

# Comparison of photosynthetic traits of codominant subalpine conifers *Abies veitchii* and *A. mariesii* in central Japan

Koichi Takahashi<sup>1,3</sup> · Sohei Otsubo<sup>1</sup> · Hajime Kobayashi<sup>2,3</sup>

Received: 10 May 2016/Revised: 20 March 2017/Accepted: 20 March 2017/Published online: 20 July 2017  
© International Consortium of Landscape and Ecological Engineering and Springer Japan 2017

**Abstract** Evergreen conifers *Abies mariesii* and *A. veitchii* codominate in the subalpine zone in central Japan. This study compared the photosynthetic light response curves and related leaf traits of 1-year-old needles between the two species to examine whether photosynthetic traits of *A. veitchii* are more favorable for growth in bright conditions than those of *A. mariesii*. Saplings of the two species were sampled at forest edge (FE) and forest understory (FU). FE saplings of the two species showed more shade-intolerant traits (i.e., lower initial slope of photosynthesis light response curve, greater light compensation point and dark respiration rate) than FU saplings. Maximum photosynthetic rate and leaf nitrogen concentration were greater in *A. veitchii* than *A. mariesii* for both FE and FU saplings. Photosynthetic nitrogen use efficiency (PNUE) was also greater in *A. veitchii* than *A. mariesii*. On the contrary, leaf mass per area was greater in *A. mariesii* than *A. veitchii* for both FE and FU saplings. Therefore, this study showed that photosynthetic traits of *A. veitchii* are more favorable for growth in bright conditions compared with *A. mariesii*, and *A. mariesii* has more robust needles at the expense of PNUE.

**Keywords** Leaf mass per area · Leaf nitrogen concentration · Photosynthetic light response curve · Photosynthetic nitrogen use efficiency · Shade tolerance

## Introduction

Evergreen conifers *Abies mariesii* Mast. and *A. veitchii* Lindl. codominate in the subalpine zone in central Japan (Takahashi 1962; Franklin et al. 1979; Aizawa and Kaji 2003; Miyajima et al. 2007). Many studies have investigated the regeneration process of the two *Abies* species. *A. veitchii* is less shade tolerant with shorter leaf lifespan than *A. mariesii*, and grows faster than *A. mariesii* in bright conditions (Kohyama 1983, 1984). Therefore, *A. veitchii* dominates after large-scale disturbances, such as wave-regenerated forests, by regenerating from newly established seedlings, while *A. mariesii* dominates forests where small-scale disturbances frequently occur by regenerating from advanced saplings existing before gaps in the forest are created (Kohyama 1984). Therefore, difference in shade tolerance between the two *Abies* species is important for their regeneration and coexistence (Kohyama 1984).

Photosynthetic characteristics and related leaf traits are important factors affecting shade tolerance of plants. In general, shade-tolerant species show higher initial slope of the light response curve of photosynthesis and lower light compensation point (LCP), but lower photosynthetic rate in light-saturated conditions, compared with shade-intolerant species (Koike 1988). Leaf mass per area (LMA) is thought to be an indicator of leaf construction cost per leaf area (Kudo 1999). Generally, LMA correlates positively with leaf lifespan (Reich et al. 1992); i.e., long-lived leaves need robust structure. Photosynthetic production during leaf lifespan must be greater than the leaf construction cost

✉ Koichi Takahashi  
koichit@shinshu-u.ac.jp

<sup>1</sup> Department of Biology, Faculty of Science, Shinshu University, Asahi 3-1-1, Matsumoto 390-8621, Japan

<sup>2</sup> Faculty of Agriculture, Education and Research Center of Alpine Field Science, Shinshu University, 8304, Minami-Minowa 399-4598, Japan

<sup>3</sup> Institute of Mountain Science, Shinshu University, Asahi 3-1-1, Matsumoto 390-8621, Japan

for positive carbon balance at the level of individual leaves. Leaf nitrogen concentration is a good indicator of maximum photosynthetic rate (or assimilative capacity), because photosynthetic enzymes, such as RuBP carboxylase, contain large quantities of nitrogen in leaves (Ellsworth and Reich 1992, 1993). In particular, photosynthetic nitrogen use efficiency (PNUE) is an important index because nitrogen is a limiting resource for plant growth (Hirose 1987; Hirose and Werger 1987). Early successional species tend to have lower LMA but greater nitrogen concentration and PNUE (Poorter et al. 1990; Reich et al. 1991, 1994; Hikosaka et al. 2002).

