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Author(s)	Kayama, Masazumi; Koike, Takayoshi
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**Full title: Differences in growth characteristics and dynamics of elements in seedlings
of two birch species grown in serpentine soil in northern Japan.**

Authors: Masazumi Kayama^{a*}, and Takayoshi Koike^b

a: Hokkaido University Forests, FSC, Sapporo 060-0809, JAPAN

b: Silviculture and Forest ecological Studies, Hokkaido University, Sapporo 060-8589,
JAPAN

* Corresponding author

Present affiliation of Masazumi Kayama: Japan International Research Center for
Agricultural Sciences. Ibaraki 305-8686, JAPAN.

Address of corresponding author:

Japan International Research Center for Agricultural Sciences.

1-1 Ohwashi, Tsukuba, Ibaraki 305-8686, JAPAN

Tel: +81-29-838-6313

Fax: +81-29-838-6654

E-mail: kayama@affrc.go.jp

Abstract

Betula ermanii and *Betula platyphylla* var. *japonica*, two typical light-demanding-deciduous trees in northern Japan, usually invade disturbed areas. *B. ermanii* can invade serpentine soil and grow in it, whereas *B. platyphylla* var. *japonica* can hardly regenerated in it. Serpentine soil is distributed throughout Japan, and it is characterized by excessive Mg and heavy metals (Ni, Cr, and Co) which can lead to suppressed plant growth. We examined the tolerance of the two *Betula* species by planting seedlings in serpentine and non-serpentine (brown forest) soils.

The dry mass of each organ was suppressed in both birches planted in serpentine soil, and the photosynthetic rate was reduced by accumulation of Ni. As well, uptakes of K and Ca were inhibited by accumulation of Mg, Ni, Cr and Co. *B. ermanii* planted in serpentine soil showed high value of net assimilation rate in second year, and maintained photosynthetic rate from June to September. The effects of Mg, Ni, Cr and Co accumulation were small for the relative growth rate of *B. ermanii*. In contrast, *B. platyphylla* var. *japonica* planted in serpentine soil showed decreased photosynthetic rate in September, and smaller net assimilation rate than that for *B. ermanii* at the same time. Even more, *B. platyphylla* var. *japonica* showed decreased relative growth rate, induced by accumulation of Mg in leaves,

and Co in roots. We conclude that *B. ermanii* has high advantage to regenerate in serpentine soil.

Key words: birch, serpentine soil, photosynthetic capacity, heavy metal, nutrient physiology.

Key message: Seedlings of two birch species were grown in serpentine soil, with *Betula ermanii* showing high tolerance.

Introduction

Edaphic traits of serpentine soils usually form a special vegetation type (Brooks 1987; Alexander et al. 2007), which may be related to special resistant capacity of the plants. Ultramafic rocks are scattered throughout Japan and, in fact, host unique patterns of vegetation (Mizuno and Nosaka 1992). A metamorphic belt exists on Hokkaido Island, northern Japan, and serpentinite occurs intermittently from north to south (Tatewaki and Igarashi 1971; Mizuno and Nosaka 1992). Erman's birch (*Betula ermanii* Cham.) can regenerate and well establish in this serpentine soil (Takikawa et al. 1994; Yamada 1999; 2001). Likewise, Japanese-white birch (*Betula platyphylla* Suk. var. *japonica* Hara., hereafter *B. platyphylla*) seedlings can be distributed at contiguous habitat of serpentinite (Tatewaki and Igarashi 1971), but seedlings of *B. platyphylla* can be rarely regenerated in serpentine soil (Takikawa et al. 1994; Yamada 1999; 2001). These birches also rapidly regenerate in disturbed areas where they synthesize secondary forests as pioneer species (Koyama and Yajima 1989; Ishibashi 1998). *B. ermanii* is distributed at higher altitude than *B. platyphylla* in central Hokkaido, but grows in cool and humid regions in northeast Asia (Miyawaki 1988; Koike 1995; Takahashi et al. 2003).

Serpentine soil is originated from weathered serpentinite rock (Brooks 1987; Brady et al. 2005), and it is characterized by excess amounts of Nickel (Ni), Chromium (Cr), Cobalt (Co), and Magnesium (Mg). It has a low Ca/Mg ratio and low levels of several essential nutrients for plants (Proctor 1971; Brooks 1987; Brady et al. 2005; Alexander et al. 2007). Excess Ni inhibits ion uptake and translocation, root growth, and photosynthetic capacity (Jones and Hutchinson 1988a; b; Yang et al. 1996; Tilstone and Macnair 1997; Miller and Cumming 2000; Kayama et al. 2005; 2006; Velikova et al. 2011). Cr in serpentine soil is mainly a form of Cr(III) (Oze et al. 2004; Alexander et al. 2007) and its toxicity was lower than that of Cr(VI) (Vernay et al. 2008; Santana et al. 2012). However, accumulation of Cr(III) suppresses photosynthetic capacity (Vernay et al. 2008; Santana et al. 2012). Co and Mg are essential elements for plant growth (Marschner 1995), however, excessive accumulation of Co by plants may inhibit growth and photosynthesis (Cocucci and Morgutti 1986; Palit et al. 1994; Ali et al. 2010; Sinha et al. 2012). Excess levels of Mg inhibit plants' growth and uptakes of K and Ca (Rao et al. 1987; Kobayashi et al. 2005; Ding et al. 2006).

Some plant species can resist to serpentine soil, and form unique vegetation on it (e.g. Brooks 1987; Alexander et al. 2007). Plants generally use two mechanisms to avoid and tolerate or adapt to high concentrations of heavy metals in serpentine soils: i) by restricting

the uptake of metals (exclusion-plants) or ii) by translocating and accumulating the metals in a non-toxic form (Baker 1987). Uptake of macronutrients, such as Potassium (K) and Calcium (Ca), are not suppressed by accumulation of toxic elements in the plants growing in serpentine soil (Alexander et al. 2007; Lazarus et al. 2011); these plants do not show decreased photosynthetic rate by accumulation of the elements in this soil (Kayama et al. 2006; Palm et al. 2012).

B. ermanii has greater tolerance of environmental stresses than *B. platyphylla* (Koike 1995; Kitao et al. 1999; Matsumura et al. 2005) and the ability to accumulate toxic metals (Manganese: Mn and Lead: Pb) in its leaves (Kitao et al. 1999; Bai et al. 2007). Still, *B. ermanii* has larger photosynthetic rate than *B. platyphylla* when Mn is accumulated (Kitao et al. 1999). Nevertheless, effects of toxic elements on growth characteristics and photosynthetic rates of birch species growing in serpentine soil remain, still, unclear.

Based on the above evidence, we hypothesized that: (a) *B. ermanii* has a higher tolerance to the presence of toxic elements in soil than *B. platyphylla*; (b) *B. ermanii* growing in serpentine soil may accumulate large amounts of toxic elements in its leaves; consequently, (c) effects of these elements on photosynthetic capacity may be less than in *B. platyphylla*; and (d) uptake of K and Ca may hardly suppress the growth of *B. ermanii* growing in

serpentine soil.

To test our hypotheses, we examined three ecophysiological traits of the two birch species seedlings grown either in serpentine or in brown forest soils: (1) growth characteristics, (2) photosynthetic rate, and (3) concentrations of various elements in plant organs.

Materials and Methods

Study site

The experimental site is located at Teshio Experimental Forest (TEF), under the administration of Hokkaido University (N45°06', E142°12', 110 m a.s.l.). Serpentine soils are distributed in eastern part of TEF, where *Picea glehnii* Masters. is dominant (Tatewaki and Igarashi 1971; Nakata and Kojima 1987). *Betula ermanii* also grows and regenerates in the same region (Nakata and Kojima 1987; Takikawa et al. 1994). The mean annual precipitation of the experimental year was about 1,200 mm yr⁻¹, and the snow-free period lasted from late May to early November. The annual mean, maximum and minimum temperatures were 5.2°C, 32.0°C and -33.5°C, respectively, over the last 30 years, as measured by a thermo recorder at the Kami-toikan meteorological station (TEF, unpublished

data) which was located at about 7 km from the experimental site.

