

# Photosynthetic rate, needle longevity, and nutrient contents in *Picea glehnii* growing on strongly acidic volcanic ash soil in northern Japan

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## Abstract

*Picea glehnii* Masters can grow in strongly acidic volcanic ash soil (pH 3.6) in northern Japan. We compared needle longevity, photosynthetic rate, and concentrations of elements in needles, in mature trees of *P. glehnii* growing in volcanic ash soil and in brown forest soil (pH 5.4). *P. glehnii* growing in volcanic ash soil showed suppressed photosynthetic rate and growth by the deficiency in nitrogen compared with its growth in brown forest soil. However, the younger needles of *P. glehnii* growing in volcanic ash soil maintained a high photosynthetic rate, as a result of large amounts of remobilized nitrogen from senesced needles. Needles of *P. glehnii* growing in volcanic ash soil did not show deficiencies in Ca, Mg, or K. Moreover, Al was at low levels in the needles, suggesting that *P. glehnii* was able to avoid Al toxicity by Al exclusion. *P. glehnii* thus exhibits great ability to adapt to an acidic environment.

*Additional key words:* needle longevity; nitrogen retranslocation; nutrient physiology; photosynthetic rate; solfataras; spruce.

## Introduction

Sakhalin spruce (*Picea glehnii*) can distribute throughout harsh environments, including swamps, serpentine regions, volcanic sands, and sand dunes (Tatewaki 1958, Matsuda 1989). *P. glehnii* appears to have great ability to adapt to different edaphic conditions; soil pH ranging from 3.6 to 7.4 (Nakata and Kojima 1987, Kayama 2004). Our previous research has mainly focused on the ecophysiology of *P. glehnii* in the high pH soil of serpentine regions, for reforestation purposes (Kayama *et al.* 2002, 2009). However, there is still little information available about the adaptive mechanisms of *P. glehnii* growing in these hot spring areas in strongly acidic soils (Tatewaki 1958, Kayama 2004). In our previous studies of *P. glehnii* planted in serpentine and volcanogenic soils, *P. glehnii* was observed to increase needle longevity to compensate for low photosynthetic productivity (Kayama *et al.* 2002, 2007). In areas around the hot springs, termed 'solfataras', the soil has a paucity of nutrients (Kayama

*et al.* 2000, Gross *et al.* 2002, Yoshitake *et al.* 2007). We have investigated the compensative abilities of *P. glehnii* in very infertile soil, with low pH.

In addition, soils around solfataras contain high levels of aluminum (Al) (Kayama *et al.* 2000, Hall *et al.* 2003, Watanabe *et al.* 2010). This is released from soil when pH falls below 5.5 (Marschner 1995, Kochian *et al.* 2004). Photosynthetic rate and chlorophyll (Chl) levels decrease when Al is transported to leaves (Ridolfi and Garrec 2000, Izuta *et al.* 2004, Chen *et al.* 2005). Some plants grown in high-Al environments have exclusion or internal detoxification mechanisms (Barceló and Poschenrieder 2002, Kochian *et al.* 2004). In the genus *Picea*, Al concentration in needles has been reported as 312  $\mu\text{g g}^{-1}$  in *P. jezoensis* var. *hondoensis*; this is considerably lower than in most woody plants ( $> 10,000 \mu\text{g g}^{-1}$ ) (Takada *et al.* 1994). Thus, we expected that *P. glehnii* would show low concentrations

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*Abbreviations:* ANCOVA – analysis of covariance; Chl – chlorophyll;  $g_s$  – stomatal conductance;  $P_N$  – net photosynthetic rate;  $P_{Nsat}$  – maximum photosynthetic rate at light saturation; PPF – photosynthetic photon flux;  $R$  – respiration rate; SLA – specific leaf area;  $\alpha$  – initial gradient of the curve.

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of Al within tissues, and would exclude Al from plant organs. We predicted that *P. glehnii* growing in an acidic environment could increase needle longevity and exhibit a high adaptability to infertile conditions.