Kuroiwa (1960) showed that the maximum photosynthetic rate and leaf nitrogen concentration were greater in *A. veitchii* than *A. mariesii*. By contrast, Kimura (1963) showed that the maximum photosynthetic rate did not differ between the two *Abies* species, although the photosynthetic rate of *A. mariesii* was greater than that of *A. veitchii* in dark conditions. No further studies have compared the photosynthetic light response curve and related leaf traits between the two *Abies* species. Therefore, the objective of this study was to examine whether photosynthetic traits of *A. veitchii* are more favorable for growth in bright conditions than those of *A. mariesii* by comparing the photosynthetic light response curve, leaf nitrogen concentration, LMA, and PNUE between the two *Abies* species.

## Materials and methods

### Study site

This study was conducted at 2000 m above sea level on the east slope of Mt. Norikura (36°06'N, 137°33'E, 3026 m a.s.l.) in central Japan. *A. veitchii* and *A. mariesii* dominate between 1600 and 2000 m a.s.l. and between 2000 and 2500 m a.s.l., respectively (Miyajima et al. 2007). The two *Abies* species codominate around 2000 m a.s.l. The mean monthly temperatures of the coldest month (January) and the hottest month (August) at the study site (2000 m a.s.l.) were estimated as −9.2 and 14.9 °C, respectively, with annual mean temperature of 2.7 °C, from temperatures recorded at Nagawa Weather Station (1068 m a.s.l.) during 1979–2011 using the standard lapse rate of −0.6 °C for each +100 m altitude. The soil type was Gleyic Cambisols (Oura 2010), covered with a thick litter layer.

The study site was dominated by *A. mariesii*, *A. veitchii*, and *Tsuga diversifolia* Mast. Although *Picea jezoensis* var. *hondoensis* Rehder also grows at this study site, its density was lower than for the other three species (Miyajima et al. 2007). Subordinate trees were all deciduous broadleaved trees: *Betula ermanii* Cham., *Sorbus commixta* Hedland,

and *Acer ukurunduense* Trantv. et Meyer. The canopy height was about 20 m. The species composition and forest structure along an altitudinal gradient of Mt. Norikura has been previously described (Miyajima et al. 2007; Miyajima and Takahashi 2007; Takahashi et al. 2012).

### Field sampling

A paved road for vehicles continues up to 2700 m a.s.l. on Mt. Norikura (Takahashi and Miyajima 2010). Only a limited number of vehicles with entry passes can use this road. Effects of the vehicle road on tree growth have not been recognized so far (Ida and Ozeki 2000). Therefore, we chose a forest edge (FE) at the roadside and the nearby intact forest understory (FU) at the same altitude (2000 m a.s.l.) to compare photosynthetic traits between sun-exposed and shaded leaves of saplings. Relative photosynthetic photon flux density (PPFD, mean ± standard deviation) values measured on a cloudy day (26 September 2013) by using a quantum sensor (model LI-190SL, Li-Cor Biosciences, Lincoln, NE, USA) were  $89.4 \pm 15.6$  and  $14.0 \pm 4.2$  % at the FE and FU sites, respectively.

About ten saplings (30–50 cm tall), including roots, were dug up at each of the FE and FU sites for each species in the morning during 23–27 August and 6–8 September 2013. Sampled saplings were put in a bucket filled with water after soil on the roots was washed out and were brought to the Norikura Observatory (2800 m a.s.l.), Institute for Cosmic Ray Research, The University of Tokyo, about 3 km from the sampling site in horizontal distance, to measure the photosynthetic rates.

