with N loading at 0 (-N) or 47 kg N ha^{-1} $year^{-1}$ (+N). The error bar shows SE (n = 8). ANOVA: ** P < 0.01; *** P < 0.001; n.s. not significant. There was no significant interaction between soil type and N load for any parameter.

Fig. 4 Relations between mass-based element content and net photosynthetic rate at 380 μmol mol^{-1} CO_2 (A_{380}) in the needles of hybrid larch F_1 seedlings. The seedlings were grown on serpentine soil (S) or brown forest soil (B) in combination with N loading at 0 (-N) or 47 kg N ha^{-1} $year^{-1}$ (+N). Pearson's correlation test: ** P < 0.01; *** P < 0.001; n.s. not significant.

Fig.1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B+N
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60
70
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2008
2009
Diameter increment (mm)
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M J J A S O N D J F M A M J J A S
2008
2009

Fig. 2

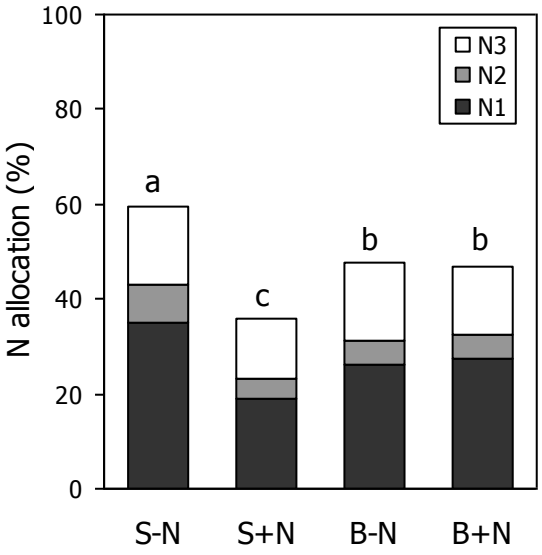
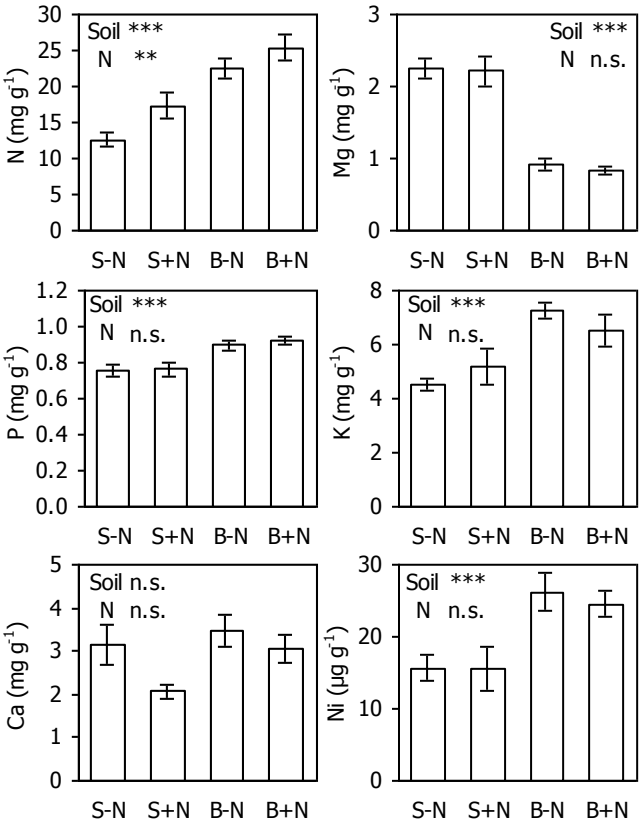