To test these predictions, we compared the ecophysiological traits of *P. glehnii* at two sites: the volcanic ash

## Materials and methods

**Study sites** were located in the town of Teshikaga (43°37'N, 144°27'E, 150 m a.s.l.), on the eastern part of Hokkaido Island, Japan. Mean annual precipitation at Kawayu Weather Station, near the study sites, is approximately 856 mm  $y^{-1}$ . Mean annual temperature is 4.3°C, the maximum is 31.0°C, and the minimum -28.3°C (Japan Meteorological Agency 2010). The Akan National Park contains active volcanoes, and Mt. Atosanupuri has erupted from several solfataras resulting in acid deposition in nearby areas. Around the solfataras, the vegetation is dominated by *Ledum palustre* L. var. *yesoense* Nakai, *Pinus pumila* (Pall.) Regel, and *Empetrum nigrum* L. (Igarashi 1986). *P. glehnii* grows in the areas surrounding this vegetation, and white volcanic ash accumulates under *P. glehnii*. We selected a study site where several *P. glehnii* trees were growing in close proximity; this volcanic ash soil site was approximately 1.5 km from the solfataras.

We also selected two sites where *P. glehnii* trees were growing in brown forest soil (pH 5.4, Kayama *et al.* 2011). One was in the public forest of Teshikaga; the other was in the National Forest. The distance between two sites was 4 km, and these were approximately 6 km from our volcanic ash soil site. Two brown forest soil sites lay behind the solfataras on Mt. Atosanupuri, and there was little evidence of damage caused by acid deposition.

**Soil chemical properties** of the topsoil (top 10 cm) of brown forest and volcanic ash soils have been well documented (Kayama and Koike 2011). The pH of volcanic ash soil (3.6) is significantly lower than that of brown forest soil (pH 5.4,  $P < 0.001$ ). N, Ca, Mg, K, and Mn concentrations are significantly higher in brown forest soil [N, 350; Ca, 153; Mg, 12.4; K, 5.78; and Mn, 2.77 mg (100 g) $^{-1}$ ] than in volcanic ash soil [N, 93; Ca, 7; Mg, 1.7; K, 2.98; and Mn, 0.10 mg (100 g) $^{-1}$ ,  $P < 0.05$ ]. In contrast, concentrations of S, Al, and Fe were significantly higher in volcanic ash soil [S, 308; Al, 34.5; and Fe, 17.2 mg (100 g) $^{-1}$ ] than in brown forest soil [S, 68; Al, 6.5; and Fe, 4.2 mg (100 g) $^{-1}$ ,  $P < 0.05$ ]. P concentration was not significantly different between brown forest [6.16 mg (100 g) $^{-1}$ ] and volcanic ash [7.17 mg (100 g) $^{-1}$ ] soils.

**Needle longevity and shoot growth:** To compare growth characteristics of *P. glehnii* at the two sites, we selected three mature trees approximately 8–10 m high at each

soil site and the brown forest soil site. Moreover, to investigate the mechanisms by which *P. glehnii* adapts to severe conditions, we measured needle longevity, photosynthetic rate, and concentrations of elements in needles in various needle age classes.

To determine needle longevity, we measured survival of needles in current and even-age classes. In September 2000, three branches from three mature trees were sampled at each site. Branches at a height of 2.0–2.5 m were selected from sunny crowns of trees at the forest edge. The main branch was segregated into shoot ages, and three shoots of each age from each of three trees were dried for 4 days at 80°C. After drying, we counted numbers of needles and needle scars on each shoot. The survival of needles was calculated by dividing needle number by the total (Kayama *et al.* 2009). We measured shoot length for every age of main shoot, and calculated annual shoot length for 5 consecutive years.