Experimental plots

We established two experimental plots in May 1999: one using serpentine soil and one using brown forest soil. According to the FAO-UNESCO systems, the serpentine and brown forest soils are classified as a Podzol and a Cambisol, respectively (Nakata and Kojima 1987). To reduce soil heterogeneity, the soil in each experimental plot was cultivated using a tractor. Each soil type was dually replicated using 2×10 m seedling beds. The distance between the experimental plots of serpentine and brown forest soils was about 100 m.

Plant materials

Seeds from *B. ermanii* and *B. platyphylla* were selected from similar habitats, from trees growing in non-serpentine soils in the central part of Hokkaido, of an area managed by the National Forestry Research Institute. This location was chosen to minimize genetic differences caused by climatic and edaphic factors. Seeds of both species were planted on

clay loam soils (Cambisol), from the central part of Hokkaido, in nursery, and they remained there until seedlings were 2-year old. Thirty-two seedlings (2-year-old) of each birch species were planted to the two experimental plots in TEF in early June 1999, just after snow had melted completely. During transportation of seedlings, the roots of each seedling were wrapped with moist paper towels to prevent dehydration (Kimtowel, Crecia Co., Tokyo, Japan). Seedlings of each birch species were planted in both serpentine and brown forest soils. Each plot was weeded periodically by hand. Soil moisture was monitored via time domain reflectometry (TRIME-FM, IMKO Micromodultechnik GmbH, Ettlingen, Germany). The water content at field capacity was 48% for both soils types; watering was carried out to prevent desiccation.

Soil chemistry

Table 1 shows the soil chemistry of the two plots. Serpentine soil had a higher pH (6.7) than brown forest soil (pH=5.2) ($P < 0.001$). The concentrations of Mg, Sodium (Na), Zinc (Zn), Ni and Cr were higher in serpentine soil than in brown forest soil ($P < 0.001$). Similarly, concentrations of Mg and Ni in serpentine soil were 19 and 78 times, respectively, higher

than in brown forest soil. In contrast, the concentrations of nitrogen (N) and iron (Fe) were lower in serpentine soil than in brown forest soil ($P < 0.05$). Concentrations of carbon (C), phosphorus (P), Ca, K, Mn and copper (Cu) did not differ significantly between the two soil types. Ca/Mg molar ratio was 0.12 for serpentine soil and 2.79 for brown forest soil.

Soil samples for Co analysis prepared as described by Kayama (2006). Solutions for Co analysis were prepared by mixing 25 mL of 0.1 M HCl with 5.0 g of dry soil, by shaking for 1 h (Kubota and Cary 1982). The temperature was maintained at 30 °C during this process. Co in the extracted solution was analyzed using an inductivity coupled plasma (ICP) analyzer (SPS4000, SII Nano Technologies Ins., Chiba, Japan).

Measurement of photosynthetic traits

Photosynthetic rates of sun-exposed leaves of the seedlings were measured. Sixteen individuals: eight planted in serpentine and eight in brown forest soils were used to measure the photosynthetic rate in early and late growing season (June and September 2000). Birch species usually develop their early leaves first in spring, and then later expand their late leaves after complete expansion of early leaves (Koike 1995). For the photosynthesis

measurements, early leaves were selected in May (for leaf age uniformity).

These measurements were taken using a portable gas analyser (H4A, ADC-Analytical Development Company, UK) under steady-state conditions, with an ambient temperature of 25–26°C and ambient CO₂ concentration of 35.5–36.0 Pa. Supplementary light was provided by a halogen lamp (WALZ, Effeltrich, Germany). The photosynthetic photon flux (PPF) was changed from high to low using shade cloths (Krory, Osaka, Japan). Light-dependent photosynthesis curves were calculated from the photosynthetic data according to the method of Thornley (1976):

$$P_n = P_{\max} [1 - e^{-(\alpha I / P_{\max})}] - R,$$

where P_n is the net photosynthetic rate, $P_{\max}$ is the maximum photosynthetic rate at light saturation, α is the initial gradient of the curve, I is the PPF, and R is the respiration rate at 0 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPF. Stomatal conductance (gs) was also measured simultaneously with the measurements of photosynthetic rates.

After the measurements of photosynthetic rates and stomatal conductance, the leaf-projected area was estimated using an image scanner (FB636U, Canon, Japan). The leaf dry mass was measured after each leaf was separately enveloped and oven-dried at 80°C for four days. Finally, the specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$) was calculated following the method

of Lambers et al. (1998).

Measurement of seedling growth

To determine the growth characteristics of seedlings of the two birch species, the dry masses of leaves, stems and branches, and roots were measured. Eight seedlings of each birch species planted in the two types of soil were harvested in June 1999, June 2000, and September 2000 (i.e. the initial, 12th, and 15th month harvest). The roots of the harvested seedlings were washed with tap water to remove soil, and then washed with distilled water in an ultrasonic washer (US-2A, As One Co., Osaka, Japan) for 15 minutes. The washed seedlings were divided into three components: (a) leaves, (b) stems and branches, and (c) roots. Each component was put in its own paper envelope and was oven-dried at 80°C for four days. The dry masses of the each components were then determined.

Growth of the birch seedlings was also analysed from data of total dry mass. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) of both birches' seedlings were examined (after Lambers et al. 1998). Total leaf area used by estimation of NAR and LAR was calculated by leaf dry mass and specific leaf area (SLA).

Analysis of nutrition in plants

The (mass-based) concentrations of N, P, K, Ca, Mg, Ni, Cr and Co in leaves and roots were determined. Leaves of the birch species used for chemical analysis were those sampled for measurement of the photosynthetic rate. The dried samples were ground to a fine powder using a sample mill (WB-1, Osaka Chemical Co., Osaka, Japan). The concentration of N was determined using a NC analyser (Sumigraph NC-22, Sumica Chemical Analysis Service, Tokyo, Japan). To determine the concentration of the elements P, K, Ca, Mg, Ni, Cr, and Co, dried samples were digested by a speed digester using sulfuric acid and hydrogen peroxide (K-424, BÜCHI Labortechnik, Flawil, Switzerland).

The concentrations of K, Ca and Mg in each sample solution were determined using an atomic absorption spectrophotometer (Z-2310, Hitachi High-Technologies Co., Tokyo, Japan). In the solutions for Ca and Mg determination, LaCl_3 was added at $1,000 \text{ mg l}^{-1}$ to prevent interference from other elements. The concentration of P was determined by a spectrophotometer (UV-2500PC, Shimadzu Co., Kyoto, Japan), using the molybdenum blue method (American Public Health Association et al. 1998). The concentrations of Ni, Cr and

Co were determined by an ICP analyser. Standard solutions were also analysed at intervals of 20 samples, to verify the reliability of the analysis.

To estimate the relationship between growth and various elements, correlation analysis was examined between RGR and the concentrations of elements in leaves or roots. In addition, correlation analysis was examined between the photosynthetic rate and the concentrations of elements in leaves. In general, the photosynthetic rate is closely related to N (Evans 1989; Lambers et al. 1998; Kayama et al. 2007), to P (Raaimakers et al. 1995; Bown et al. 2007) and to K (Baillon et al. 1988; Pettigrew 2008; Kanai et al. 2011). Photosynthetic rate is decreased by accumulation of Ni (Jones and Hutchinson 1988a; b; Miller and Cumming 2000; Molas 2002; Kayama et al. 2006; Velikova et al. 2011), Cr(III) (Vernay et al. 2008; Santana et al. 2012), and Co (Cocucci and Morgutti 1986; Ali et al. 2010). Area-based concentrations of elements were calculated from the SLA, as photosynthetic rates of broadleaf trees were based on the leaf surface area (e.g. Koike 1995).