### Leaf measurements

The photosynthesis of the two *Abies* species was measured by using a portable photosynthesis system (model LI-6400, Li-Cor Biosciences, Lincoln, NE, USA) equipped with a conifer chamber, including an RGB light source (6400-22L). Incoming CO<sub>2</sub> gas concentration from a CO<sub>2</sub> cartridge was set to 380 ppm. The leaf temperature was maintained at 20 °C, and the relative humidity ranged between 50 and 75% during the measurement. The number of shoots used for the measurement was 9 and 10 for FE and FU saplings of *A. veitchii*, respectively, and 13 and 8 for FE and FU saplings of *A. mariesii*, respectively. In this study, only 1-year-old shoots were used to measure the photosynthesis of each sapling because current-year shoots are immature in midsummer, and photosynthetic rate and leaf survival rate decrease with leaf age (Kajimoto 1990; Kitajima et al. 1997). Photosynthesis was measured at 12 light levels of PPFD of 2000, 1750, 1500, 1250, 1000, 750, 500, 250, 150, 100, 50, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

The needles used to measure the photosynthesis were scanned, then the total area of needles was measured from the image using the NIH image program (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). Needles were oven-dried at 80 °C for at least 48 h then weighed. The LMA was calculated as the needle dry mass divided by the needle area. After the measurements of needle dry mass, the needles were ground into fine powder. The leaf nitrogen concentration was measured by using an elemental analyzer (Thermo Finnigan Flash EA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA) after oven-drying the powder samples for 24 h.

We determined the maximum photosynthetic rate, dark respiration rate, light compensation point, and initial slope of each light response curve of photosynthesis on leaf area basis. The dark respiration rate was measured as the net photosynthetic rate at PPFD 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Because there was a decline in the net photosynthetic rate when the PPFD exceeded 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , the maximum net photosynthetic rate was defined as the highest measured photosynthesis rate. The net photosynthetic rate increased linearly from PPFD 0 to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Therefore, the LCP and initial slope of the light response curve were estimated using linear regression in the PPFD range from 0 to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The photosynthetic nitrogen use efficiency (PNUE) was calculated by dividing the maximum photosynthetic rate by the leaf nitrogen content per leaf area.

### Statistical analyses

The maximum net photosynthetic rate, leaf nitrogen concentration, dark respiration rate, light compensation point, initial slope of the light response curve, LMA, and PNUE were compared between FE and FU saplings of the two species by two-way analysis of variance (ANOVA) with species and light condition (FE and FU) as independent variables. Statistical analysis was carried out using free statistical software R 2.14.2 (R development core team 2012).

### Results

The net photosynthetic rate declined when PPFD exceeded 750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in both *A. veitchii* and *A. mariesii* saplings (Fig. 1). The net photosynthetic rate was highest between PPFD 500 and 750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for both FE and FU saplings of *A. veitchii*, while it was highest between PPFD 250 and 750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for saplings of *A. mariesii*. The net photosynthetic rate above PPFD 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  tended to be slightly greater for FE

than FU saplings, while the net photosynthetic rate below PPFD 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  tended to be greater for FU than FE saplings in both species.

The maximum net photosynthetic rate ( $P_{\text{max}}$ ) was greater in *A. veitchii* than in *A. mariesii* for both FE and FU saplings ( $P < 0.001$ , Figs. 1, 2a; Table 1). The dark respiration rate ( $R$ ) was greater for FE than FU saplings of the two *Abies* species ( $P < 0.05$ ), and was greater in *A. mariesii* than *A. veitchii* for each of FE and FU saplings ( $P < 0.05$ , Fig. 2b; Table 1). The light compensation point (LCP) showed a pattern similar to the dark respiration rate; i.e., the LCP was greater for FE than FU saplings of the two *Abies* species ( $P < 0.01$ ), and was greater in *A. mariesii* than *A. veitchii* for each of FE and FU saplings ( $P < 0.05$ , Fig. 2c; Table 1). The initial slope of the light response curve (IS) tended to be lower in FE than FU saplings for the two species ( $P < 0.05$ ), and there was no statistical significant difference between the two *Abies* species for each of FE and FU saplings (Fig. 2d; Table 1).