**Photosynthetic rate:** In September 2000, we measured the mass-based photosynthetic rate of 2-year-old needles at a height of 2.0–2.5 m, in the sunny crowns of trees at the forest edge. We selected 2-year-old needles for analysis because it takes two years for needles to reach their maximum photosynthetic rate (Hom and Oechel 1983). The photosynthetic rate of needles was measured for three trees at each site. Measurements were made using a portable gas analyzer (*H4A, ADC-Analytical Development Company*, Hoddesdon, U.K.) under steady-state conditions (ambient temperature 20–25°C; ambient CO<sub>2</sub> concentration 35.5–36.0 Pa). The mean relative humidity at the time of measurements was 62.4%. Supplementary light was provided by a halogen lamp (Walz, Effeltrich, Germany). We changed the photosynthetic photon flux (PPF) from high to low one using shade cloth (*Krory*, Osaka, Japan). After measuring photosynthetic rate, the needle projected area was determined using an image scanner, and then used to calculate the net photosynthetic rate per unit area. From the photosynthetic data, light photosynthesis curves were defined as follows (Thornley 1976):

$$P_N = P_{N_{\text{sat}}} [1 - e^{-(\alpha I / P_{N_{\text{sat}}})}] - R,$$

where  $P_N$  is the net photosynthetic rate,  $P_{N_{\text{sat}}}$  is the maximum photosynthetic rate at light saturation,  $\alpha$  is the initial gradient of the curve,  $I$  is PPF, and  $R$  is the respiration rate at 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF.

We measured the mass-based net photosynthetic rate at light saturation ( $P_{N_{\text{sat}}}$ ) and stomatal conductance ( $g_s$ ) for current and even-aged needles, in three trees at each site (September 2000). For these analyses, we selected shoots in the sunny crowns of trees at the forest edge, at a height of 2.0–2.5 m. Measurements were made using a

portable gas analyzer under the same steady-state conditions (ambient temperature 20–25°C; ambient CO<sub>2</sub> concentration 35.5–36.0 Pa). The mean relative humidity at the time of measurements was 62.4%, and PPF was established at 1,700  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After measuring the  $P_{\text{Nsat}}$ , the projected needle area was determined using an image scanner, and calculated the net photosynthetic rate per unit area. The needle samples were dried at 80°C for 4 days. Needle dry mass was then weighed. We calculated specific leaf area (SLA, Koike 1988, Reich *et al.* 1992).

**Chl and elements concentrations in needles:** We determined the concentrations of pigments and elements (Chl, N, P, K, S, Ca, Mg, Al, Mn, and Fe) in current and even-aged green needles. Needles were divided into age classes for analyses, and were obtained from three branches from each of three trees at each site (September 2000). Chl was extracted from needles using dimethyl sulfoxide (DMSO). Absorbance of the solution was measured using a spectrophotometer (*Type 100-50, Hitachi Co.*, Tokyo, Japan), and concentrations of Chl *a* and *b* were calculated (Barnes *et al.* 1992).

The remaining needle samples were dried at 80°C for 4 days and then ground into a fine powder using a vibrating sample mill (*TI-100; Tester Industry Co.*,

## Results

**Survival of needles** decreased as needles aged for both soil types (Table 1,  $P < 0.001$ ); and thus showed no significant differences between soil types. However, there was significant interaction between soil type and needle age ( $P < 0.001$ ). Thus, the inclination of regression line between survival of needles and needle age was steeper for *P. glehnii* in volcanic ash soil than in brown forest soil. Aged needles were shed more quickly at volcanic ash soil sites. At both sites, almost all needles were shed by the time they were 10 years old.

Annual shoot length of *P. glehnii* was significantly longer in brown forest soils than in volcanic ash soils ( $6.9 \pm 0.2$  vs.  $3.5 \pm 0.2$  cm;  $P < 0.05$ ). A similar trend was observed for the mean number of needles per shoot ( $200 \pm 17$  vs.  $99 \pm 17$ ;  $P < 0.05$ ).