The interrelation was examined among N, P, K, Ca, Mg, Ni, Cr and Co in leaves or roots of the two birch species, because various elements in plant organ, such as Ca and Mg, affect mutually (Proctor 1971; Brooks 1987; Alexander et al. 2007).

Statistical analyses

Stat View 5.0 (SAS Institute Inc.) was used for statistical analyses of all parameters. The mean dry mass of each organ, growth parameters (RGR, NAR, and LAR), the photosynthetic rate and stomatal conductance at light saturation, and the concentrations of elements in leaves and roots were tested using two-way ANOVA. The independent variables were the soil type and the birch species. Correlation analysis was used between physiological parameters and the elements' concentrations (see Table 7, 8 and 9). To select the main elements that affect RGR and photosynthetic rate, stepwise multiple regression analysis was performed.

All mean values of soil chemistry measurements taken at the two nurseries were examined by a single ANOVA; there were no significant differences between the two nurseries.

Results

Soil chemistry

The concentration of Co was 23 times higher in serpentine soil than in brown forest soil

(Table 1, $P < 0.001$). In serpentine soil, concentration of Co was higher than that of Cr, and lower than that of Ni.

Growth characteristics

The dry masses of each organ of the two birch seedlings grown in serpentine soil were smaller than those of seedlings grown in brown forest soil (Table 2; $P < 0.001$). Compared with brown forest soil, the total dry mass of seedlings in serpentine at the 15th month was reduced to 12 % for *B. ermanii* and to 9 % for *B. platyphylla*. The leaf dry mass of *B. ermanii* at the 15th month was significantly smaller than that of *B. platyphylla* ($P < 0.05$). There was a significant interaction between soil types and species ($P < 0.05$). The leaf dry mass was almost identical for the two birches in serpentine soil, at the 15th month. A clear trend appeared in *B. platyphylla*, in which leaves of seedlings in brown forest soil had a large dry mass. *B. platyphylla* in brown forest soil showed increased leaf dry mass significantly from 12th to 15th month ($P < 0.001$, t-test), but *B. ermanii* did not. Both species did not show significant increase of leaf dry mass from 12th to 15th month when grown in serpentine soil.

The average SLA for the two birches was $140 \text{ cm}^2 \text{ g}^{-1}$ at the 12th month and $126 \text{ cm}^2 \text{ g}^{-1}$ at

the 15th month. Consequently, no significant difference in SLA was found between the two birch species or between the two soil types.

RGR, NAR and LAR of two birch seedlings in serpentine soil were smaller than those in brown forest soil (Table 3; $P < 0.05$). RGR and NAR showed significantly increased values from 12th to 15th month, as compared with those from initial to 12th month ($P < 0.01$). NAR was significantly higher in *B. ermanii* than in *B. platyphylla* ($P < 0.05$), from 12th to 15th month. Similarly, NAR showed a significant interaction between soil type and species ($P < 0.01$) from initial to 12th month. A trend of NAR for *B. platyphylla* was almost identical between two types of soil. LAR showed significantly high values at the 12month, as compared with that at the 15th month ($P < 0.01$). LAR at the 15th month was significantly higher in *B. platyphylla* than in *B. ermanii* ($P < 0.001$). A significant interaction was observed between soil type and species in LAR, at the 12th month ($P < 0.001$), but LAR of *B. ermanii* was almost identical between the two soils.

Photosynthetic characteristics

The net photosynthetic rate (P_n) of leaves of *B. ermanii* and *B. platyphylla* saturated at

approximately $1700 \mu\text{mol m}^{-2}\text{s}^{-1}$ PPF in each type of soil (Fig. 1). The photosynthetic rate of seedlings in serpentine soil was notably lower than that of seedlings in brown forest soil, for the two birches. For both birches the initial gradients of the light photosynthetic curve of seedlings in serpentine soil were less than that of seedlings in brown forest soil ($P<0.05$).

The photosynthetic rate at light saturation (P_{sat}) of seedlings in serpentine soil was significantly lower than that of seedlings in brown forest soil (Table 4; $P<0.001$), both at early and late growth seasons. We found that P_{sat} at the late growth season was significantly higher in *B. ermanii* than in *B. platyphylla*, and that the interaction between soil types and birch species in P_{sat} was significant at late season ($P<0.05$). P_{sat} was almost identical for two birches in brown forest soil at the late growth season, but in serpentine soil P_{sat} was higher for *B. ermanii*. The P_{sat} value for *B. ermanii* grown in serpentine soil was maintained until September, whereas two birches in brown forest soil showed decreased P_{sat} at that time ($P<0.001$, t-test).

Stomatal conductance (gs) at the early growth season did not differ significantly between species or soil types, but gs of seedlings in serpentine soil was significantly lower than that of seedlings in brown forest soil at the late growth season ($P<0.001$). We found that gs at the late growth season was significantly higher for *B. ermanii* than for *B. platyphylla* ($P<0.001$).

The gs value for *B. platyphylla* in serpentine soil significantly decreased at the late growth season, as compared with that at the early growth season ($P<0.001$, t-test). In contrast, gs value for *B. ermanii* in serpentine soil did not show significant decrease.

Element concentrations

Tables 5 and 6 show the concentrations of elements in leaves and roots, respectively. The N concentration in leaves and roots did not differ significantly between the two types of soil or between the two species. K and Ca concentrations in leaves and roots were significantly lower in plants grown in serpentine soil ($P<0.01$). The Ca concentration in leaves at the 15th month was significantly higher in *B. ermanii* than *B. platyphylla*. There was a significant interaction between the soil type and the species in the leaf concentrations of K at the 15th month and of Ca at the 12th month ($P<0.05$).

P concentration was significantly lower in roots of seedlings grown in serpentine soil ($P<0.01$). Leaf P concentration at the 15th month exhibited a similar trend, although at the 12th month two types of soil gave rise to no significant difference. There was a significant interaction between soil types and birch species in leaves' P concentration at the 12th month

($P < 0.01$). This interaction could explain why P concentration in *B. ermanii* seedlings in serpentine soil was lower than that in seedlings in brown forest soil, while the P concentration in *B. platyphylla* seedlings was slightly different between the soils.

Mg concentrations in leaves and roots of two birches grown in serpentine soil were significantly higher than in birches grown in brown forest soil ($P < 0.001$). In serpentine soil, leaf Mg concentration was higher than in roots, and it was significantly higher in *B. ermanii* than in *B. platyphylla* ($P < 0.01$). Obviously, the interaction between soil types and birch species was significant ($P < 0.05$). In *B. ermanii*, Ca/Mg ratios in leaves and roots in serpentine soil were 0.055 and 0.064, respectively, at 12th month, and 0.076 and 0.104, respectively, at 15th month. Ca/Mg ratios of leaves and roots of *B. platyphylla* in serpentine soil were 0.078 and 0.066, respectively, at 12th month and 0.085 and 0.112, respectively, at 15th month. Ca/Mg ratio of leaves at 12th month was significantly lower in *B. ermanii* than in *B. platyphylla* ($P < 0.01$). The Ca/Mg ratio of leaves and roots at 15th month showed no significant difference.

Ni and Cr concentrations in leaves and roots of the two species grown in serpentine soil were significantly higher than in those grown in brown forest soil ($P < 0.001$). Ni concentration tended to be higher in roots than in leaves of birches grown in serpentine soil.

Root Ni concentration at the 15th month was significantly higher in *B. platyphylla* than in *B. ermanii* ($P<0.05$). The interaction of soil types and birch species was significant ($P<0.05$). Ni in roots in brown forest soil was broadly similar for the two birches.

Co was detected in leaves of two birches grown in serpentine soil. However, Co was not detected in leaves of two birches grown in brown forest soil. Co concentrations in roots of two birches grown in serpentine soil were significantly higher than those in roots of seedlings grown in brown forest soil ($P<0.001$).

