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Author(s)	Dong-Gyu, Kam; Shi, Cong; Watanabe, Makoto et al.
Citation	Journal of agricultural meteorology, 71(3), 239-244 https://doi.org/10.2480/agrmet.D-14-00029
Issue Date	2015-09
Doc URL	https://hdl.handle.net/2115/60224
Type	journal article
File Information	71290(koike).pdf



Growth of Japanese and hybrid larch seedlings grown under free-air O₃ fumigation—an initial assessment of the effects of adequate and excessive nitrogen

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Abstract

Ground-surface ozone (O₃) and nitrogen (N) deposition in forests are increasing rapidly in East Asia. The Japanese larch (JL) has been transplanted from central Japan to northern areas. Because the JL suffers from shoot blight disease and grazing by voles, the hybrid larch (F₁) was developed to overcome these weak points. For sustainable use of these larches, we should know the growth responses of larches to elevated O₃ (eO₃) and excessive N deposition in northern Japan. Two-year-old JL and F₁ seedlings were potted in well-weathered immature volcanic ash soil with basal dressing of balanced fertilizer containing 47 kgN ha^{−1} yr^{−1}, due to a lack of nutrients in the soil. To simulate eO₃, seedlings were grown in a free-air O₃ fumigation system (60 nmol mol^{−1} in daytime). Also, to simulate acid rain, NH₄NO₃ (50 kgN ha^{−1} yr^{−1} in total) was applied to the plants. Independent of the N loading, the dry mass of needle litter in both JL and F₁ was less at eO₃ than at ambient O₃. Re-translocation of N before the needle shedding of both larches was lower under eO₃. As a result, the aboveground biomass per needle (*i.e.* productive efficiency) was higher at eO₃.

Key words: Ground surface ozone, Hybrid larch F₁, Japanese larch, Nitrogen deposition, Re-translocation.

1. Introduction

Ground surface ozone (O₃) has been continuing to rise especially in the northern hemisphere (Akimoto, 2003; Izuta, 2006; Sitch *et al.*, 2007; Galloway *et al.*, 2008). In the current decade, nitrogen (N) deposition is expected to exceed the maximum rate of 50 kgN ha^{−1} yr^{−1} in central Japan (Kimura *et al.*, 2009). Indeed, the threshold of N loading for significant impact to plant growth is regarded as 20–30 kgN ha^{−1} yr^{−1} (Watanabe *et al.*, 2011; Mao *et al.*, 2014).

Larches are a vital tree species under sustainable forest management in northeast Asia today (*e.g.* Ryu *et al.*, 2009; Japanese Forestry Agency, 2010). A hybrid larch F₁ (hereafter F₁) was developed to overcome the weak points of the Japanese larch (*Larix kaempferi*) (Kita *et al.*, 2009; Ryu *et al.*, 2009). However, the responses of the F₁ to combination effects of elevated O₃ (eO₃) and excessive N deposition are still unknown (Akimoto, 2003; Ohara *et al.*, 2007; Watanabe and Yamaguchi, 2011; Koike *et al.*, 2012).

In general, eO₃ accelerates foliar senescence (*e.g.* Matyssek and Sandermann, 2003) and reduces the re-translocation rate of N from aged leaves (Uddling *et al.*, 2005). However, these phenomena may be affected by N conditions (*e.g.* Koike *et al.*, 1992; Schulze *et al.*, 2005). What about the effects of excessive N deposition on needle function and the re-translocation of N from larch

needles?

To assess these questions, we monitored seasonal changes in the growth of the main stem of seedlings and compared the productive efficiency of needles in two larch species, *L. kaempferi* (hereafter JL) and the F₁, grown under free-air O₃ fumigation with excessive N loading. Based on the needle function and N status, we discuss the combined effect of eO₃ and excessive N loading on the two larch species.