**$P_{\text{N}}$ ,  $g_{\text{s}}$ , and SLA:**  $P_{\text{N}}$  for 2-year-old needles of *P. glehnii* was saturated at approximately 1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF at the two types of soil sites (Fig. 1). The values of  $\alpha$  showed that the initial gradient of the curves was significantly steeper for *P. glehnii* in brown forest soil than that in volcanic ash soil ( $0.0084 \pm 0.0004$  vs.  $0.0064 \pm 0.0004$ ;  $P < 0.05$ ).  $P_{\text{Nsat}}$  was 3.34  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  in brown forest soil, and 2.64  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  in volcanic ash soil (Table 1). However,  $P_{\text{Nsat}}$  showed no significant difference between the two sites. Neither was  $R$  significantly different between the sites ( $0.557 \pm 0.037$  in brown forest vs.  $0.431 \pm 0.010$   $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  in volcanic ash soils).

Tokyo, Japan). We determined N and S levels using a CHNS/O analyzer (*PE 2400 series II, Perkin-Elmer*, Norwalk, CT, USA). To determine levels of the other elements (P, K, Ca, Mg, Al, Mn, and Fe), dried samples were digested using a microwave digestion system (*O-I Analytical, College Station, TX, USA*), and analyzed using an inductively coupled plasma (ICP) analyzer (*IRIS, Jarrel Ash, Franklin, MA, USA*).

**Statistical analysis:** Mean growth and photosynthetic values, and element concentrations of 2-year-old needles were analyzed by *Mann-Whitney U*-tests using *Stat View 5.0 (SAS Institute Inc., Cary, NC)*, and comparing values for brown forest and volcanic ash soils.

Moreover, needle survival values,  $P_{\text{Nsat}}$ , SLA, Chl, and element levels were analyzed using *ANCOVA*. In Table 1, we show the above-mentioned parameters as dependent variables, and needle age for covariate. We analyzed the effects of differences in soil type, needle age, and the interaction between soil type and needle age.

We used the *Mann-Whitney U*-test to compare our two plots of brown forest soil. Since there was no significant difference between these, we pooled mean values for growth and photosynthesis, and Chl and element levels in needles.

The  $P_{\text{Nsat}}$  of 2-year-old needles showed the highest value for both soil types (Table 1).  $P_{\text{Nsat}}$  significantly decreased with increasing needle age ( $P < 0.001$ ).  $P_{\text{Nsat}}$  was significantly higher in brown forest soil than in volcanic ash soil ( $P < 0.05$ ).  $g_{\text{s}}$  showed highest value for 2-year-old needles in two types of soils. SLA showed highest value for current needles in two types of soils.  $g_{\text{s}}$  and SLA significantly decreased with increasing needle age ( $P < 0.001$ ). However,  $g_{\text{s}}$  and SLA was not significantly different between the sites.

**Concentrations of Chl and elements in needles:** The total concentration of Chl (*a+b*) and S in needles of *P. glehnii* growing both soil types showed no significant variation corresponding to needle aging (Table 1). Needle Chl levels were not significantly different between the sites.

In both soil types, concentrations of N, P, and K in needles significantly decreased as needles aged (Table 1,  $P < 0.001$ ). Needle N concentration was significantly lower in volcanic ash soil than in brown forest soil ( $P < 0.05$ ). In contrast, needle K concentration was significantly higher in volcanic ash soil than in brown forest soil ( $P < 0.05$ ). P concentrations were not significantly different between the two soil types. In 2-year-old needles, N and P concentrations were not significantly different between the two soil types. In contrast, K concentration was significantly higher in volcanic ash soil than in brown forest soil ( $P < 0.05$ ).

Table 1. Survival of needles, photosynthetic rate at light saturation ( $P_{\text{Nsat}}$ ), stomatal conductance ( $g_s$ ), specific leaf area (SLA), and concentrations of chlorophyll (Chl) and elements for current and even-aged needles of *P. glehnii* growing on two types of soil mean  $\pm$  SE,  $n = 6$  for brown forest soil, and  $n = 3$  for volcanic ash soil). Effects of soil type, needle age, and interaction between soil type and needle age were analyzed using ANCOVA. Asterisks indicate significant effects: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; and n.s. – not significant. DM – dry mass; FM – fresh mass. Note: “Brown” and “Volcanic” mean “Brown forest soil” and “Volcanic ash soil”. “S  $\times$  A” means the interaction between soil type and age of needles.