Relationship between relative growth rate (RGR) and element concentrations

As to the elements in leaves, there were a significant positive correlation between P, K and Ca concentrations and RGR for the two birch species (Table. 7, $P<0.05$). In addition, there was a significant positive correlation between root P concentration and RGR ($P<0.01$). In *B. ermanii*, there was a significant positive correlation between root K concentration and RGR ($P<0.01$). In contrast, there were significant and negative correlations between Mg and Ni concentrations and RGR, respectively, in leaves and roots of the two birch species ($P<0.05$).

As to the elements in roots, we found significant negative correlations between N, Cr and

Co concentrations and RGR for both species ($P<0.05$). In *B. platyphylla*, we found significant negative correlations between Cr and Co concentrations in leaves and RGR.

Based on stepwise multiple regression analysis between eight elements and RGR, only P was selected for *B. ermanii*. The models about equation of RGR for *B. ermanii* were given as follows:

$$\text{RGR} = 6.639 + 0.031P_{(\text{leaf})}, R^2=0.33^*$$

$$\text{RGR} = 4.203 + 0.050P_{(\text{root})}, R^2=0.52^{**}$$

In *B. platyphylla*, only Mg and Co were selected, for leaves and roots, respectively. The models for the equation of RGR for *B. platyphylla* were given as follows:

$$\text{RGR} = 22.56 - 0.045\text{Mg}_{(\text{leaf})}, R^2=0.59^{***}$$

$$\text{RGR} = 18.15 - 26.79\text{Co}_{(\text{root})}, R^2=0.57^{***}$$

Relationship between photosynthetic rate and element concentrations

We found a significant positive correlation between K concentration and P_{sat} for both species (Table 8, $P<0.001$). In *B. ermanii* alone, we found a significant positive correlation between N and P concentrations and P_{sat} ($P<0.01$). In contrast, there were significant

negative correlations between Ni, Cr and Co concentrations and P_{sat} ($P<0.001$).

Based on stepwise multiple regression analysis between six elements and P_{sat} , N and Ni were selected for *B. ermanii*. The equation of P_{sat} for *B. ermanii* was given as follows:

$$P_{\text{sat}} = 6.949 + 0.030N_{(\text{leaf})} - 9.950Ni_{(\text{leaf})}, R^2=0.82^{***}$$

In *B. platyphylla*, N, K and Ni were selected by this analysis. Equation of P_{sat} for *B. platyphylla* was given as follows:

$$P_{\text{sat}} = 6.515 + 0.023N_{(\text{leaf})} + 0.078K_{(\text{leaf})} - 15.90Ni_{(\text{leaf})}, R^2=0.93^{***}$$

Among six elements, F value was the highest for Ni (98.1 for *B. ermanii*, and 247.8 for *B. platyphylla*).

Interrelation among elements

Among Mg, Ni, Cr and Co, there were positive relationships for leaves and roots of both species (Table 9; $P<0.001$). A positive relationship was observed among N, P and K of the leaves of *B. ermanii* ($P<0.05$). Positive relationships were also revealed for K and Ca in leaves and roots of *B. ermanii* and in leaves of *B. platyphylla*, and for P and K, N and Ca in roots of two birch species.

In contrast, we found many negative correlations: (1) K with Mg, Ni, Cr, and Co in leaves and roots of the two birch species ($P<0.05$); (2) P in *B. ermanii* and in root of two birch species with Mg, Ni, Cr, and Co; (3) Ca in leaves and roots of both species with Mg and Ni (except for Mg in roots of *B. platyphylla*, $P<0.05$); (4) Ca with Cr or with Co in leaves of *B. platyphylla*, and in roots of *B. ermanii* ($P<0.05$); (5) between N and P or K in roots of both species ($P<0.05$).

Discussion

Total dry masses of the two birches planted in serpentine soil were markedly less than those in brown forest soil (Table 2). This is in accordance with leaf dry mass of seedlings in serpentine soil (9.4 % for *B. ermanii*, 6.1 % for *B. platyphylla*). It is clear that production of leaves was particularly suppressed in both birches planted in serpentine soil. Additionally, photosynthetic rate was less in two birches grown in serpentine soil (Fig. 1, Table 4), and it was related to low biomass productivity. The photosynthetic rates of the two species were markedly decreased by Ni accumulation, as it is described by the equation of P_{sat} (Table 8). We consider that the major factor for growth inhibition of both species grown in serpentine

soil was the Ni accumulation in their leaves.

When Ni is translocated to leaves, it is accumulated in the chloroplasts (Molas 2002; Seregin and Kozhevnikova 2006), inhibiting photosynthetic capacity and activity of various enzymes (Jones and Hutchinson 1988a; b; Miller and Cumming 2000; Molas 2002; Kayama et al. 2006; Seregin and Kozhevnikova 2006; Velikova et al. 2011). We compared the effects of photosynthetic rate and Ni with other species. *Populus nigra* grown in hydroponic culture containing 200 µg Ni solution had decreased photosynthetic rate ($1.5 \mu\text{mol m}^{-2}\text{s}^{-1}$); Ni concentration in leaves was $2.81 \mu\text{mol g}^{-1}$ for matured leaves (Velikova et al. 2011). *Picea abies* seedlings planted in serpentine nurseries contained $0.79 \mu\text{mol g}^{-1}$ Ni in their leaves, and their photosynthetic rate was greatly reduced to $1.0 \mu\text{mol m}^{-2}\text{s}^{-1}$ (Kayama 2006). Ni concentration in leaves of *B. ermanii* and *B. platyphylla* was high (4.14 and $3.16 \mu\text{mol g}^{-1}$ at 15th month) in our observations. Thus, Ni concentration of the two birch species could decrease the photosynthetic rates, and probably to inhibit the activity of various enzymes.

Moreover, the two birch species grown in serpentine soil showed low Ca concentration (Tables 5 and 6), and the values of Ca/Mg ratio in their plant organs ranged from 0.055 to 0.112. These values were quite low as compared with data of other plants grown in serpentine soil (Brooks 1987; Alexander et al. 2007). Thus, both species were suffering from

direct accumulation of Mg. Besides, uptakes of P, K and Ca were suppressed by accumulations of Mg, Ni, Cr and Co (except for leaves in *B. platyphylla*; Table 9). Uptakes of these toxic elements probably caused deficiencies of P, K and Ca. Other researchers reported that accumulations of Mg and Ni suppressed uptakes of P, K and Ca (Pandolfini et al. 1992; Baccouch et al. 1998; Millar and Cumming 2000; Kayama et al. 2005; Kobayashi et al. 2005; Ding et al. 2006), and we can suggest similar trends for the present species. However, uptake of N was not suppressed by accumulation of Mg, Ni, Cr and Co (Table 9). Previous evidence shows that accumulation of Ni and Cr(III) suppressed uptakes of N (Miller and Cumming 2000; Kayama et al. 2005; Singh et al. 2013). Our results disagree with the latter, but an explanation could be that the two birch species may have a trait that makes uptake of N hard to interfere by accumulation of toxic elements.

NAR of *B. ermanii* was higher than that of *B. platyphylla* (Table 3) from 12th month to 15th month, as compared between the two birches grown in serpentine soil. *B. ermanii* showed high productivity per unit leaf area in this period. Similarly, LAR at 12th month had higher value in *B. ermanii* compared with that in *B. platyphylla* (Table 3). Hence, growth of *B. ermanii* at 12th month was probably allocated to leaves. In general, leaf extension in early growing season was affected by stored nitrogen in plant organs (Millard 1996; Neilsen et al.