2. Materials and Methods

2.1 Plant materials

In late May 2013, two-year-old JL and F₁ seedlings were planted in 15 L pots filled with Kanuma pumice and Akadama soil (1:1 in volume, almost no nutrient), simulating well-weathered immature volcanic ash soil. The JLs were obtained from Naganuma near Sapporo and F₁s were from the Hokkaido Forestry Research Institute, Hokkaido Research Organization in Bibai, also near Sapporo. Prior to planting the seedlings in the pots, the mean stem diameter (at 2 cm from the bottom) was 2.51 ± 0.05 mm for JL and 2.20 ± 0.04 mm for F₁, and the mean height was 17.0 ± 0.56 cm for JL and 19 ± 0.72 cm for F₁, respectively. Before the N treatment, we applied 47 kgN ha^{−1} yr^{−1} as liquid balanced fertilizer (1:1000 (v/v) Hyponex N:P:K = 6:10:5; Hyponex Japan, Osaka, Japan) to each of the pots in early June 2013, as Kanuma and Akadama soils have almost no nutrients.

2.2 Treatments

For the O₃ treatment, we used a free-air O₃ fumigation system (DALTON Ltd, Sapporo, Japan), which is described in detail by Watanabe *et al.* (2013). The mean O₃ concentration from May to August, in East Asia, is expected to increase consistently up to 60

Received; July 2, 2014.

Accepted; June 9, 2015.

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DOI: 10.2480/agrm.14-00029

nmol mol⁻¹ over the next 10 years (ADORC, 2006; Izuta, 2006). The O₃ concentration of 60 nmol mol⁻¹ was therefore applied during the daytime from June to October. The photoperiod was regulated by a PPF sensor (Hamamatsu Photonics, Japan) by detecting the light compensation point of the larches (60 μmol m⁻² s⁻¹), as determined by previous studies (Watanabe *et al.*, 2012; Mao *et al.*, 2012). During this period of O₃ exposure, ambient daytime O₃ concentration was 26.5 nmol mol⁻¹.

In order to simulate the acid rain of current conditions in Japan (Izuta *et al.* 2005; Izuta, 2006), NH₄NO₃ solution was applied to the potted soil. The total amount of N applied was 50 kgN ha⁻¹ yr⁻¹, and four differing applications were made in the order of 15, 20, 10 and 5 kg N ha⁻¹ yr⁻¹ from June to late September, according to the seasonal N deposition pattern (Environmental Science Center, HRO, 2012). For the N treatment, we set four tables with four potted plants using water-traps. The trapped water was reused to maintain the total amount of N applied to the seedlings. Overall, 32 seedlings were used for each ambient and O₃ treatment, making a total of 64 plants in May 2013.

2.3 Measurements

The stem diameter of the seedlings was measured using digital vernier calipers (Mitsutoyo, Kanagawa, Japan) and the height was measured using a scale, four times at one-month intervals from July to October. We calculated the volume of the stem by diameter and height.

In order to quantify the photosynthetic traits of needles, the in-

tercellular CO₂ concentration (C_i) response curve for the net (=apparent) photosynthetic rate (*A*-C_i curve) in fully mature needles was measured in early September 2013 by using an open gas exchange system (Li-6400, Li-Cor Inc., Lincoln, NE, USA). From the *A*-C_i curve, we calculated the maximum rate of carboxylation (*V*_{max}), the maximum rate of electron transport (*J*_{max}), the light and CO₂ saturated photosynthetic rate (*A*_{max}), the net photosynthetic rate at 380 μmol mol⁻¹ CO₂ (*A*₃₈₀) and stomatal conductance (*G*_s) (Long and Bernacchi, 2003). Needles used for measurement were collected and their leaf mass per unit area (LMA) was estimated. The leaf temperature and photosynthetic photon flux density during the measurement were maintained at 25.0 ± 0.5°C and 1500 ± 0.5 μmol m⁻² s⁻¹ at the needle surface, as determined previously (Koike *et al.*, 2012). All collected needles, after measurement, were dried at 80°C. All photosynthetic rates were then calculated based on the needles' dry mass.

For collecting litterfall, the lower 2/3 of height of the seedlings was covered with 2 mm mesh cloth (Krary, Osaka, Japan) from mid-October to late November 2013, by which time the needles were visibly senescent from their changing color. The litterfall was dried at 80°C for a week. The volume increment (cm³) of aboveground per needle litter dry mass (g) was estimated as the needle production efficiency (NPE).