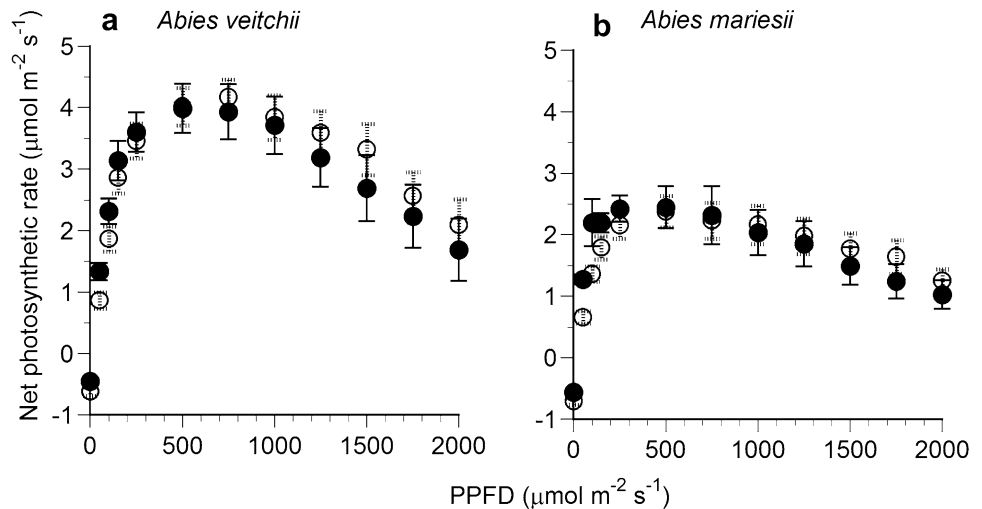
LMA was greater for FE than FU saplings of the two *Abies* species ( $P < 0.001$ ), and was greater in *A. mariesii* than *A. veitchii* for each of FE and FU saplings ( $P < 0.001$ , Fig. 2e; Table 1). Leaf nitrogen content per mass ( $N_{\text{mass}}$ ) was greater in *A. veitchii* than *A. mariesii* ( $P < 0.001$ ), especially for FU saplings [Fig. 2f; Table 1,  $P < 0.001$  ( $S \times L$ )]. Leaf nitrogen content per area ( $N_{\text{area}}$ ) was slightly greater in *A. veitchii* than *A. mariesii* ( $P < 0.05$ ), and showed no statistically significant difference between FE and FU saplings for each species (Fig. 2g; Table 1). The species difference of  $N_{\text{area}}$  was apparently smaller than that of  $N_{\text{mass}}$ , because greater LMA counterbalanced lower  $N_{\text{mass}}$  for *A. mariesii* compared with *A. veitchii* (Fig. 2e–g). Therefore, photosynthetic nitrogen use efficiency (PNUE) was greater in *A. veitchii* than *A. mariesii* ( $P < 0.01$ ), and showed no statistical significant difference between FE and FU saplings (Fig. 2h; Table 1).

LMA was negatively correlated with  $N_{\text{mass}}$  for the pooled data of the FE and FU saplings of the two *Abies* species ( $R = -0.605$ ,  $P < 0.001$ , Fig. 3a). Although PNUE was weakly negatively correlated with LMA for the pooled data of the FE and FU saplings of the two *Abies* species ( $R = -0.308$ ,  $P = 0.053$ ), PNUE was clearly negatively correlated with LMA only for the FE saplings of the two *Abies* species ( $R = -0.595$ ,  $P < 0.01$ , Fig. 3b).

### Discussion

This study showed that the photosynthetic traits of *A. veitchii* were more favorable for growth in bright conditions compared with *A. mariesii*. Greater maximum photosynthetic rates of *A. veitchii* than *A. mariesii* coincided with the result of Kuroiwa (1960). Species with

**Fig. 1** Light response curves of net photosynthetic rate of *Abies veitchii* (a) and *A. mariesii* (b) for forest edge (FE) saplings (open symbols, dotted lines) and forest understory (FU) saplings (solid symbols, solid lines). Mean ( $\pm$ standard error, SE) value is shown at each PPFD for each species. The number of samples was 9 and 10 for FE and FU saplings of *A. veitchii*, respectively, and 13 and 8 for FE and FU saplings of *A. mariesii*, respectively



