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<td>Author(s)</td>
<td>Hoshika, Yasutomo; Tatsuda, Shinpei; Watanabe, Makoto; Wang, Xiao-na; Watanabe, Yoko; Saito, Hideyuki; Koike, Takayoshi</td>
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Effect of ambient ozone at the somma of Lake Mashu on growth and leaf gas exchange in *Betula ermanii* and *B. platyphylla* var. *japonica*

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Abstract
Serious die-back of mountain birch have been observed at the somma of Lake Mashu in northern Japan where ozone concentration has been recently elevated in early growing season. We examined effects of ambient ozone \textit{in situ}, on growth and photosynthetic traits of two common birch species in Japan (mountain birch and white birch). Seedlings of the two birch species were grown in open-top chambers and were exposed to charcoal-filtered ambient air (CF) or non-filtered ambient air (NF) at the somma of Lake Mashu during the growing season in 2009. Ambient ozone increased the ratio of above-ground wood (branch + stem) dry mass to root dry mass (T/R ratio) and decreased the maximum photosynthetic rate ($A_{\text{max}}$) in mountain birch, although no difference in the whole-plant dry mass was observed between the treatments. In white birch, in contrast, ozone exposure at ambient level slightly increased the whole plant dry mass, but had no effect on the T/R ratio and photosynthetic variables. Moreover, an increase in individual leaf size and total leaf area was observed as main O$_3$ effect in birches. These results suggest that ambient O$_3$ at the somma of Lake Mashu may have shifted the allocation of biomass to above-ground rather than below-ground in the mountain birch.

Key words: tropospheric ozone; birch; forest decline; biomass allocation; photosynthesis
1. INTRODUCTION

Tropospheric ozone (O$_3$) is a major secondary air pollutant and causes damage to plants (Bytnerowicz et al. 2007; Serengil et al., 2011; Yamaguchi et al., 2011). In Japan the ground surface O$_3$ concentration has been increasing since the late 1980s, and this trend is expected to continue (Yamaji et al., 2008). Ohara et al. (2001) reported that this increase in O$_3$ concentration may be influenced by trans-boundary air pollution. The present O$_3$ concentration in Japan may have a negative effect on the growth of forest tree species (Watanabe et al., 2010; 2012).

Lake Mashu is located in Akan National Park in northern Japan. This lake was formed in the caldera of a potentially active volcano, and has been called the clearest lake in the world. Mountain birch (Betula ermanii) forest covers the somma at Lake Mashu. Birches are typical light demanding species (Koike, 1988; Mao et al., 2010), and these species comprise 12% of the total forest stock in northern Japan (Kawaguchi et al., 2012). Birch forests are natural resources that also constitute tourist attractions as a result of the beautiful scenery of northern Japan. A serious decline or dieback of mountain birch has been recently observed in the somma of Lake Mashu (Yamaguchi and Noguchi, 2011). Ozone might be one of the factors involved in this decline, as a relatively high O$_3$ concentration of at least 60 nmol mol$^{-1}$ (monthly average) is recorded in spring in northern Japan, including the Lake Mashu area (Watanabe, 2011; Yamaguchi and Noguchi, 2011), while O$_3$ concentration in summer is relatively low (about 20 nmol mol$^{-1}$) (Yamaguchi and Noguchi, 2011). This implies that relatively high O$_3$ concentrations are recorded at the beginning of growing
season of birches.

Ozone is known to cause a reduction in photosynthetic rate (e.g., Reich, 1987; Matyssek and Sandermann, 2003). Ozone-induced compensatory leaf growth may be initially stimulated in birch to prevent O₃-induced decline of whole-plant carbon gain, although subsequent O₃ exposure may cause reductions in leaf and stem biomass (Pääkkönen et al., 1996). Also, O₃ may limit photosynthate allocation to roots (Anderson, 2003). We hypothesized that compensatory leaf growth and limitation of root growth may occur simultaneously under O₃ stress in birches at this location, and thus may shift the allocation of biomass to above-ground rather than below-ground. To test this prediction, we examined the effects of ambient O₃ at the somma of Lake Mashu on the growth and photosynthetic traits of the mountain birch (B. ermanii, the dominant tree species in the somma of Lake Mashu), and another common birch species in Japan (the white birch, B. platyphylla var. japonica).