The N concentration in needle litter was measured by an NC analyzer (Model NC 900, Sumica-Shimadzu, Kyoto, Japan). From the difference between the N contents of needles in early

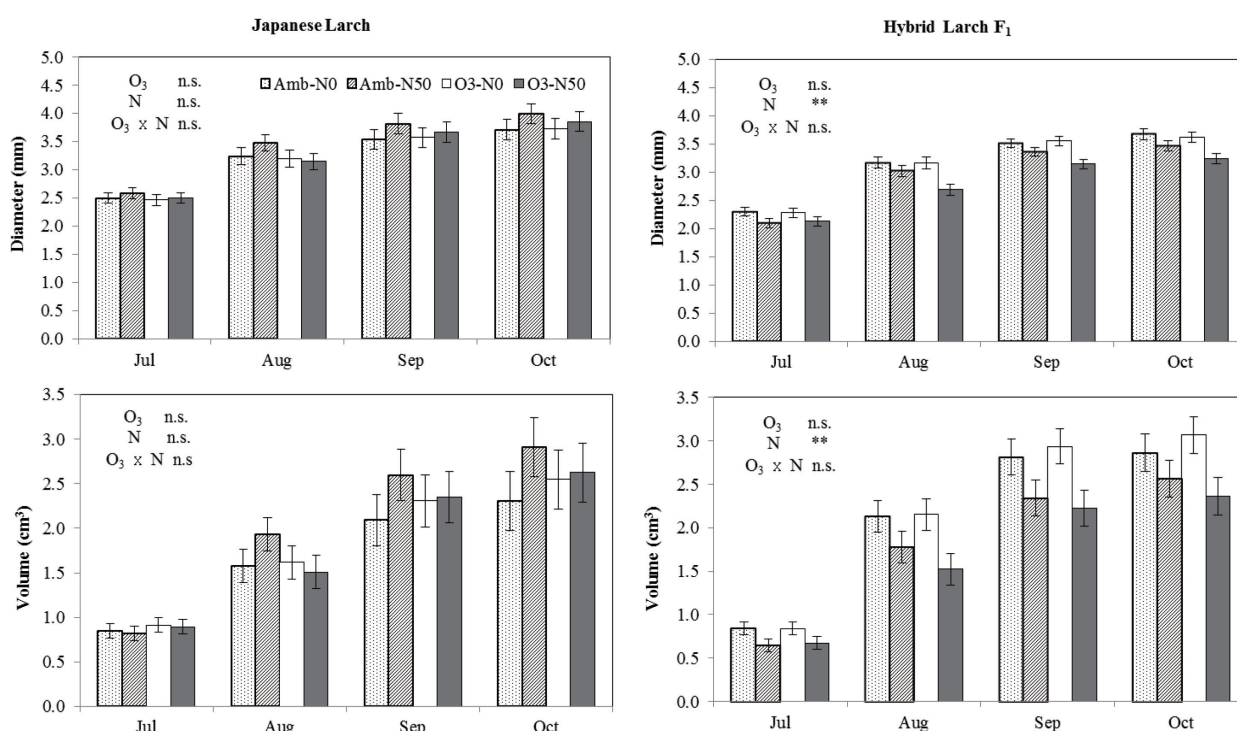


Fig. 1. Stem volume and diameter of Japanese larch and hybrid larch F₁ under O₃ and N loading, measured 4 times, in July (Jul), August (Aug), September (Sep) and October (Oct) 2013. The gas regimes were ambient air (Amb) and elevated O₃ treatment (O₃). The N was supplied as 0 (N0) and 50 (N50) kgN ha⁻¹ yr⁻¹. Repeated measures two-way ANOVA analysis was applied to the values for each species: ***P* < 0.01, n.s. not significant. Each value is the mean of 6 determinations; the error bar indicates the standard error.

September and of needle litter, we estimated the re-translocation rate of N in needle to plant body.