1997). N concentration in leaves and roots at 12th month was not low in *B. ermanii* grown in serpentine soil (Table 5, 6); stored N was probably sufficient even in serpentine soil, resulting in ability of *B. ermanii* to extend leaves early in growing season. Moreover, growth and photosynthetic productivity of *B. ermanii* are projected in early growing season (Koike 1995). High value of LAR of *B. ermanii* in June is probably reflected by its inherent growth characteristics and stored N. Also, *B. ermanii* grown in serpentine soil could maintain almost the same value of P_{sat} from June to September (Table 4). Therefore, *B. ermanii* probably attains large photosynthetic production in second year; as a result, NAR shows high value.

Effects of accumulation of toxic elements (Mg, Ni, Cr and Co) were small in RGR of *B. ermanii*, according to the regression model of RGR. The regression model of P_{sat} revealed that coefficient of Ni was smaller in *B. ermanii* than in *B. platyphylla*. Effect of Ni accumulation on the growth and photosynthetic rate was, therefore, smaller for *B. ermanii* than for *B. platyphylla*. The maximum concentrations of Ni and Cr in leaves of *B. ermanii* grown in serpentine soil were $6.8 \mu\text{mol g}^{-1}$ ($399 \mu\text{g g}^{-1}$) and $5.0 \mu\text{mol g}^{-1}$ ($260 \mu\text{g g}^{-1}$), respectively. In general, elements in leaves of plants not endemic to serpentine soil range between 1 and $100 \mu\text{g g}^{-1}$ for Ni, and $<70 \mu\text{g g}^{-1}$ for Cr (Brooks 1987; Alexander et al. 2007). The concentrations of Ni and Cr in leaves of *B. ermanii* are much greater than those reported

as general ranges, and *B. ermanii* has inherent characteristics for accumulations of these elements. The mechanisms of detoxification of Ni and Cr within leaves are: (1) binding to cell wall components, and (2) transport in vacuoles (Seregin and Kozhevnikova 2006; Liu et al. 2009). There is a possibility that leaves of *B. ermanii* may have mechanisms for detoxification of Ni and Cr. Meanwhile, suppressed uptake of P in *B. ermanii* by accumulation of Mg, Ni, Cr, and Co (Table 8), and RGR was regulated by P concentration in leaves and roots (Table 7). Thus, a trade-off for *B. ermanii* between accumulations of toxic elements and uptake of P may be happened.

As to the roots, there was less Ni at the 15th month in *B. ermanii* than in *B. platyphylla* (Table 6). Nickel uptake was suppressed higher in *B. ermanii* than in *B. platyphylla*. The characteristics of Ni uptake from roots differ between tolerant and non-tolerant species. According to Kayama et al. (2005), *P. glehnii*, a tolerant species against Ni, eliminated Ni uptake through the progress of growth, whereas the non-tolerant species *P. abies* did not eliminate Ni uptake. *B. ermanii* appears to behave similarly to *P. glehnii*, and may have the capability to exclude Ni. Symbiosis with ectomycorrhizal fungi is significant in excluding toxic metals such as Ni (Jones and Hutchinson, 1988b; Gabbrielli *et al.*, 1990; Kayama et al. 2005). Ni concentrations in leaves of matured trees of *B. ermanii* and *B. platyphylla* grown

in serpentine soil showed different values ($0.09 \mu\text{mol g}^{-1}$ for *B. ermanii* and $0.76 \mu\text{mol g}^{-1}$ for *B. platyphylla*; reported by Blandon et al. 1994). It is possible that roots of *B. ermanii* take part in enhanced ectomycorrhizal symbiosis, and uptake of Ni is suppressed during progress of growth.

Compared with *B. ermanii*, *B. platyphylla* showed high value of LAR at 15th month (Table 3). However, NAR was lower for *B. platyphylla* than for *B. ermanii* (Table 3) from 12th to 15th month. Number of late leaves of *B. platyphylla* developed from July was higher than that of *B. ermanii*. This can be explained by higher growth rate and photosynthetic productivity of *B. platyphylla* at late growing season (Koike 1995). However, leaf dry mass of *B. platyphylla* grown in serpentine soil was not increased from 12th to 15th month (Table 1). So, *B. platyphylla* cannot produce late leaves, and its growth characteristics cannot contribute for the acceleration of productivity under the serpentine condition.

B. platyphylla grown in serpentine soil showed decreased P_{sat} and g_s at late growing season (Table 4). Decrease of stomatal conductance is concerned with stomatal closure; as a result, photosynthetic rate probably decreased at late growing season. To examine if an element induces stomatal closure, we compared concentration of elements in leaves of *B. platyphylla* grown in serpentine soil between 12th and 15th months (Table 5). Phosphorus

concentration was significantly decreased, and Mg concentration was significantly increased at 15th month ($P < 0.01$, t-test). There was no significant relationship for *B. platyphylla* between P concentration and P_{sat} (Table 8). Accumulation of Mg does not directly decrease the photosynthetic rates (Rao et al. 1987; Ding et al. 2006).

However, there is a possibility a plant grown in non-serpentine habitat having sensitivity for accumulation of Mg, so, photosynthetic rates and growth to decrease by Mg accumulation (Palm et al. 2012). Accumulation of Mg induces the decrease of leaf water potential, which may result to decreased photosynthetic rate (Rao et al. 1987). Given this, *B. platyphylla* grown in serpentine soil is probably sensitive in accumulation of Mg, and may suffer from water deficit; this resulted to closed stomata and decreased photosynthetic rate in late growing period. Accumulation of Mg in leaves also decrease RGR of *B. platyphylla*. There is a possibility that water deficit caused by accumulation of Mg may affect decrease of growth of *B. platyphylla*.

Accumulation of Co in roots decreased RGR drastically for *B. platyphylla* (Table 7). Compared with previous studies, concentration of $200 \mu\text{g g}^{-1}$ Co ($3.4 \mu\text{mol g}^{-1}$) in roots of *Brassica campestris* suppressed growth (Sinha et al. 2012). Concentration of Co in roots of *B. platyphylla* was much lower than those of previous data (Table 6). Based on our results,

there is a possibility that slight accumulation of Co in roots may suppress the tree growth, and *B. platyphylla* may be sensitive to Co. As to the photosynthetic rate of *B. platyphylla*, K concentration was also concerned with the model of P_{sat} (Table 8). Potassium deficiency induces a decrease in photosynthetic rate (Baillon et al. 1988; Pettigrew 2008; Kanai et al. 2011). *B. platyphylla* is rather easy to decrease photosynthetic rate by deficiency of K compared with *B. ermanii*.

Conclusion

When seedlings of *B. ermanii* and *B. platyphylla* were planted in serpentine soil, their growths were suppressed due to decreased photosynthetic rates. The photosynthetic rates were decreased by accumulation of Ni in leaves. Uptakes of nutrients, such as K and Ca were also suppressed by accumulation of Mg, Ni, Cr and Co. *B. ermanii* tended to accumulate large amount of Ni in leaves, so, the effect on the photosynthetic rates was less than the effect on those of *B. platyphylla*. In contrast, accumulation of Mg in leaves and of Co in roots of *B. platyphylla* led to suppressed growth. *B. ermanii* planted in serpentine soil showed high leaf area ratio in early growth season and maintained photosynthetic rate from June to

September. These traits of *B. ermanii* are relevant to high net assimilation rate in second year.

The above characteristics of *B. ermanii* are considered as high advantages to regenerate and grow in serpentine soil. We conclude that *B. ermanii* had a tolerance to serpentine soil, but to enhance its growth in serpentine soil, adequate uptake of nutrients -especially P- is needed.

Author Contribution Statement

M.K. and T.K. designed the experiments and grew seedlings of birch species. M.K. conducted the experiments, measured the photosynthetic rates, and analyzed the various nutrients. Both analyzed the data, discussed the results, and co-authored the paper.

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Conflict of Interest

The source support for this study is a non-profit organization (Japan Society for the Promotion of Science, and Japan Science Society). We declare that our research has no conflict of interest.