2. MATERIALS AND METHODS

2.1 Plant materials

We studied 2-year-old seedlings of mountain birch (B. ermanii) and white birch (B. platyphylla var. japonica) obtained from the nursery in the town of Naganuma in central part of Hokkaido, i.e. the main area of distribution for these birch species, in order to adjust the acclimation of day length for these birches (e.g. Evans, 1963; Larcher, 2004). The seedlings were grown outdoors in the nursery. The seeds were collected around central Hokkaido, northern Japan. In May 2009, these birch seedlings were planted in 7-liter pots filled with 1:1 (v/v)
mixture of Kanuma pumice soil and clay soil. These soils are nutrient poor, and originate from volcanic ash that is very common in Japan. Diluted liquid fertilizer (N:P:K=6:10:5, Hyponex, Ohio, U.S.A.) was supplied to all potted soils, for a total nitrogen (N) application of 96 mg N pot$^{-1}$. Watering was carried out at 3-7 day intervals to prevent desiccation.

2.2 \textit{O}_3 treatments

The experiment was carried out in open-top chambers (OTC) located in the somma of Lake Mashu, in northern Japan (43°56' N, 144°51' E, 683 m a.s.l., annual mean temperature: 5.0 °C, total precipitation: 1393 mm in 2009). The snow-free period is from late May to late September. The hourly mean ambient \textit{O}_3 concentration has been continuously monitored by commercial \textit{O}_3 analyzer (Dylec, model 1150, Japan) at the measurement site. Fig. 1 shows a seasonal variation of ambient \textit{O}_3 concentration in measurement site. Monthly mean concentration of nitrogen dioxide (NO$_2$) and sulfur dioxide (SO$_2$) were 0.7 nmol mol$^{-1}$ and 0.2 nmol mol$^{-1}$ from May to October, respectively (T. Yamaguchi, personal comm.). All birch seedlings were exposed to the following treatments, from June 2nd to September 1st, 2009: charcoal-filtered ambient air (CF, \textit{O}_3=14.1 nmol mol$^{-2}$ as monthly average value during the experimental period) and non-filtered ambient air (NF, \textit{O}_3=18.5 nmol mol$^{-2}$ as monthly average value during the same period). We measured monthly mean \textit{O}_3 concentrations in OTCs by passive sampler (Ogawa Co., Kobe, Japan) and estimated daytime mean \textit{O}_3 concentration in both treatments as (a ratio between monthly mean \textit{O}_3 concentration in OTCs and those in the ambient condition) × (daytime
hourly mean O₃ concentration in the ambient condition) (Table 1). The CF or NF air was supplied into the chambers from the chamber bottom. Sirocco fan (Mitsubishi, BF23-S, Tokyo, Japan) was attached to OTCs to provide an air of 0.216 m³ s⁻¹, so that the air in the OTC changed 7.5 times a minute. The theoretical wind speed was 0.15 m s⁻¹ at the top of chamber. There were 6 OTCs (1.2m × 1.2m × 1.2m) with three replicate OTCs for both gas treatment. The OTCs were made of a steel frame covered with polyvinyl chloride film in the side panel. The resulting transmittance of sunlight was 88%. Five potted plants per each birch species were set in each OTC.

2.3 Measurement of plant growth

The seedling height at the beginning of the experiment was 12.3±3.4 cm in CF and 12.9±3.7 cm in NF for the mountain birch, and 24.3±4.3 cm in CF and 23.2±4.1 cm in NF for the white birch. The initial stem basal diameter was 2.9±0.6 mm in CF and 3.1±0.7 cm in NF for the mountain birch, and 3.7±0.4 cm in CF and 3.7±0.6 cm in NF for the white birch. There was no statistically difference in height and diameter between CF and NF for the mountain birch and the white birch at the beginning of the experiment (t-test, \( p = 0.76 \) in height and \( p = 0.53 \) in diameter for the mountain birch, \( p = 0.28 \) in height and \( p = 0.92 \) in diameter for the white birch). We measured the height and diameter of the seedlings every month (July 14th, August 6th and September 2nd, 2009). At the end of the experiment (September 2nd, 2009), all seedlings were harvested and separated by organ (i.e., stem, branch, leaf and root). The plant organs were dried at 70°C for 1 week, weighed, and a ratio of branch and stem wood dry mass to root dry
mass (T/R ratio) was calculated. Although the roots reached the bottom of the pot and circled a little, we did not find intertwining roots at the end of the experiment.