Fig. 3


N (mg g-1)
Soil ***
N **
Mg (mg g-1)
Soil ***
N n.s.
P (mg g-1)
Soil ***
N n.s.
K (mg g-1)
Soil ***
N n.s.
Ca (mg g-1)
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N n.s.
Ni (µg g-1)
Soil ***
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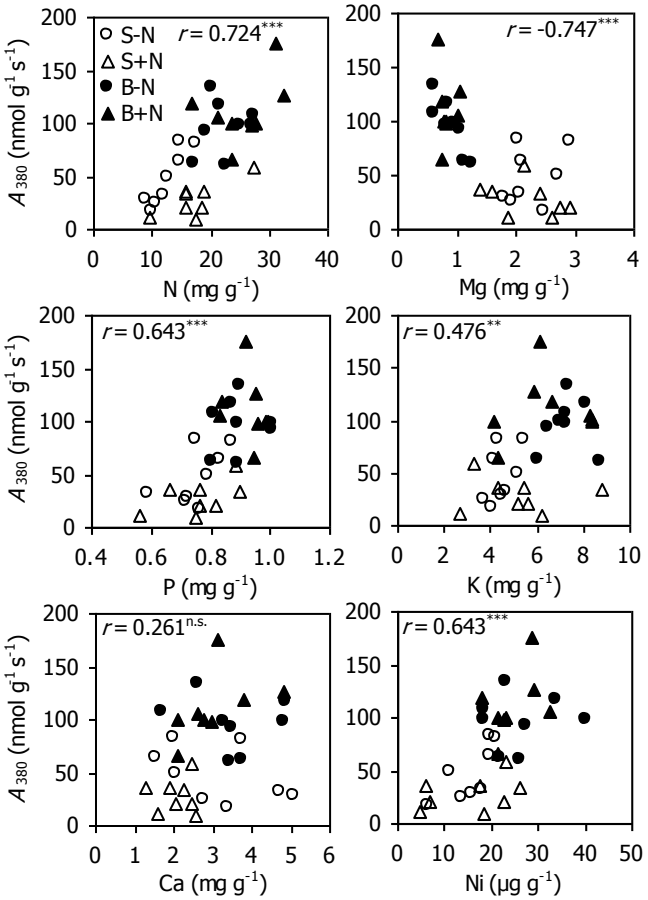
S-N
S+N
B-N
B+N

Fig. 4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S+N
B-N
B+N
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r = 0.643***
r = 0.476**
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r = 0.643***
A380 (nmol g-1 s-1)
N (mg g-1)
Mg (mg g-1)
P (mg g-1)
K (mg g-1)
Ca (mg g-1)
Ni (μg g-1)

Table 1 Physical properties of serpentine soil and brown forest soil

	Serpentine soil	Brown forest soil	*t*-test
Dry bulk density (Mg m^{-3})	0.85 (0.02)	0.87 (0.02)	n.s.
Soil water content (g g^{-1})	0.61 (0.03)	0.42 (0.01)	***
Maximum water holding capacity (g g^{-1})	0.57 (0.01)	0.51 (0.01)	**

Each value is the mean of 8 determinations; the standard error is shown in parentheses
t-test: ** $P < 0.01$; *** $P < 0.001$; n.s. not significant

Table 2 Effect of nitrogen loading on the chemical properties of serpentine soil and brown forest soil

	Serpentine soil		Brown forest soil		Two-way ANOVA		
	-N	+N	-N	+N	Soil	N	Soil×N
pH	6.17 (0.04) a	5.86 (0.11) b	5.54 (0.07) c	4.84 (0.10) d	***	***	*
Total C (%)	2.21 (0.55)	2.10 (0.23)	2.94 (0.33)	2.50 (0.17)	n.s.	n.s.	n.s.
Total N (%)	0.11 (0.01)	0.17 (0.02)	0.14 (0.02)	0.17 (0.01)	n.s.	*	n.s.
NO_3^- (mg kg^{-1})	3.96 (0.72)	9.91 (1.66)	3.82 (0.70)	6.01 (1.63)	n.s.	*	n.s.
NH_4^+ (mg kg^{-1})	6.19 (0.35) b	10.16 (1.30) a	8.21 (0.50) ab	8.61 (0.57) a	n.s.	*	*
P (mg kg^{-1})	6.01 (1.76)	3.67 (0.73)	11.43 (1.75)	13.94 (2.37)	**	n.s.	n.s.
Ca (mg kg^{-1})	686 (38)	758 (53)	304 (75)	339 (85)	***	n.s.	n.s.
Mg (mg kg^{-1})	1490 (32)	1350 (195)	51 (10)	12 (2)	***	n.s.	n.s.
K (mg kg^{-1})	113 (17)	103 (9)	114 (15)	67 (5)	n.s.	*	n.s.
Cr (AA) (mg kg^{-1})	2.25 (0.28)	3.48 (0.48)	3.07 (0.49)	2.47 (0.26)	n.s.	n.s.	n.s.
Ni (AA) (mg kg^{-1})	3.98 (0.58) b	6.96 (0.38) a	0.06 (0.01) c	0.05 (0.01) c	***	**	***
Cr (HA) (mg kg^{-1})	2.04 (0.55)	1.37 (0.62)	0.49 (0.04)	0.57 (0.07)	**	n.s.	n.s.
Ni (HA) (mg kg^{-1})	39.11 (1.99)	40.08 (4.12)	0.19 (0.08)	0.13 (0.06)	***	n.s.	n.s.