		Age of needles (year)					Statistical test ( $F$ )		
		1	2	4	6	8	Soil	Age	S $\times$ A
Survival of needles [%]									
Brown		94.4 $\pm$ 1.3	91.6 $\pm$ 1.4	83.5 $\pm$ 1.9	81.8 $\pm$ 2.9	54.1 $\pm$ 3.1			
Volcanic		93.5 $\pm$ 1.7	91.0 $\pm$ 2.6	70.1 $\pm$ 2.6	69.1 $\pm$ 2.3	28.4 $\pm$ 6.9	1.0 <sup>n.s.</sup>	116 <sup>***</sup>	13 <sup>***</sup>
$P_{\text{Nsat}}$ [ $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ]									
Brown		2.81 $\pm$ 0.10	3.34 $\pm$ 0.25	2.66 $\pm$ 0.06	1.77 $\pm$ 0.19	1.14 $\pm$ 0.14			
Volcanic		2.39 $\pm$ 0.08	2.64 $\pm$ 0.35	1.38 $\pm$ 0.17	1.17 $\pm$ 0.18	0.58 $\pm$ 0.07	7.0*	112 <sup>***</sup>	<0.1 <sup>n.s.</sup>
$g_s$ [ $\text{mmol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$ ]									
Brown		41.5 $\pm$ 2.4	49.7 $\pm$ 3.4	37.5 $\pm$ 3.3	31.6 $\pm$ 1.9	26.3 $\pm$ 3.7			
Volcanic		40.8 $\pm$ 1.8	47.8 $\pm$ 0.2	37.0 $\pm$ 3.3	29.7 $\pm$ 1.3	25.5 $\pm$ 0.4	<0.1 <sup>n.s.</sup>	44 <sup>***</sup>	<0.1 <sup>n.s.</sup>
SLA [ $\text{cm}^2 \text{ g}^{-1}(\text{DM})$ ]									
Brown		51.6 $\pm$ 1.3	50.6 $\pm$ 2.0	47.7 $\pm$ 2.1	46.9 $\pm$ 2.2	45.4 $\pm$ 0.7			
Volcanic		53.0 $\pm$ 2.6	52.2 $\pm$ 1.1	48.6 $\pm$ 4.3	47.3 $\pm$ 1.9	46.4 $\pm$ 2.9	0.4 <sup>n.s.</sup>	234 <sup>***</sup>	<0.1 <sup>n.s.</sup>
Chl ( $a+b$ ) [ $\mu\text{mol g}^{-1}(\text{FM})$ ]									
Brown		0.65 $\pm$ 0.03	1.04 $\pm$ 0.04	1.03 $\pm$ 0.06	1.05 $\pm$ 0.07	0.75 $\pm$ 0.10			
Volcanic		0.68 $\pm$ 0.03	0.85 $\pm$ 0.02	0.83 $\pm$ 0.07	0.66 $\pm$ 0.03	0.57 $\pm$ 0.04	0.2 <sup>n.s.</sup>	0.7 <sup>n.s.</sup>	1.5 <sup>n.s.</sup>
Elements [ $\mu\text{mol g}^{-1}(\text{DM})$ ]									
N	Brown	938 $\pm$ 38	910 $\pm$ 34	798 $\pm$ 53	728 $\pm$ 44	586 $\pm$ 27			
	Volcanic	872 $\pm$ 37	791 $\pm$ 23	638 $\pm$ 12	636 $\pm$ 43	603 $\pm$ 11	6.8*	67 <sup>***</sup>	1.3 <sup>n.s.</sup>