2.4 Measurement of leaf gas exchange

To assess the accumulative effect of O₃ on leaf photosynthesis, gas exchange was measured for fully expanded sun leaves at the end of the experimental period (September 1st, 2009) before the first frost came. The measurements were carried out using a portable infra-red gas analyzer (Model 6400, Li-Cor instruments, Lincoln, NE, USA), at controlled values of the leaf temperature (25 °C) and the leaf-to-air vapour pressure deficit (VPD, 1.2 kPa); details are the same as in Watanabe et al. (2011a). For the measurements, three seedlings per treatment-chamber combination were randomly selected for the measurements. The intercellular CO₂ concentration (Cᵢ) response curve of the net photosynthetic rate (A), i.e., the A/Cᵢ curve, was obtained by measurements over 12 steps of CO₂ concentration in the leaf chamber (Cₐ, 50-1700 μmol mol⁻¹). We determined A at growth CO₂ concentration (i.e., 380 μmol mol⁻¹ for CF and NF treatment, A₉ᵢ) and at 1700 μmol mol⁻¹ (Aₘₐₓ), and determined the stomatal conductance at growth CO₂ concentrations (Gₛ). The maximum rate of carboxylation (Vₑₘₐₓ) and the maximum rate of electron transport (Jₚₚ) were calculated from the A/Cᵢ curve (Farquhar et al., 1980; Long and Bernacchi, 2003). The Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) Michaelis constants for CO₂ (Kₑ) and O₂ (Kₒ), and the CO₂ compensation point in the absence of dark respiration (Γ⁺) for the analysis of the A/Cᵢ curve were derived as according to
Bernacchi et al. (2001). All gas exchange measurements were carried out on a day between 9:00 and 15:00.

2.5 Measurement of leaf traits

After the gas exchange measurement, leaves were collected and dried in an oven at 70°C for 1 week and then weighed. Leaf mass per area (LMA) was calculated as the ratio of dry mass to the area of the leaves. Leaf nitrogen (N) contents were determined by gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) after combustion with circulating O₂ using a NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Osaka, Japan).

2.6 Statistics

The main and interactive effects of O₃ and species on leaf traits, gas exchange, growth and plant dry mass were tested via two-way analysis of variance (ANOVA). The effects of O₃ on the relationship between leaf or root dry mass and whole plant dry mass were tested via analysis of covariance for standardized major axes (SMA; model II type regression); results were considered significant at p<0.05. All statistical analyses were performed with SPSS software (20.0, SPSS, Chicago, USA).

3. Results

3.1 Growth and biomass of seedlings

No differences were found in the height and diameter increment in either birch species between the treatments (Table 2). The attached total leaf area was larger in NF than CF (+21% in the mountain birch
and +22% in the white birch) (Table 3).

A significant increase in leaf total dry mass was also found in NF compared to CF (Table 3). A larger whole-plant dry mass of the white birch was recorded under NF than CF, whereas the whole-plant dry mass of the mountain birch did not differ between the treatments. Interactive effect of O₃ and species on T/R ratio of birch seedlings was found with lower T/R ratio in CF than NF for the mountain birch, while O₃ did not affect T/R ratio of the white birch.

Fig. 2 shows a relationship between leaf dry mass or root dry mass and whole plant dry mass of the mountain birch. For this species, the leaf dry mass increased linearly with the whole-plant dry mass (Fig. 2A). A larger ratio of the leaf dry mass to the whole-plant dry mass was recorded in NF than CF. The root dry mass of the mountain birch also increased linearly with the whole-plant dry mass (Fig. 2B). A different y-intercept in the linear relationship was found between the treatments.