2.4 Statistical analysis

The effects of O₃ and N on stem diameter and volume were analyzed statistically by repeated measures of two-way analysis of variance (ANOVA). Two-way ANOVA was used to test the effects of O₃ and N loading on the dry mass of needle litter, NPE, the N content of needles and the re-translocation rate of N in needles. For photosynthetic parameters, split-plot ANOVA was applied to test the interaction of O₃, N loading and species. All statistical analyses were performed using PASW software (18.0, SPSS Inc., USA). No statistical difference was found between the two sets of potted plants in each ambient or in each O₃ treatment, so we merged the statistically identical samples.

3. Results

3.1 Plant growth

Figure 1 shows seasonal changes in the diameter and volume of the stem of JL and F₁ seedlings. For JLs, there was no significant effect of O₃ and N loading on the diameter and stem volume. In contrast, N loading significantly suppressed the growth of the diameter of F₁s, so that its volume was also suppressed after August. No significant interaction of O₃ and N loading was observed. In all treatments, the two larches flushed their short-shoot needles at 5–6 days after the transplanting and their long shoot needles 20–25 days after the complete expansion of short-shoot needles.

3.2 Needle dry mass and needle production efficiency (NPE)

Figure 2 shows the effects of O₃ and N loading on the dry mass

of needle litter, collected in November 2013, and the stem NPE of the seedlings. The dry mass of needle litter of both larches was lowered significantly by O₃. Regarding the JL at ambient concentration, the litter of needle mass was slightly larger with N loading (Fig. 2 left). NPE was larger for both JLs and F₁s at eO₃. However, there was no significant interaction between O₃ and N loading for either JLs or F₁s.

3.3 Nitrogen in needle litter and re-translocation rate of N

The N concentration in needle litter of both JLs and F₁s was significantly increased by N application (Fig. 3 upper) but it was significantly increased by eO₃ only in F₁s. Also, there was no significant interaction between O₃ and N loading for N concentration in needle litter of either JLs or F₁s. In addition, the re-translocation of N for both species was significantly suppressed by eO₃. However, no significant interaction of O₃ and N loading on N re-translocation was found.

3.4 Photosynthetic traits in needles

Table 1 shows the effects of eO₃ and N loading on the mass-based photosynthetic parameters of JLs and F₁s. The photosynthetic parameters (V_{cmax} , J_{max} and A_{max}) for JLs at eO₃ and for F₁s at ambient O₃ were significantly increased by N loading. The photosynthetic parameters of JLs were not suppressed by O₃ when N50 was applied. The G_s of JLs were marginal larger than of F₁s. However, there was no significant effect of O₃ and N loading on A_{380} and LMA.