P	Brown	45.9 $\pm$ 2.1	37.6 $\pm$ 3.0	29.4 $\pm$ 1.9	29.7 $\pm$ 3.4	28.4 $\pm$ 4.9			
	Volcanic	47.2 $\pm$ 4.4	36.2 $\pm$ 1.3	27.0 $\pm$ 2.6	24.3 $\pm$ 3.3	19.9 $\pm$ 1.9	0.2 <sup>n.s.</sup>	39 <sup>***</sup>	1.9 <sup>n.s.</sup>
K	Brown	146 $\pm$ 10	121 $\pm$ 6	102 $\pm$ 6	99 $\pm$ 7	94 $\pm$ 7			
	Volcanic	179 $\pm$ 11	151 $\pm$ 11	115 $\pm$ 8	120 $\pm$ 12	109 $\pm$ 18	6.4*	38 <sup>***</sup>	0.8 <sup>n.s.</sup>
S	Brown	177 $\pm$ 14	156 $\pm$ 8	146 $\pm$ 6	159 $\pm$ 11	167 $\pm$ 12			
	Volcanic	144 $\pm$ 13	147 $\pm$ 4	149 $\pm$ 7	171 $\pm$ 9	166 $\pm$ 16	2.8 <sup>n.s.</sup>	1.1 <sup>n.s.</sup>	2.2 <sup>n.s.</sup>
Ca	Brown	56 $\pm$ 2	134 $\pm$ 7	232 $\pm$ 16	274 $\pm$ 20	316 $\pm$ 19			
	Volcanic	65 $\pm$ 13	125 $\pm$ 17	189 $\pm$ 30	243 $\pm$ 34	272 $\pm$ 50	<0.1 <sup>n.s.</sup>	135 <sup>***</sup>	1.5 <sup>n.s.</sup>
Mg	Brown	30.7 $\pm$ 0.7	30.9 $\pm$ 0.9	34.3 $\pm$ 2.2	33.2 $\pm$ 2.9	29.3 $\pm$ 2.9			
	Volcanic	37.6 $\pm$ 2.9	38.1 $\pm$ 1.4	40.0 $\pm$ 2.0	45.8 $\pm$ 4.7	50.6 $\pm$ 4.6	0.6 <sup>n.s.</sup>	7.9 <sup>**</sup>	9.0 <sup>**</sup>
Al	Brown	2.71 $\pm$ 0.09	3.35 $\pm$ 0.13	4.16 $\pm$ 0.26	5.00 $\pm$ 0.45	5.39 $\pm$ 0.41			
	Volcanic	3.22 $\pm$ 0.32	4.03 $\pm$ 0.08	5.36 $\pm$ 0.31	6.20 $\pm$ 0.31	8.75 $\pm$ 1.51	<0.1 <sup>n.s.</sup>	106 <sup>***</sup>	11 <sup>**</sup>
Mn	Brown	4.1 $\pm$ 0.3	8.8 $\pm$ 0.5	11.9 $\pm$ 1.2	12.4 $\pm$ 1.3	7.1 $\pm$ 1.0			
	Volcanic	5.3 $\pm$ 1.4	8.0 $\pm$ 1.5	9.4 $\pm$ 2.2	10.2 $\pm$ 2.3	9.3 $\pm$ 2.2	0.2 <sup>n.s.</sup>	4.8*	0.1 <sup>n.s.</sup>
Fe	Brown	0.42 $\pm$ 0.01	0.62 $\pm$ 0.02	0.69 $\pm$ 0.03	0.72 $\pm$ 0.03	1.14 $\pm$ 0.02			
	Volcanic	0.58 $\pm$ 0.06	0.60 $\pm$ 0.06	0.67 $\pm$ 0.12	0.80 $\pm$ 0.13	1.08 $\pm$ 0.21	1.3 <sup>n.s.</sup>	70 <sup>***</sup>	1.0 <sup>n.s.</sup>