3.2 Leaf traits and gas exchange parameters

A significantly smaller value of $A_{\text{max}}$ for the mountain birch was found in NF relative to CF (Table 4). It is noted that $J_{\text{max}}$ for the mountain birch was lower in NF than CF when analysed using a t-test ($p=0.044$). The gas exchange parameters of the white birch did not differ between the treatments.

Individual leaves were larger in the NF than CF treatment (+18% in the mountain birch and +25% in the white birch) (Table 5). There was no difference in LMA and N contents between the treatments and neither between the species.
4. Discussion

We found that ambient O₃ at the somma of Lake Mashu changed an allocation to leaves and roots of birch seedlings; there was an O₃-related increase in leaf dry mass, and T/R ratio also increased in the mountain birch. Studies on European birch (Betula pendula) also reported that O₃ induced compensatory leaf development (Karlsson et al., 2003; Wittman et al., 2007). Pääkkönen et al. (1996) reported that O₃ exposure initially stimulated an increase in leaf area of B. pendula, although subsequent exposure to O₃ caused a reduction in attached leaf area. Shoot development of birch species has been classified as heterophyllous (Koike, 1995), which may facilitate leaf growth as a strategy in compensation for chronic O₃ stress. Such stimulation of leaf growth by O₃ was enhanced in larger plant size in the mountain birch (Fig. 2A). However, no stimulation in root growth was found in NF. Moreover, a lower T/R ratio was found in NF than CF treatment for the mountain birch. The result suggests that seedlings of the mountain birch allocated their photosynthates to above-ground rather than below-ground under ambient O₃ levels at the somma of Lake Mashu.

Leaf gas-exchange measurements revealed that ambient O₃ at the somma of Lake Mashu significantly reduced the value of A_max and J_max for the mountain birch, although the treatment had no effect on the A_growth (Table 4). This result suggests that O₃ may inhibit the Ribulose-1,5-bisphosphate (RuBP) regeneration in the Calvin cycle for the mountain birch, depending on the electron transport rate in the thylakoid membrane and/or the supply or utilization of inorganic
phosphate (e.g., Farquhar et al., 1980; Sharkey, 1985).

Ambient O₃ at the somma of Lake Mashu stimulated the growth of the white birch. There was no reduction in photosynthetic capacity of the white birch leaves exposed to O₃ at the ambient level and the individual leaves were larger in the NF than CF treatment, thereby increasing the total attached leaf area. Gross carbon assimilation per plant may therefore have been enhanced due to the increase in total leaf area. In general, O₃ however reduces leaf size and area in deciduous tree species (e.g., Matyssek et al., 1993; Oksanen, 2003). Matsumura et al. (2005) reported that exposure to O₃ of 66 nmol mol⁻¹ (an average during daytime) for two years led to adverse effects on the growth of the white birch in their OTC experiments at central Japan. The ambient level of O₃ concentration in Lake Mashu was not enough to be harmful for growth of the white birch. The present response may be interpreted as stimulation at low levels of stress, known as a hormetic response (Calabrese, 2005).

The white birch had lower Gₛ than the mountain birch, which may lead to lower stomatal O₃ uptake and stress. Similar findings were reported in a previous OTC experiment for the 3 kinds of Japanese birch species by Hoshika et al. (2012). They reported that O₃ exposure of 60 nmol mol⁻¹ caused a reduction in branch biomass and net photosynthetic rate and an increase in the number of defoliated leaves in the mountain birch, while no adverse effect caused by O₃ was recorded in the white birch. Although Gₛ might have been altered by irrigation and the OTC climate (Ashenden et al., 1992), a previous study also reported that the white birch had lower Gₛ than the mountain birch in chamberless conditions (Watanabe et al., 2011b).
Koike (1995) reported a difference in growth pattern between the mountain birch and the white birch. The mountain birch exhibited a determinate-like growth pattern, i.e., shoot development was ceased at the early summer. On the other hand, the white birch showed an indeterminate growth pattern, i.e., shoot development was continued until autumn. Manninen et al. (2009a, b) pointed out that rapid phenological development and determinate growth pattern may limit the ability to produce new leaves to compensate for the O$_3$ damage. Compared with the white birch, the mountain birch with determinate growth pattern may thus be more susceptible to cumulative effects of O$_3$ on photosynthetic capacity.