4. Discussion

Elevated O₃ suppresses plant growth, but this effect may be in-

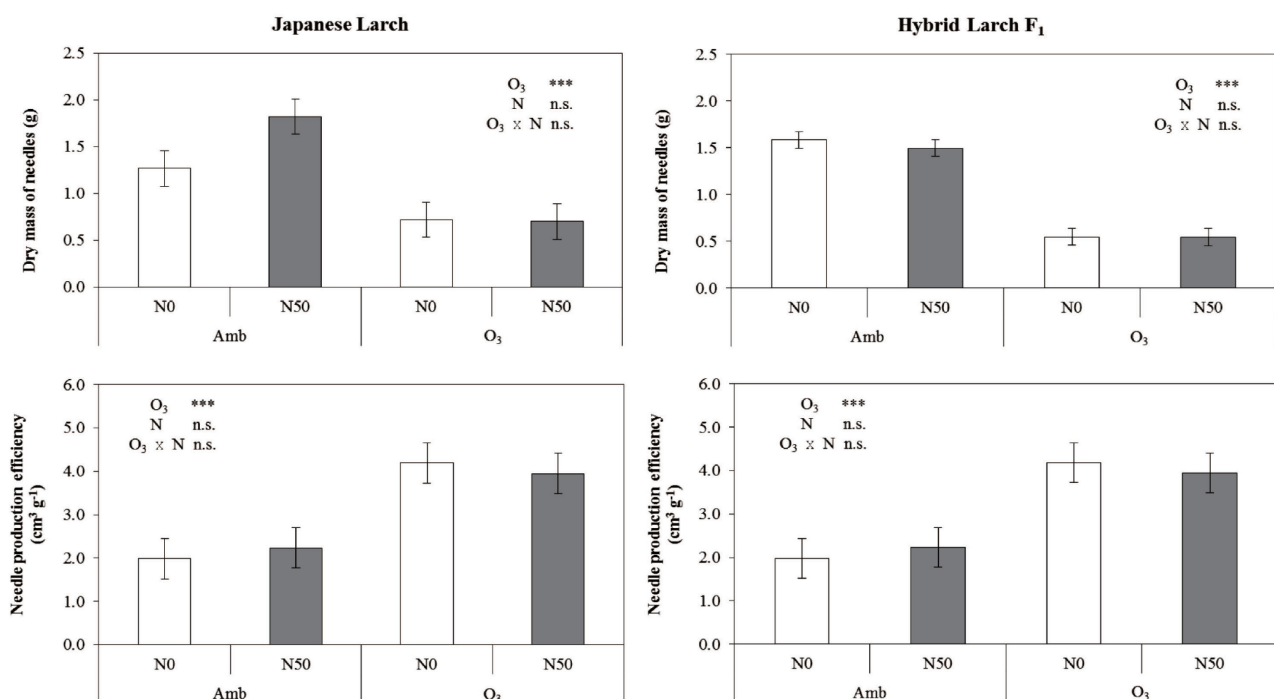


Fig. 2. Dry mass of needle litter and the needle production efficiency of aboveground volume based on the dry mass of needle litter, collected in November 2013. The abbreviations are referred to Figure 1. Two-way ANOVA was applied to the values for each species: *** $P < 0.001$, n.s. not significant. Each value is the mean of 6 determinations; the error bar indicates the standard error.