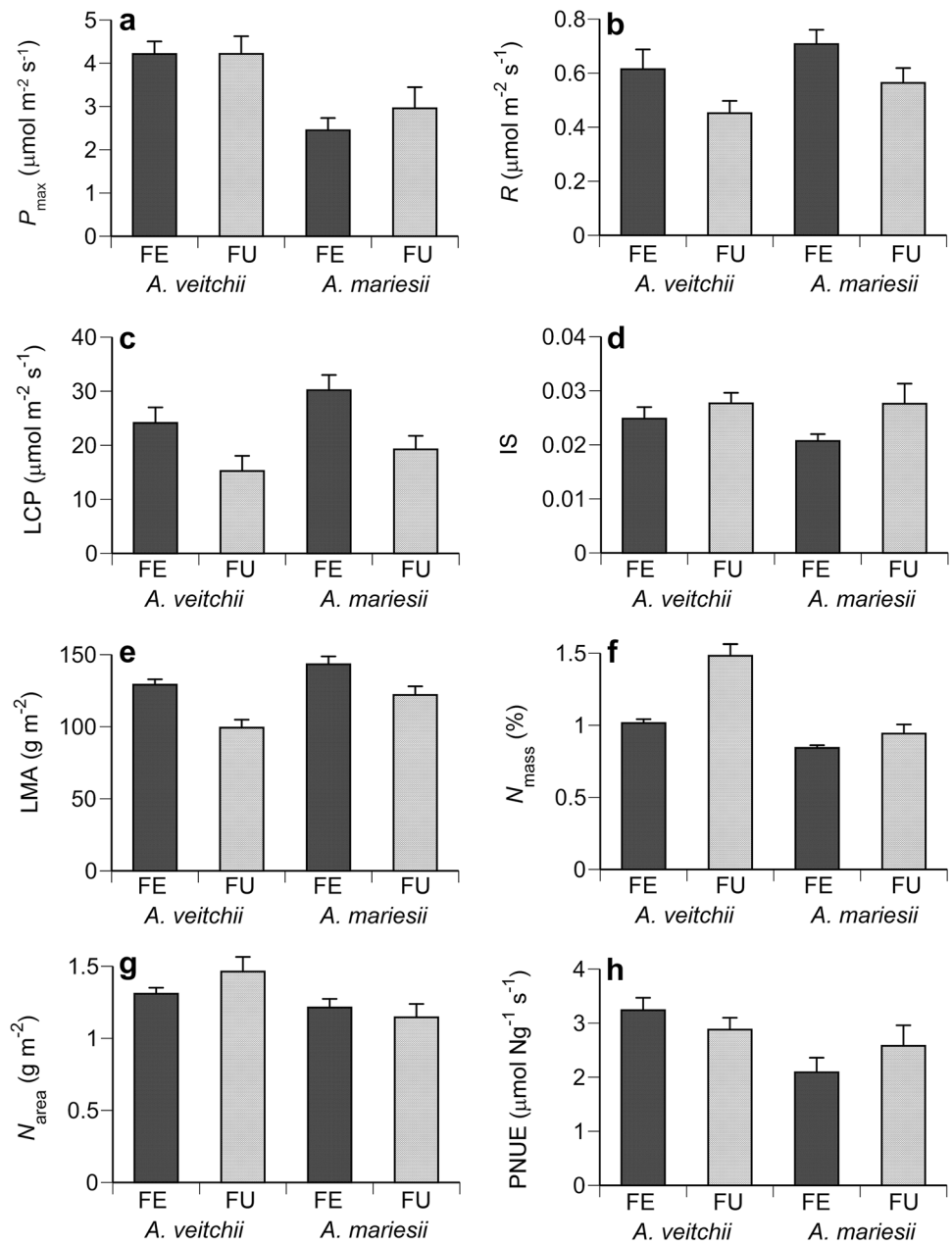
greater maximum photosynthetic rates tend to have greater growth rates at whole plant level (Reich et al. 1992). Actually, *A. veitchii* grows faster than *A. mariesii* in bright conditions (Kohyama 1983). Greater leaf nitrogen concentration and PNUE of *A. veitchii* probably contribute to the greater maximum photosynthetic rate compared with *A. mariesii*. The difference in PNUE between the two *Abies* species can be caused by the species difference in LMA. First, more nitrogen is used for leaf construction in leaves with greater LMA (Hikosaka 2004). Second, the increase in LMA lengthens the internal diffusion pathway to chloroplasts, which decreases the  $\text{CO}_2$  supply to chloroplast (Sparks and Ehleringer 1997; Niinemets et al. 1999; Takahashi and Mikami 2006; Takahashi and Miyajima 2008; Takahashi and Otsubo 2017). Generally, LMA is positively and negatively correlated with leaf lifespan and leaf nitrogen concentration, respectively (Reich et al. 1992). This indicates that long-lived leaves with greater LMA need more carbon investment to increase mechanical stiffness. Mean leaf lifespans (50 % survival) of *A. veitchii* and *A. mariesii* are about 6–7 and 7–8 years, respectively (Mori and Takeda 2004; Takahashi and Obata 2014). Probably, *A. mariesii* needs to increase the mechanical stiffness (i.e., LMA) of needles to maintain its longer-lived needles compared with *A. veitchii*. Therefore, PNUE was lower in *A. mariesii* probably because of its greater LMA than in *A. veitchii*.