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Table 1. Chemical properties of the serpentine and non-serpentine (brown forest) soils, sampled at depth 0-10 cm (Mean $\pm$ SD, n=16). Data are quoted from Kayama (2006) except for concentration of Co. Asterisks indicate significant effects according to t-test. $*$ = P <0.05, $**$ = P <0.01, $***$ = P <0.001.

	pH	C (g kg ⁻¹)	N (g kg ⁻¹)	P (mg 100g ⁻¹)	Ca (mg 100g ⁻¹)
Serpentine	6.73 $\pm$ 0.09	27.4 $\pm$ 5.1	1.85 $\pm$ 0.25	1.11 $\pm$ 0.12	25.5 $\pm$ 3.1
Brown forest	5.18 $\pm$ 0.06	35.3 $\pm$ 9.3	2.43 $\pm$ 0.39	0.97 $\pm$ 0.34	32.2 $\pm$ 7.9
Statistical test	***	n.s.	*	n.s.	n.s.
	Mg (mg 100g ⁻¹)	K (mg 100g ⁻¹)	Na (mg 100g ⁻¹)	Mn (mg 100g ⁻¹)	Fe (mg 100g ⁻¹)
Serpentine	135 $\pm$ 35	6.44 $\pm$ 0.89	7.25 $\pm$ 1.06	0.89 $\pm$ 0.04	0.06 $\pm$ 0.03
Brown forest	7 $\pm$ 2	5.24 $\pm$ 1.03	4.89 $\pm$ 0.44	1.33 $\pm$ 0.01	4.68 $\pm$ 2.94
Statistical test	***	n.s.	**	n.s.	*
	Cu (mg 100g ⁻¹)	Zn (mg 100g ⁻¹)	Ni (mg 100g ⁻¹)	Cr (mg 100g ⁻¹)	Co (mg 100g ⁻¹)
Serpentine	0.051 $\pm$ 0.015	0.350 $\pm$ 0.041	8.61 $\pm$ 1.52	0.342 $\pm$ 0.035	1.14 $\pm$ 0.12
Brown forest	0.070 $\pm$ 0.021	0.163 $\pm$ 0.018	0.11 $\pm$ 0.04	0.137 $\pm$ 0.016	0.05 $\pm$ 0.01
Statistical test	n.s.	***	***	***	***

Table 2. Dry mass of each organ (leaf, stem and branch, and root) and total dry mass for seedlings of two birch species planted in serpentine and non-serpentine (brown forest) soils (Mean $\pm$ SE, n=8). Asterisks indicate significant effects of two-factor ANOVA. $*$ = P <0.05, $**$ = P <0.01, $***$ = P <0.001.

Organs Months	<i>B. ermanii</i>		<i>B. platyphylla</i>		Statistical test		
Leaf (g)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.04 $\pm$ 0.01		0.04 $\pm$ 0.01		—	n.s.	—
12	0.43 $\pm$ 0.06	1.98 $\pm$ 0.46	0.37 $\pm$ 0.05	1.95 $\pm$ 0.18	***	n.s.	n.s.
15	0.43 $\pm$ 0.07	4.57 $\pm$ 1.17	0.57 $\pm$ 0.10	9.29 $\pm$ 1.79	***	*	*
Stem and branch (g)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.24 $\pm$ 0.02		0.36 $\pm$ 0.03		—	**	—
12	0.66 $\pm$ 0.04	2.97 $\pm$ 0.65	0.84 $\pm$ 0.08	2.47 $\pm$ 0.28	***	n.s.	n.s.
15	2.1 $\pm$ 0.1	17.9 $\pm$ 3.3	2.0 $\pm$ 0.2	22.6 $\pm$ 4.4	***	n.s.	n.s.
Root (g)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.32 $\pm$ 0.04		0.38 $\pm$ 0.07		—	n.s.	—
12	0.72 $\pm$ 0.08	2.79 $\pm$ 0.55	1.01 $\pm$ 0.11	2.45 $\pm$ 0.34	***	n.s.	n.s.
15	1.5 $\pm$ 0.2	11.9 $\pm$ 1.9	1.6 $\pm$ 0.1	14.3 $\pm$ 2.3	***	n.s.	n.s.
Total (g)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.60 $\pm$ 0.06		0.78 $\pm$ 0.09		—	n.s.	—
12	1.80 $\pm$ 0.17	7.74 $\pm$ 1.59	2.31 $\pm$ 0.24	6.87 $\pm$ 0.78	***	n.s.	n.s.
15	4.0 $\pm$ 0.3	34.3 $\pm$ 5.8	4.2 $\pm$ 0.3	46.2 $\pm$ 8.3	***	n.s.	n.s.

Note: “S” indicates serpentine soil, and “B” indicates brown forest soil. Values for 0 month refer to data immediately before transplanting. “So $\times$ Sp” in the Statistical test refers to interaction between soil type and birch species.

Table 3. Relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) for seedlings of two birch species planted in serpentine and non-serpentine (brown forest) soils (Mean $\pm$ SE, n=8). Asterisks indicate significant effects by two-factor ANOVA.

*= $P<0.05$, **= $P<0.01$, ***= $P<0.001$.

Parameters Months	<i>B. ermanii</i>		<i>B. platyphylla</i>		Statistical test		
RGR ($\text{mg g}^{-1} \text{ day}^{-1}$)	S	B	S	B	Soil	Species	So $\times$ Sp
0-12	3.0 $\pm$ 0.4	6.6 $\pm$ 0.2	2.9 $\pm$ 0.3	5.8 $\pm$ 0.2	***	n.s.	n.s.
12-15	7.8 $\pm$ 0.5	13.6 $\pm$ 1.6	5.7 $\pm$ 3.4	17.4 $\pm$ 2.7	***	n.s.	n.s.
NAR ($\text{g m}^{-2} \text{ day}^{-1}$)	S	B	S	B	Soil	Species	So $\times$ Sp
0-12	0.94 $\pm$ 0.20	1.95 $\pm$ 0.03	1.28 $\pm$ 0.15	1.48 $\pm$ 0.10	***	n.s.	**
12-15	6.09 $\pm$ 0.15	9.11 $\pm$ 1.45	4.20 $\pm$ 0.15	7.06 $\pm$ 1.12	**	*	n.s.
LAR ($\text{cm}^2 \text{ g}^{-1}$)	S	B	S	B	Soil	Species	So $\times$ Sp
12	32.8 $\pm$ 2.0	34.5 $\pm$ 0.9	22.0 $\pm$ 0.6	40.2 $\pm$ 2.1	***	n.s.	***
15	12.8 $\pm$ 1.1	15.0 $\pm$ 0.6	17.6 $\pm$ 2.8	25.5 $\pm$ 1.1	*	***	n.s.

Note: RGR and NAR were calculated by total dry masses in 0 and 12th months or 12th and 15th months, respectively. “S” indicates serpentine soil, and “B” indicates brown forest soil.

“So $\times$ Sp” in the Statistical test refers to interaction between soil type and birch species.

Table 4. Photosynthetic rate at light saturation (P_{sat}) and stomatal conductance (gs) for seedlings of two birch species planted in serpentine and non-serpentine (brown forest) soils in early and late growth periods (Mean $\pm$ SE, n=8). Asterisks indicate significant effects of two-factor ANOVA. **= $P<0.01$, ***= $P<0.001$.

Seasons	<i>B. ermanii</i>		<i>B. platyphylla</i>		Statistical test		
P_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	S	B	S	B	Soil	Species	So $\times$ Sp
Early	6.5 $\pm$ 0.3	10.2 $\pm$ 0.1	6.9 $\pm$ 0.4	10.1 $\pm$ 0.2	***	n.s.	n.s.
Late	6.6 $\pm$ 0.3	9.4 $\pm$ 0.2	5.4 $\pm$ 0.1	9.5 $\pm$ 0.1	***	**	**
gs ($\text{mol m}^{-2} \text{s}^{-1}$)	S	B	S	B	Soil	Species	So $\times$ Sp
Early	0.73 $\pm$ 0.11	0.64 $\pm$ 0.16	0.57 $\pm$ 0.07	0.72 $\pm$ 0.11	n.s.	n.s.	n.s.
Late	0.45 $\pm$ 0.08	0.95 $\pm$ 0.09	0.17 $\pm$ 0.02	0.83 $\pm$ 0.11	***	*	n.s.