Cr and Ni were extracted by two solutions, ammonium acetate (AA) and hydrochloric acid (HA).
Each value is the mean of four measurements; the standard error is shown in parentheses
ANOVA: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant
For each parameter, values with different letters attached differ significantly, with $P < 0.05$

Table 3 Effect of nitrogen loading on the dry mass of plant organs and the ratio of aboveground dry mass to belowground dry mass (T/R) of hybrid larch F_1 seedlings grown on serpentine soil and brown forest soil

	Serpentine soil				Brown forest soil				Two-way ANOVA		
	-N		+N		-N		+N		Soil	N	Soil×N
Needle	9.5 (2.0)		3.0 (0.7)		36.2 (4.0)		40.2 (6.1)		***	n.s.	n.s.
Stem	10.6 (1.9)	b	4.3 (0.7)	b	52.7 (6.9)	a	67.1 (8.6)	a	***	n.s.	*
Root	6.9 (1.2)	c	3.2 (0.5)	c	23.8 (3.7)	b	33.8 (4.4)	a	***	n.s.	**
Whole-plant	27.0 (5.0)	b	10.5 (1.9)	b	112.6 (14.3)	a	141.0 (18.6)	a	***	n.s.	*
T/R	2.5 (0.4)		2.1 (0.3)		3.7 (0.3)		3.2 (0.2)		***	n.s.	n.s.

Each value is the mean of 16 measurements; the standard error is shown in parentheses

ANOVA: * $P < 0.05$; *** $P < 0.001$; n.s. not significant

For each parameter, values with different letters attached differ significantly, with $P < 0.05$

Table 4 Effect of nitrogen loading on the needle traits of hybrid larch F_1 seedlings grown on serpentine soil and brown forest soil

	Serpentine soil				Brown forest soil				Two-way ANOVA		
	-N		+N		-N		+N		Soil	N	Soil×N
A_{380} (μmol m^{-2} s^{-1})	3.9 (0.7)		2.4 (0.5)		7.3 (0.7)		8.5 (0.7)		***	n.s.	n.s.
A_{max} (μmol m^{-2} s^{-1})	16.3 (2.4)	b	10.3 (1.3)	c	19.6 (2.0)	ab	22.0 (1.4)	a	***	n.s.	*
G_s (mol m^{-2} s^{-1})	0.07 (0.01)		0.04 (0.01)		0.12 (0.02)		0.14 (0.02)		***	n.s.	n.s.
E (mmol m^{-2} s^{-1})	1.02 (0.13)	b	0.63 (0.09)	c	1.17 (0.14)	ab	1.44 (0.11)	a	***	n.s.	*
Ls	0.44 (0.03)		0.49 (0.05)		0.36 (0.04)		0.37 (0.03)		*	n.s.	n.s.
V_{cmzx} (μmol m^{-2} s^{-1})	51 (6)	b	36 (5)	c	56 (4)	ab	67 (4)	a	**	n.s.	*
J_{mzx} (μmol m^{-2} s^{-1})	127 (14.6)	b	85 (10.7)	c	127 (9.7)	ab	147 (10.5)	a	*	n.s.	*
PNUE (μmol mol^{-1} s^{-1})	48 (7)	b	22 (3)	c	61 (6)	ab	63 (7)	a	***	n.s.	*
LMA (g m^{-2})	83.4 (1.5)		84.1 (2.0)		75.7 (1.2)		77.5 (2.2)		***	n.s.	n.s.
N_{area} (g m^{-2})	1.04 (0.08)		1.45 (0.13)		1.70 (0.10)		1.94 (0.11)		***	**	n.s.
Chl (g m^{-2})	0.27 (0.01)		0.27 (0.02)		0.42 (0.02)		0.42 (0.03)		***	n.s.	n.s.

A_{380}, net photosynthetic rate at 380 μmol mol^{-1} CO_2; A_{max}, net photosynthetic rate at 1700 μmol mol^{-1} CO_2; G_s, stomatal conductance to water vapor; E, transpiration rate; L_s, stomatal limitation of photosynthesis; V_{cmax}, maximum rate of carboxylation; J_{max}, maximum rate of electron transport; PNUE. photosynthetic nitrogen use efficiency; LMA, leaf mass per area; N_{area}, nitrogen content per unit needle area; Chl, chlorophyll content

Each value is the mean of eight measurements; the standard error is shown in parentheses

ANOVA: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant

For each parameter, values with different letters attached differ significantly, with $P < 0.05$