Concentrations of Ca, Mg, Al, Mn, and Fe in *P. glehnii* needles increased as needles aged ( $P < 0.05$ ). These elements were not significantly different between the two soil types. However, concentrations of Mg and Al showed a significant interaction between soil type and

needle age ( $P < 0.01$ ). The inclination of regression line between Mg or Al concentrations and needle age was steeper for *P. glehnii* growing in volcanic ash soil than in brown forest soil. Accumulations of Mg and Al in aged needles were obvious at the volcanic ash soil site.

## Discussion

*P. glehnii* growing in volcanic ash soil suppressed annual shoot growth, and decreased number of needles per shoot, compared with trees growing in brown forest soil. Comparing all needle age classes,  $P_{Nsat}$  and N in needles were significantly lower in volcanic ash soil than in brown forest soil (Table 1). This tendency was found generally in many plant species (Evans 1989, Reich *et al.* 1992, Lawlor 2001, Ripullone *et al.* 2003, Kayama *et al.* 2007). Nitrogen uptake was probably less for *P. glehnii* growing in volcanic ash soil than in brown forest soil. As a result, *P. glehnii* growing in volcanic ash soil may suppress photosynthetic rate, and exhibit low annual shoot growth. We predicted that *P. glehnii* growing in volcanic ash soil might increase needle longevity to compensate for lack of photosynthetic productivity as found in this spruce on the infertile soils (Kayama *et al.* 2002). However, *P. glehnii* in volcanic ash soil exhibited accelerated shedding of aged needles (Table 1), and maximum needle age was 9 years. This was different from what we have previously found (Kayama *et al.* 2002, 2007). The cause of this difference is probably lower concentrations of soil N in volcanic ash soil. N concentration in topsoil of the volcanic ash soil was  $93 \text{ mg } (100 \text{ g})^{-1}$  (Kayama and Koike 2011). This value is quite low compared with its concentrations in serpentine soil [ $183 \text{ mg } (100 \text{ g})^{-1}$ , Kayama *et al.* 2002] and volcanogenic soil [ $350 \text{ mg } (100 \text{ g})^{-1}$ , Kayama *et al.* 2007]. *P. glehnii* can increase needle longevity to compensate for the small nitrogen deficiency in serpentine soil (Kayama *et al.* 2002). However, nitrogen deficiency was more serious in volcanic ash soil, and, at this site, increase in needle longevity may not sufficiently compensate for low photosynthetic productivity.

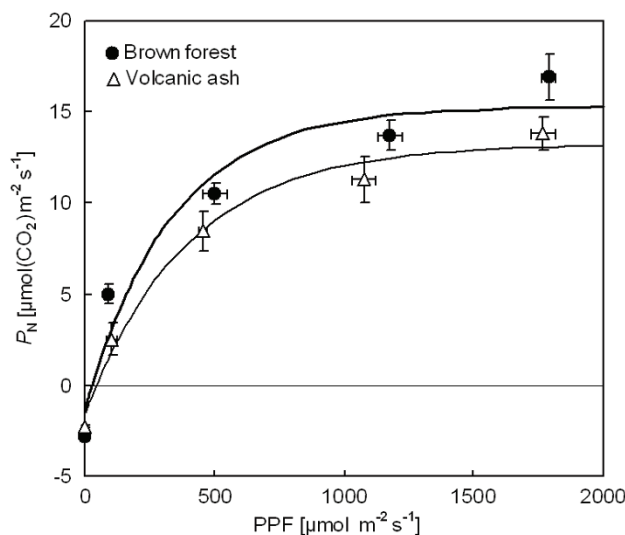


Fig. 1. Photosynthetic light-response curves of 2-year-old needles of *P. glehnii* growing on two types of soil (mean  $\pm$  SE,  $n = 6$  for Brown forest soil, and  $n = 3$  for volcanic ash soil).