In general, species with greater maximum photosynthetic rate tend to have greater dark respiration rate and light compensation point (Koike 1988). However, *A. mariesii* with lower maximum photosynthetic rate tended to have greater dark respiration rate and light compensation point compared with *A. veitchii* for both FE and FU saplings. If this result were true, it would be expected that *A. mariesii* would be an inferior species to *A. veitchii* in both dark and bright conditions, and *A. mariesii* would be competitively

excluded by *A. veitchii*. However, this expectation is not plausible because *A. mariesii* and *A. veitchii* actually coexist (Kohyama 1984). Probably, physiological stresses at sampling caused the unusual increase of the dark respiration rate and LCP of *A. mariesii*. Therefore, we should reexamine the photosynthetic light response curves of the two *Abies* species. On the contrary, Takahashi and Obata (2014) examined the net production and carbon allocation patterns of saplings of four subalpine conifers including *A. veitchii* and *A. mariesii* at 2000 m a.s.l. on Mt. Norikura (i.e., the same site as in this study). They showed that understory saplings of *A. mariesii* can survive with less net production per sapling leaf mass compared with those of *A. veitchii* because of greater sapling leaf mass and longer leaf lifespan in *A. mariesii* than *A. veitchii*. Kohyama (1983) also showed that *A. mariesii* can survive with smaller critical growth rate than *A. veitchii* for seedlings after age 3 because of longer leaf lifespan in *A. mariesii*. The results of these two previous studies indicate that less requirement of photosynthetic production enables seedlings (older than age 3) and saplings of *A. mariesii* to survive in the forest understory for longer time compared with *A. veitchii*. Therefore, it is suggested that not only photosynthetic traits but also leaf lifespan and sapling leaf mass at whole plant level largely reflect the species difference in shade tolerance between the two *Abies* species (Takahashi and Obata 2014).

This study showed that the photosynthetic traits of *A. veitchii* were more favorable for growth in bright conditions by having greater maximum photosynthetic rate, leaf nitrogen content per both leaf mass and area, and PNUE compared with *A. mariesii*, and that *A. mariesii* has more robust needles at the expense of PNUE to increase the mechanical stiffness. Therefore, it is suggested that photosynthesis and related leaf traits are important for the regeneration traits of the two *Abies* species.

**Fig. 2** Photosynthetic and leaf traits of *Abies veitchii* and *A. mariesii* for forest edge saplings (FE, *lightly shaded bars*) and forest understory saplings (FU, *darkly shaded bars*): maximum net photosynthetic rate ( $P_{max}$ , **a**), dark respiration rate ( $R$ , **b**), light compensation point (LCP, **c**), initial slope of light response curve of net photosynthesis (IS, **d**), leaf mass per area (LMA, **e**), leaf nitrogen content per leaf mass ( $N_{mass}$ , **f**), leaf nitrogen content per leaf area ( $N_{area}$ , **g**), and photosynthetic nitrogen use efficiency (PNUE, **h**). Mean values are shown with positive standard errors. Table 1 summarizes the two-way ANOVA