Note: Season of “Early” indicates measurement in May 2000, and “Late” indicates measurement in September 2000. “S” indicates serpentine soil, and “B” indicates brown forest soil. “So $\times$ Sp” in the Statistical test refers to interaction between soil type and birch species. Data of gs are the values when P_{sat} are measured.

Table 5. Concentrations of elements (N, P, K, Ca, Mg, Ni, Cr and Co) in leaves of seedlings of two birch species planted in serpentine and non-serpentine (brown forest) soils (Mean $\pm$ SE, n=8). Asterisks indicate significant effects of two-factor ANOVA. *= P <0.05, **= P <0.01, ***= P <0.001.

Elements Months	<i>B. ermanii</i>		<i>B. platyphylla</i>		Statistical test		
N ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	2570 $\pm$ 139		2564 $\pm$ 98		—	n.s.	—
12	1261 $\pm$ 56	1426 $\pm$ 93	1357 $\pm$ 74	1487 $\pm$ 60	n.s.	n.s.	n.s.
15	1058 $\pm$ 43	1141 $\pm$ 63	1299 $\pm$ 84	1138 $\pm$ 52	n.s.	n.s.	n.s.
P ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	343 $\pm$ 52		233 $\pm$ 44		—	n.s.	—
12	179 $\pm$ 9	316 $\pm$ 36	284 $\pm$ 31	243 $\pm$ 37	n.s.	n.s.	**
25	65 $\pm$ 6	198 $\pm$ 34	143 $\pm$ 16	233 $\pm$ 45	***	n.s.	n.s.
K ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	648 $\pm$ 41		476 $\pm$ 20		—	**	—
12	175 $\pm$ 13	330 $\pm$ 17	151 $\pm$ 7	261 $\pm$ 15	***	n.s.	n.s.
15	89 $\pm$ 7	269 $\pm$ 26	135 $\pm$ 4	241 $\pm$ 12	***	n.s.	*
Ca ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	57.1 $\pm$ 4.4		55.0 $\pm$ 5.1		—	n.s.	—
12	21.5 $\pm$ 0.8	43.5 $\pm$ 2.4	22.6 $\pm$ 0.7	36.8 $\pm$ 2.0	***	n.s.	*
15	39.2 $\pm$ 1.9	81.5 $\pm$ 4.1	31.5 $\pm$ 1.3	62.6 $\pm$ 3.7	***	***	n.s.
Mg ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	120 $\pm$ 6		113 $\pm$ 4		—	n.s.	—
12	404 $\pm$ 30	109 $\pm$ 4	304 $\pm$ 18	102 $\pm$ 3	***	**	*
15	528 $\pm$ 29	115 $\pm$ 7	369 $\pm$ 12	117 $\pm$ 4	***	***	***
Ni ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.84 $\pm$ 0.18		0.38 $\pm$ 0.21		—	n.s.	—
12	3.14 $\pm$ 0.18	0.62 $\pm$ 0.08	2.70 $\pm$ 0.31	0.51 $\pm$ 0.09	***	n.s.	n.s.
15	4.14 $\pm$ 0.50	0.17 $\pm$ 0.09	3.16 $\pm$ 0.23	0.33 $\pm$ 0.07	***	n.s.	n.s.
Cr ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.21 $\pm$ 0.12		0.13 $\pm$ 0.03		—	n.s.	—
12	1.33 $\pm$ 0.36	0.22 $\pm$ 0.04	0.70 $\pm$ 0.14	0.25 $\pm$ 0.11	***	n.s.	n.s.
15	2.01 $\pm$ 0.73	0.15 $\pm$ 0.04	0.82 $\pm$ 0.17	0.13 $\pm$ 0.04	***	n.s.	n.s.
Co ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	N.D.		N.D.		—	—	—
12	0.28 $\pm$ 0.07	N.D.	0.35 $\pm$ 0.07	N.D.	—	n.s.	—
15	0.37 $\pm$ 0.15	N.D.	0.45 $\pm$ 0.08	N.D.	—	n.s.	—

Note. “S” indicates serpentine soil, and “B” indicates brown forest soil. Values for 0 month refer to data immediately before transplanting (so that soil comparison is inapplicable). “So $\times$ Sp” in Statistical test refers to interaction between soil type and birch species. “N.D.” in concentration of Co means not detected. For the concentration of Co, we could not apply statistical tests in several parameters because data are missing.

Table 6. Concentrations of elements (N, P, K, Ca, Mg, Ni, Cr and Co) in seedlings' roots of two birch species planted in serpentine and non-serpentine (brown forest) soils (Mean $\pm$ SE, n=8). Asterisks indicate significant effects by two-factor ANOVA. *= P <0.05, **= P <0.01, ***= P <0.001.

Elements Months	<i>B. ermanii</i>		<i>B. platyphylla</i>		Statistical test		
N ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	611 $\pm$ 38		733 $\pm$ 90		—	n.s.	—
12	567 $\pm$ 23	565 $\pm$ 73	604 $\pm$ 43	658 $\pm$ 56	n.s.	n.s.	n.s.
15	704 $\pm$ 19	623 $\pm$ 90	650 $\pm$ 30	583 $\pm$ 63	n.s.	n.s.	n.s.
P ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	487 $\pm$ 28		502 $\pm$ 55		—	n.s.	—
12	92 $\pm$ 10	182 $\pm$ 37	96 $\pm$ 11	152 $\pm$ 31	**	n.s.	n.s.
25	88 $\pm$ 3	175 $\pm$ 31	99 $\pm$ 8	222 $\pm$ 37	***	n.s.	n.s.
K ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	78.5 $\pm$ 6.2		82.4 $\pm$ 8.0		—	n.s.	—
12	48.5 $\pm$ 3.0	71.4 $\pm$ 7.3	57.5 $\pm$ 3.5	69.8 $\pm$ 5.6	**	n.s.	n.s.
15	56.5 $\pm$ 3.7	83.1 $\pm$ 5.1	63.2 $\pm$ 3.0	73.2 $\pm$ 4.1	***	n.s.	n.s.
Ca ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	33.0 $\pm$ 2.3		35.8 $\pm$ 4.5		—	n.s.	—
12	17.2 $\pm$ 0.6	24.5 $\pm$ 1.1	18.6 $\pm$ 1.2	23.6 $\pm$ 2.7	***	n.s.	n.s.
15	16.3 $\pm$ 0.3	25.5 $\pm$ 2.7	17.4 $\pm$ 1.1	23.0 $\pm$ 2.7	***	n.s.	n.s.
Mg ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	71 $\pm$ 5		51 $\pm$ 7		—	*	—
12	273 $\pm$ 15	37 $\pm$ 3	284 $\pm$ 11	39 $\pm$ 2	***	n.s.	n.s.
15	162 $\pm$ 11	36 $\pm$ 2	158 $\pm$ 11	32 $\pm$ 2	***	n.s.	n.s.
Ni ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.30 $\pm$ 0.06		0.17 $\pm$ 0.07		—	n.s.	—
12	7.07 $\pm$ 0.37	0.16 $\pm$ 0.09	7.34 $\pm$ 0.82	0.22 $\pm$ 0.08	***	n.s.	n.s.
15	4.11 $\pm$ 0.31	0.21 $\pm$ 0.05	5.33 $\pm$ 0.34	0.18 $\pm$ 0.06	***	*	*
Cr ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.08 $\pm$ 0.05		0.01 $\pm$ 0.01		—	n.s.	—
12	1.81 $\pm$ 0.37	0.62 $\pm$ 0.21	1.91 $\pm$ 0.24	0.53 $\pm$ 0.12	***	n.s.	n.s.
15	1.14 $\pm$ 0.30	0.15 $\pm$ 0.02	0.97 $\pm$ 0.16	0.17 $\pm$ 0.04	***	n.s.	n.s.
Co ($\mu\text{mol g}^{-1}$ DM)	S	B	S	B	Soil	Species	So $\times$ Sp
0	0.01 $\pm$ 0.01		<0.01		—	n.s.	—
12	1.11 $\pm$ 0.23	0.10 $\pm$ 0.04	0.99 $\pm$ 0.13	0.19 $\pm$ 0.04	***	n.s.	n.s.
15	0.52 $\pm$ 0.20	0.03 $\pm$ 0.01	0.42 $\pm$ 0.06	0.07 $\pm$ 0.02	***	n.s.	n.s.