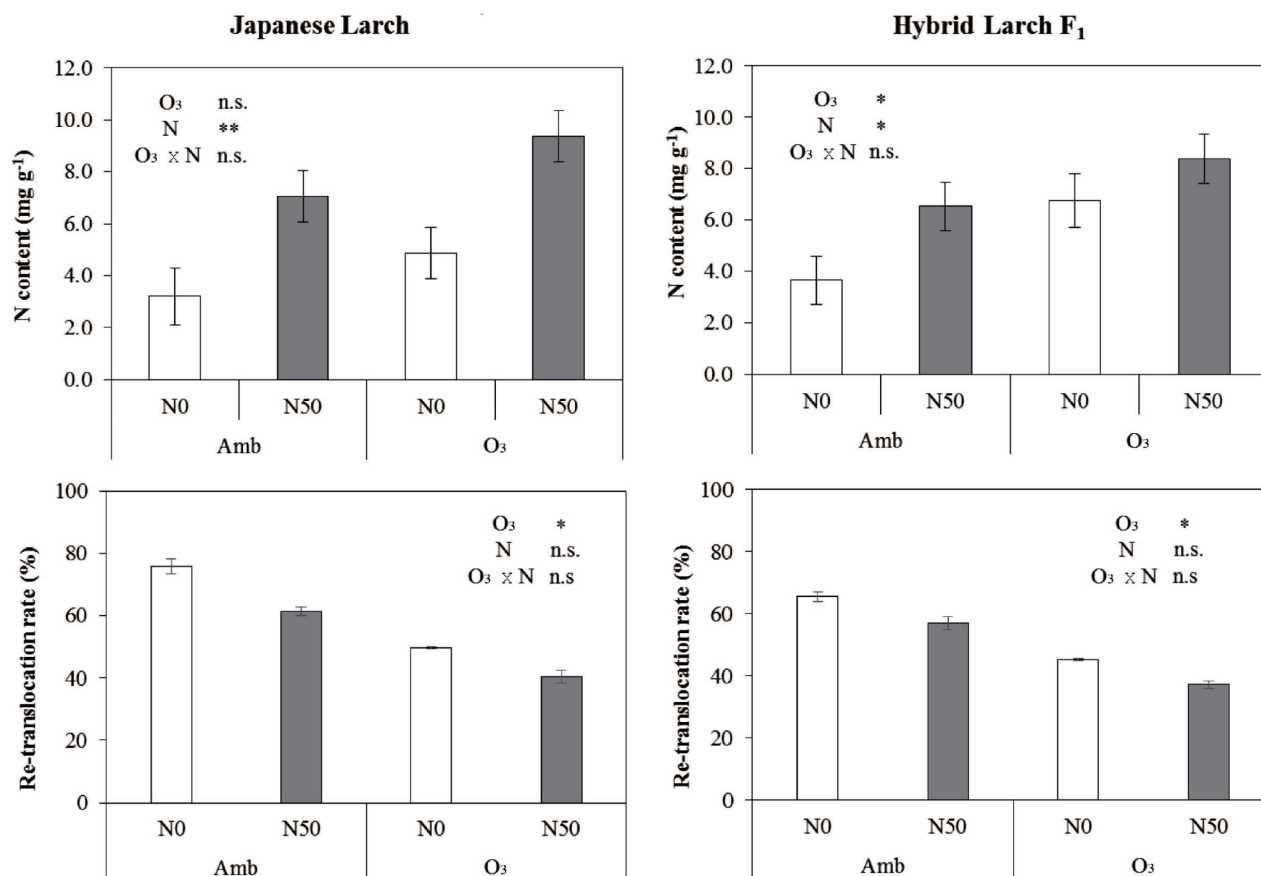


Fig. 3. Dry mass-based nitrogen content of litter and nitrogen re-translocation rate of needles for the Japanese larch and hybrid larch F₁. The abbreviations are the same of Figure 1. Two-way ANOVA was applied to the values for each species: * $P < 0.05$, ** $P < 0.01$, n.s. not significant. Each value is the mean of 6 replications for N content in litter and 3 replication for re-translocation rate; the error bar indicates the standard error.

fluenced by N loading (e.g. Karnosky *et al.*, 2003; Izuta, 2006). We expected N loading to reduce the sensitivity of F₁ seedlings to eO₃, as it has been found in JL seedlings (Watanabe *et al.*, 2006). In fact, many plants increase their susceptibility to O₃ with increasing N loading because physiological activity is usually enhanced by N as a fertilizer to some extent (e.g. Lambers *et al.*, 2008; Schulze *et al.*, 2005). In the present study, we did not find any tendency on the sensitivity to O₃, as neither eO₃ nor N loading influenced the stem growth of the JLs, and N loading decreased that of the F₁s irrespective of O₃ treatments (Fig. 1). The uptake of nutrients may be reduced by the poor development of fine roots due to high N loading as well as the reduction of photosynthate allocation to roots by eO₃ (e.g. Aber *et al.*, 1989; Izuta, 2006). Accordingly, F₁ may suffer a negative effect of excessive N loading through the limited growth of roots.

The N content of needle litter of F₁s at the two N levels was larger at eO₃ than at ambient O₃ (Fig. 3). With leaf senescence, the re-translocation of N is reduced by the excessive N loading, leading to high N content in leaf litter (e.g. Aber *et al.*, 1989; Uddings *et al.*, 2006). The re-translocation of N from needles to non-photosynthetic organs may be suppressed by eO₃ (Fig. 3) because eO₃ accelerates leaf senescence as found in many species

(Matyssek and Sandermann, 2003; Izuta, 2006; Hoshika *et al.*, 2013).

When JLs and F₁s were subjected to eO₃, the dry mass of needle litter was significantly reduced and the NPE was increased (Fig. 2, 3). We consider that small amount of needle litter under eO₃ might be attributed to the small-sized needles formed under eO₃ and/or the suppression in production of long-shoot needles under eO₃ as summarized as heterophyllous leaf development of birch (Kikuzawa and Lechowicz, 2011). Although we did not find any physiological reason why NPE was high in eO₃, NPE in ambient O₃ might be underestimated in the case where root biomass is reduced by eO₃, as reported by previous studies (Izuta, 2006). We should confirm this point by measuring the total plant biomass in future.

In conclusion, as an initial assessment on the interactive effects of eO₃ and N loading on two larch species, the stem growth in F₁s was suppressed by excessive N loading, while the re-translocation of N decreased and NPE increased in both larch species by eO₃. As perennial traits of larch, eO₃-suppressed N re-translocation may aggravate the cumulative imbalance in nutrient status in larches via the altered N cycle. To elucidate the combined effects of eO₃ and N loading on the growth of JLs and F₁s, further con-

Table 1. Effects of elevated O₃ and N loading on the photosynthetic parameters based on needle dry mass of the Japanese larch and hybrid larch F₁ in September 2013.