When 2-year-old needles were compared, differences in photosynthetic traits between the trees grown in the two soil types were small (Fig. 1).  $P_{Nsat}$  and N concentration in 2-year-old needles was not significantly different between the soil types (Table 1). This suggests that *P. glehnii* growing in volcanic ash soil is able to concentrate nitrogen in its younger needles. In general, woody species mobilize nutrients to younger leaves via a leaf turnover mechanism: nitrogen is remobilized to younger leaves with foliar senescence (Killingbeck 2004, Hikosaka 2005, Kitaoka and Koike 2005). To examine differences in remobilization, we analyzed nitrogen from senescent (brown) 8-year-old needles just prior to shedding. The content-based nitrogen retranslocation rate (Fife *et al.* 2008) was calculated by comparison with green, 8-year-old needles. N content per senescent needle was significantly lower in volcanic ash soil than in brown forest soil ( $0.49 \pm 0.03$  vs.  $0.59 \pm 0.01 \text{ } \mu\text{mol needle}^{-1}$ ,  $P < 0.05$ ). N content of green, 8-year-old needles was  $1.32 \pm 0.02 \text{ } \mu\text{mol needle}^{-1}$  in volcanic ash soil, and  $1.28 \pm 0.06 \text{ } \mu\text{mol needle}^{-1}$  in brown forest soil. The nitrogen retranslocation rate (the ratio of reduced N from senescent needle) was significantly higher in volcanic ash soil than in brown forest soil ( $63 \pm 2$  vs.  $53 \pm 1\%$ ,  $P < 0.05$ ). Thus, it is likely that *P. glehnii* growing in volcanic ash soil has a high capacity to remobilize nutrients from senescent needles to young active ones; as a result, the  $P_{Nsat}$  of these 2-year-old needles may be almost the same as in brown forest soil. Our observations suggest that internal re-allocation of nutrients may occur in trees growing in nutrient-poor soil (Kayama *et al.* 2009).

Al in *P. glehnii* needles was observed to accumulate over the course of needle aging, especially when grown in volcanic ash soils (Table 1). The maximum value of Al in needles was  $11.8 \text{ } \mu\text{mol g}^{-1}$  ( $319 \text{ } \mu\text{g g}^{-1}$ ) for 8-year-old needles in volcanic ash soil. This value is almost the same that reported for the current needles of *P. jezoensis* var. *hondoensis* ( $312 \text{ } \mu\text{g g}^{-1}$ ) (Takada *et al.* 1994), and Al was not accumulated in *P. glehnii* needles. In addition, concentration of Chl decreased as Al accumulated (Ridolfi and Garrec 2000, Chen *et al.* 2005), and was not lower in *P. glehnii* growing in volcanic ash soil than in brown forest soil (Table 1). The volcanic ash soil contained higher concentrations of S and Fe (Kayama and Koike 2011), but these elements were not found at high levels in *P. glehnii* needles (Table 1). Thus, we did not detect any effects of Al, S or Fe, on *P. glehnii* growth, suggesting that this tree has a strong ability to exclude Al. The volcanic ash soil contained low levels of K, Ca, Mg, and Mn (Kayama and Koike 2011), but these nutrients were not low in *P. glehnii* needles compared with ones in brown forest soil (Table 1). *P. glehnii* growing in volcanic ash soil may not suffer deficiency of these nutrients. It is remarkable that *P. glehnii* growing in volcanic ash soil was able to accumulate high

concentrations of K compared with in brown forest soil. For woody species, ectomycorrhizal fungal symbioses are important for exclusion of Al. Inoculation with ectomycorrhizae can reduce Al uptake, and protect plants from deficiencies in Ca, Mg, and K (Hentschel *et al.* 1993, Lux and Cumming 2001, Choi *et al.* 2008, van Schöll *et al.* 2008). Based on our microscope observation, *P. glehnii* growing in volcanic ash soil had numerous ectomycorrhizal hyphae around their roots, showing that they have active symbioses with ectomycorrhizal fungi. This may be one important means by which *P. glehnii* has the ability to reduce Al uptake, and increase uptake of Ca, Mg, and K.

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