Note. “S” refers to serpentine soil, and “B” refers to brown forest soil. Values for 0 month refer to data before transplanting (so that soil comparison is inapplicable). “So $\times$ Sp” in Statistical test refers to interaction between soil type and birch species.

Table 7. Pearson's correlation coefficients (r) between RGR (dependent variable) and concentrations of elements in leaves or roots (independent variable) of two birch species (n=16). *= P <0.05, **= P <0.01, ***= P <0.001.

Elements	Leaves		Roots	
	<i>B. ermanii</i>	<i>B. platyphylla</i>	<i>B. ermanii</i>	<i>B. platyphylla</i>
N	-0.35	-0.42	-0.69*	-0.58*
P	0.57*	0.57*	0.72**	0.67**
K	0.54*	0.64**	0.70**	0.38
Ca	0.55*	0.72**	0.06	0.18
Mg	-0.53*	-0.77***	-0.57*	-0.75***
Ni	-0.53*	-0.73**	-0.59*	-0.75**
Cr	-0.36	-0.50*	-0.50*	-0.66**
Co	-0.39	-0.61*	-0.51*	-0.66**

Table 8. Pearson's correlation coefficients (r) between area-based photosynthetic rate at light saturation (P_{sat}) (dependent variable) and area-based concentrations of N, P, K, Ni, Cr and Co in leaves (independent variable) of two birch species (n=32). **= $P<0.01$, ***= $P<0.001$.

Elements	<i>B. ermanii</i>	<i>B. platyphylla</i>
N	0.48**	0.10
P	0.59***	0.23
K	0.80***	0.84***
Ni	-0.88***	-0.94***
Cr	-0.65***	-0.64***
Co	-0.66***	-0.79***

Table 9. Pearson's correlation coefficients (r) of elements analyzed in the leaves and roots of two birch species ($n=32$). Asterisks indicate significant correlation between two elements.

*= $P<0.05$, **= $P<0.01$, ***= $P<0.001$.

	N	P	K	Ca	Mg	Ni	Cr
Leaves of <i>B. ermanii</i>							
P	0.36*						
K	0.48*	0.82***					
Ca	-0.16	0.15	0.35*				
Mg	0.48**	-0.64***	-0.86***	-0.56***			
Ni	-0.29	-0.56***	-0.78***	-0.58***	0.88***		
Cr	-0.22	-0.37*	-0.54**	-0.30	0.65***	0.78***	
Co	-0.21	-0.35*	-0.56**	-0.31	0.65***	0.80***	0.77***
Leaves of <i>B. platyphylla</i>							
P	0.25						
K	0.05	0.24					
Ca	-0.28	0.09	0.53**				
Mg	0.09	-0.15	-0.88***	-0.58***			
Ni	0.06	-0.09	-0.83***	-0.63***	0.89***		
Cr	0.25	-0.03	-0.61***	-0.48**	0.72***	0.68***	
Co	0.14	-0.05	-0.77***	-0.54**	0.86***	0.80***	0.68***
Roots of <i>B. ermanii</i>							
P	-0.65***						
K	-0.43*	0.72***					
Ca	0.38*	0.04	0.37*				
Mg	0.01	-0.51**	-0.67***	-0.61***			
Ni	0.01	-0.50**	-0.64***	-0.62***	0.96***		
Cr	0.13	-0.49**	-0.45*	-0.36*	0.63***	0.65***	
Co	0.03	-0.39*	-0.39*	-0.40*	0.69***	0.72***	0.64***
Roots of <i>B. platyphylla</i>							
P	-0.50**						
K	-0.38*	0.64***					
Ca	0.51**	-0.25	0.31				
Mg	0.04	-0.47**	-0.50**	-0.31			
Ni	0.09	-0.46**	-0.48**	-0.40*	0.92***		
Cr	0.09	-0.46**	-0.56***	-0.12	0.86***	0.70***	
Co	0.07	-0.43*	-0.53**	-0.18	0.88***	0.72***	0.70***

Figure legends

Fig. 1. Light-photosynthetic curves of two birch-species' seedlings planted in serpentine and brown forest soils, in late growing season (September 2000, Mean $\pm$ SE, n=8).

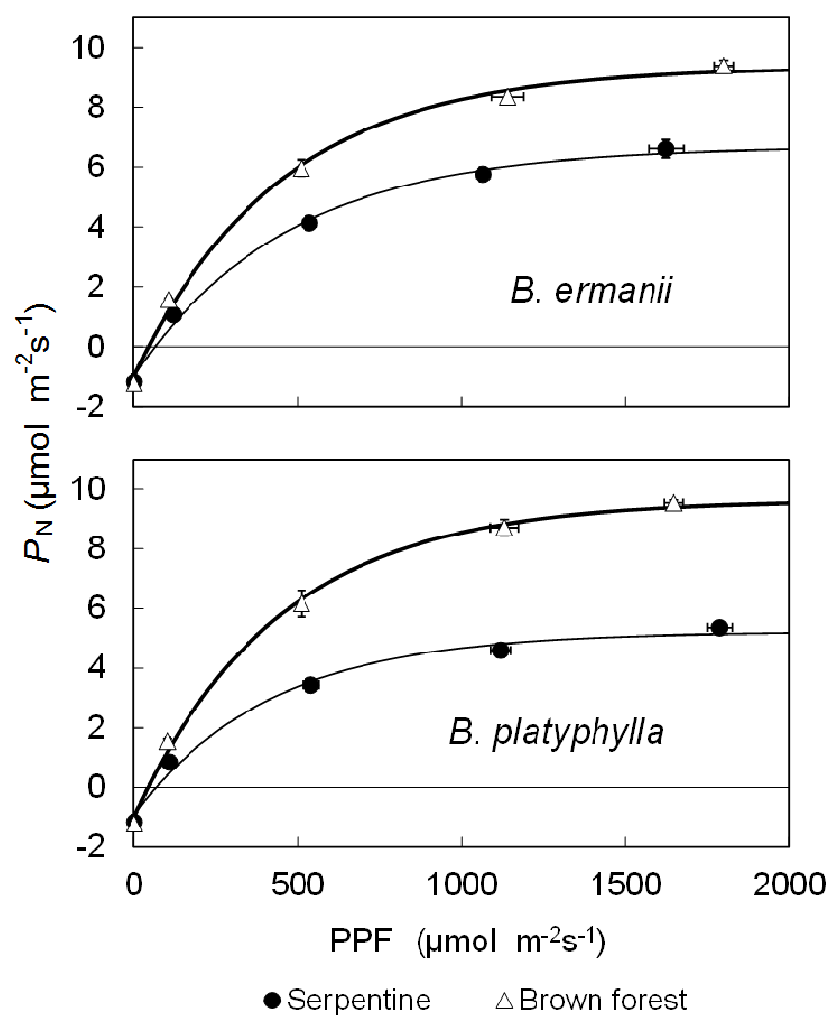


Fig. 1.