Parameters	Japanese larch				Hybrid larch F ₁			
	Ambient		Elevated O ₃		Ambient		Elevated O ₃	
	N0	N50	N0	N50	N0	N50	N0	N50
A_{380} (nmol g ⁻¹ s ⁻¹)	63.8 (19.7)	59.0 (10.9)	46.9 (10.6)	82.7 (23.8)	32.6 (3.3)	67.7 (6.6)	36.2 (8.9)	33.2 (7.2)
G_s (nmol g ⁻¹ s ⁻¹)	1.4 (0.2)	1.4 (0.4)	0.6 (0.2)	0.9 (0.3)	0.6 (0.1)	1.1 (0.2)	0.8 (0.1)	0.5 (0.1)
V_{cmax} (nmol g ⁻¹ s ⁻¹)	435.3 (119.7)	419.2 (66.3)	280.0 (41.3)	747.4 (129.4)	222.4 (19.1)	472.5 (59.7)	295.6 (44.2)	246.2 (12.2)
J_{max} (nmol g ⁻¹ s ⁻¹)	1187.6 (248.7)	1269.4 (207.4)	792.1 (48.9)	1523.4 (172.9)	638.5 (75.8)	1188.9 (105.5)	847.2 (68.2)	729.3 (94.3)
A_{max} (nmol g ⁻¹ s ⁻¹)	204.9 (60.8)	256.5 (45.3)	132.9 (19.5)	261.1 (19.5)	117.2 (20.0)	202.2 (11.2)	139.5 (14.5)	119.1 (17.0)
LMA (g m ⁻²)	62.2 (1.5)	79.1 (12.4)	71.0 (1.1)	68.6 (1.3)	95.1 (4.4)	75.6 (11.4)	75.0 (4.7)	83.0 (2.5)
N_{mass} (mg g ⁻¹)	10.8 (1.6)	18.9 (1.6)	11.3 (0.7)	14.3 (2.5)	10.5 (1.4)	15.6 (1.0)	13.5 (0.3)	12.6 (1.8)
ANOVA for Japanese larch and hybrid larch F ₁								
	A_{380}	G_s	V_{cmax}	J_{max}	A_{max}	LMA	N_{mass}	
Air (ambient/O ₃)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Nitrogen	n.s.	n.s.	***	**	*	n.s.	*	
Species	n.s.	*	n.s.	*	*	n.s.	n.s.	
Air x N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Air x species	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
N x species	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Air x N x Species	n.s.	n.s.	*	*	n.s.	*	n.s.	

A_{380} , net photosynthetic rate at 380 $\mu\text{mol mol}^{-1}$ CO₂; G_s , stomatal conductance to water vapor; V_{cmax} , maximum rate of carboxylation; J_{max} , maximum rate of electron transport; A_{max} , maximum net photosynthesis rate; LMA, leaf mass per unit area, N_{mass} , nitrogen content of needle dry mass. Split-plot ANOVA was applied: * P < 0.05, ** P < 0.01, *** P < 0.001, n.s. not significant. Each value is means of three replications for each species and treatment. The stand error is indicated in parenthesis.

secutive study is needed on the morphological and physiological responses over several years, taking into account their storage capability of nutrients and photosynthates.

Acknowledgements

This study was partly supported by the Earth Environmental Research Fund of the Ministry of Environment (B1105) of Japan, and a Grant-in-aid from the JSPS fund (Type B: 26292075, Young Scientists B: 15K16136). We thank to Mr. T. Ueda of DALTON Co. LTD for planning and maintaining our free air O₃ fumigation system and Dr. T. Nakaji in FSC Hokkaido University for NC analysis in the needles. Thanks are also due to Dr. Anthony Garrett of SCITEXT of Cambridge, UK for the intensive English proofreading, and Mr. E. Agathokleous of JSPS fellow for discussion.

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