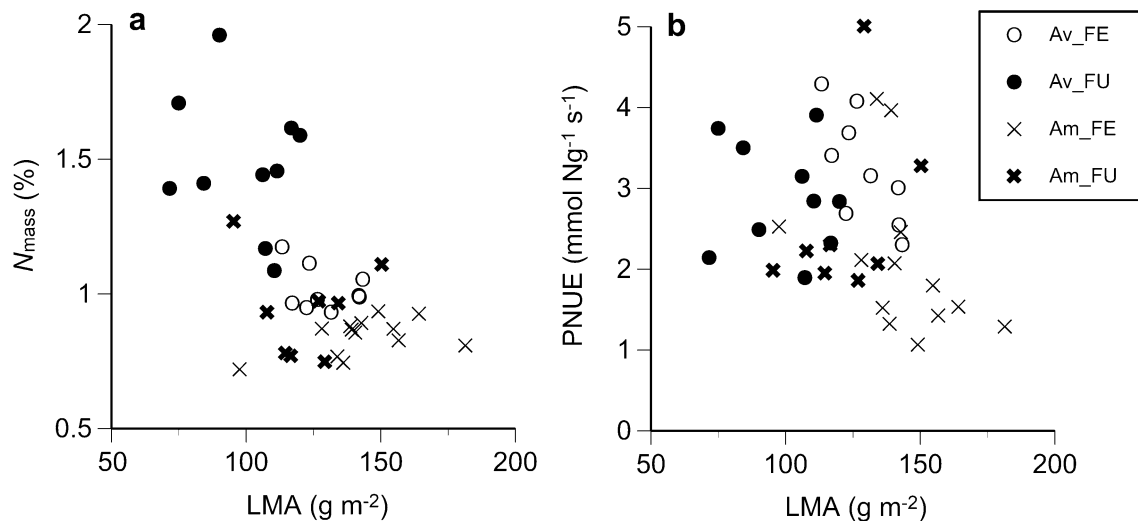


**Table 1** Summary of two-way ANOVA of effects of species ( $S$ ), light condition ( $L$ ), and their interaction ( $S \times L$ ) on photosynthesis and leaf traits

Factor	Dependent variable							
	$P_{max}$	$R$	LCP	IS	LMA	$N_{mass}$	$N_{area}$	PNUE
$S$	18.77***	4.67*	5.32*	1.88	16.05***	56.71***	6.94*	7.79**
$L$	0.5	6.94*	11.84**	4.93*	21.39***	29.57***	0.27	0.79
$S \times L$	0.47	0.03	0.13	0.84	0.6	12.85***	2.03	0.14

$F$  values are shown with the level of statistical significance. Abbreviations of dependent variables are as in Fig. 2

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$



**Fig. 3** Relationships of leaf mass per area (LMA) with leaf nitrogen concentration ( $N_{\text{mass}}$ , **a**) and with photosynthetic nitrogen use efficiency (PNUE, **b**) for forest edge (FE) and forest understory (FU) saplings of *A. veitchii* (Av) and *A. mariesii* (Am)

**Acknowledgements** We are grateful to the Norikura Observatory, Institute for Cosmic Ray Research, The University of Tokyo, for logistical support of field research. This study was partially supported by a grant (#26292081) from the Japan Society for the Promotion of Science.

## References

- Aizawa M, Kaji M (2003) The natural distribution patterns of subalpine conifers in central Japan. *Bull Univ For Fac Agric Univ Tokyo* 110:27–70 (in Japanese)
- Ellsworth DS, Reich PB (1992) Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer saccharum* seedlings in contrasting forest light environments. *Funct Ecol* 6:23–435
- Ellsworth DS, Reich PB (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* 96:169–178
- Franklin JF, Maeda T, Ohsumi Y, Matsui M, Yagi H, Hawk GM (1979) Subalpine coniferous forests of central Honshu, Japan. *Ecol Monogr* 49:311–334
- Hikosaka K (2004) Interspecific difference in the photosynthesis–nitrogen relationship: patterns, physiological causes, and ecological importance. *J Plant Res* 117:481–494
- Hikosaka K, Nagamatsu D, Ishii HS, Hirose T (2002) Photosynthesis–nitrogen relationships in species at different altitudes on Mount Kinabalu, Malaysia. *Ecol Res* 17:305–313
- Hirose T (1987) A vegetative plant growth model: adaptive significance of phenotypic plasticity in matter partitioning. *Funct Ecol* 1:195–202
- Hirose T, Werger MJA (1987) Nitrogen use efficiency instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiol Plant* 70:215–222
- Ida H, Ozeki M (2000) Shoot dynamics of *Pinus pumila* Regel along the road in Mt. Norikura, central Japan. *Bull Nagano Nat Conserv Res Inst* 3:1–7 (in Japanese)
- Kajimoto T (1990) Photosynthesis and respiration of *Pinus pumila* needles in relation to needle age and season. *Ecol Res* 5:333–340
- Kimura M (1963) Dynamics of vegetation in relation to soil development in northern Yatsugatake mountains. *Jpn J Bot* 18:255–287
- Kitajima K, Mulkey SS, Wright SJ (1997) Decline of photosynthetic capacity with leaf age in relation to leaf longevities for five tropical canopy tree species. *Am J Bot* 84:702–708
- Kohyama T (1983) Seedling stage of two subalpine *Abies* species in distinction from sapling stage: a matter-economic analysis. *Bot Mag Tokyo* 96:49–65
- Kohyama T (1984) Regeneration and coexistence of two *Abies* species dominating subalpine forests in central Japan. *Oecologia* 62:156–161
- Koike T (1988) Leaf structure and photosynthetic performance as related to the forest succession of deciduous broad-leaved trees. *Plant Species Biol* 3:77–87
- Kudo G (1999) A review of ecological studies on leaf-trait variations along environmental gradients—in the case of tundra plants. *Jpn J Ecol* 49:21–35 (in Japanese)
- Kuroiwa S (1960) Ecological and physiological studies on the vegetation of Mt. Shimagare IV. Some physiological functions concerning matter production in young *Abies* trees. *Bot Mag Tokyo* 73:133–141
- Miyajima Y, Takahashi K (2007) Changes with altitude of the stand structure of temperate forests on Mount Norikura, central Japan. *J For Res* 12:187–192
- Miyajima Y, Sato T, Takahashi K (2007) Altitudinal changes in vegetation of tree, herb and fern species on Mount Norikura, central Japan. *Veg Sci* 24:29–40
- Mori A, Takeda H (2004) Functional relationships between crown morphology and within-crown characteristics of understory saplings of three codominant conifers in a subalpine forest in central Japan. *Tree Physiol* 24:661–670
- Niinemets Ü, Kull O, Tenhunen JD (1999) Variability in leaf morphology and chemical composition as a function of canopy light environment in coexisting deciduous trees. *Int J Plant Sci* 160:837–848
- Oura N (2010) Effect of nitrogen deposition on nitrogen cycling in forested ecosystems and N<sub>2</sub>O emission from the forest floor. *Bull Nat Inst Agro-Environ Sci* 27:1–84 (in Japanese)
- Poorter H, Remke C, Lambers H (1990) Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiol* 94:621–627
- R development core team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>. ISBN:3-900051-07-0

- Reich PB, Uhl C, Walters MB, Ellsworth DS (1991) Leaf lifespan as a determinant of leaf structure and function among 23 amazonian tree species. *Oecologia* 86:16–24
- Reich PB, Walters MB, Ellsworth DS (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecol Monogr* 62:365–392
- Reich PB, Walters MB, Ellsworth DS, Uhl C (1994) Photosynthesis-nitrogen relations in Amazonian tree species. I. Patterns among species and communities. *Oecologia* 97:62–72
- Sparks JP, Ehleringer JR (1997) Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. *Oecologia* 109:362–367
- Takahashi K (1962) Studies on vertical distribution of the forest in middle Honshu. *Bull Gov For Exp Stn* 142:1–171 (in Japanese)
- Takahashi K, Mikami Y (2006) Effects of canopy cover and seasonal reduction in rainfall on leaf phenology and leaf traits of the fern *Oleandra pistillaris* in a tropical montane forest, Indonesia. *J Trop Ecol* 22:599–604
- Takahashi K, Miyajima Y (2008) Relationship among leaf life span, leaf mass per area and leaf nitrogen causes different altitudinal changes in leaf  $\delta^{13}\text{C}$  between deciduous and evergreen species. *Botany* 86:1233–1241
- Takahashi K, Miyajima Y (2010) Effects of roads on alpine and subalpine plant species distribution along an altitudinal gradient on Mount Norikura, central Japan. *J Plant Res* 123:741–749
- Takahashi K, Obata Y (2014) Growth, allometry and shade tolerance of understory saplings of four subalpine conifers in central Japan. *J Plant Res* 127:329–338
- Takahashi K, Otsubo S (2017) How *Betula ermanii* maintains a positive carbon balance at the individual leaf level at high elevations. *Am J Plant Sci* 8:482–494
- Takahashi K, Hirokawa T, Morishima R (2012) How the timberline formed: altitudinal changes in stand structure and dynamics around the timberline in central Japan. *Ann Bot* 109:1165–1174