In conclusion, our hypothesis was supported by responses we found in the mountain birch. Ambient O$_3$ at the somma of Lake Mashu may shift the allocation of biomass to above-ground rather than below-ground in the mountain birch. At the somma of Lake Mashu, poor root system for the mountain birch was observed and nutrients such as phosphorus are usually deficient in the volcanic ash soils that comprise the surface soil layer (Sakuma et al., 2012). A long-term monitoring on tree growth including above- and below-ground processes in the field is needed, in addition to continuous measurements of O$_3$ concentrations and other environmental factors, to clarify a cause of the decline of trees.

**Acknowledgements**

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Figure captions

Fig. 1 Monthly mean ambient O$_3$ concentration (nmol mol$^{-1}$) at somma of Lake Mashu in 2009.

Fig. 2 Ratio of leaf dry mass (A) and root dry mass (B) to whole plant biomass in mountain birch. The seedlings were grown in charcoal-filtered air (CF, open circle and gray line) and non-filtered air (NF, closed circle and black line) at somma of Lake Mashu.
Fig. 1

Ozone concentration (nmol mol⁻¹)

Fig. 2

(A) Log (leaf dry mass) vs. Log (whole plant dry mass) (g)

Slope: $\mu=0.042$

(B) Log (root dry mass) vs. Log (whole plant dry mass) (g)

y-intercept: $\mu=0.017$
Table 1: Daytime hourly mean O₃ concentrations (nmol mol⁻¹) in charcoal filtered air (CF) and non-filtered air (NF) open-top chambers at somma of Lake Mashu during the experiment.

<table>
<thead>
<tr>
<th>Month</th>
<th>NF</th>
<th>CF</th>
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<tbody>
<tr>
<td>June</td>
<td>27.4 (8.8)</td>
<td>18.4 (5.9)</td>
</tr>
<tr>
<td>July</td>
<td>14.5 (5.4)</td>
<td>10.1 (3.7)</td>
</tr>
<tr>
<td>August</td>
<td>17.0 (5.5)</td>
<td>12.6 (4.1)</td>
</tr>
</tbody>
</table>

Each value is the mean (±SD).
Table 2 Height and diameter increment of mountain birch and white birch seedlings grown in charcoal filtered air (CF) and non-filtered air (NF) open-top chambers at somma of Lake Mashu

<table>
<thead>
<tr>
<th></th>
<th>Mountain birch</th>
<th>White birch</th>
<th>p value for two-way ANOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CF</td>
<td>NF</td>
<td>CF</td>
</tr>
<tr>
<td>Height increment (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 14th</td>
<td>7.6 (0.5)ab</td>
<td>6.1 (3.4)a</td>
<td>12.1 (1.2)bc</td>
</tr>
<tr>
<td>August 6th</td>
<td>11.0 (0.9)ab</td>
<td>9.4 (3.7)a</td>
<td>16.5 (2.0)bc</td>
</tr>
<tr>
<td>September 2nd</td>
<td>11.4 (0.6)ab</td>
<td>11.0 (5.5)a</td>
<td>18.9 (1.2)bc</td>
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Diameter increment (mm)

<table>
<thead>
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<th>White birch</th>
<th>p value for two-way ANOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CF</td>
<td>NF</td>
<td>CF</td>
</tr>
<tr>
<td>July 14th</td>
<td>0.67 (0.19)a</td>
<td>0.37 (0.10)a</td>
<td>1.30 (0.08)b</td>
</tr>
<tr>
<td>August 6th</td>
<td>1.37 (0.31)ab</td>
<td>1.05 (0.13)a</td>
<td>2.00 (0.13)c</td>
</tr>
<tr>
<td>September 2nd</td>
<td>1.53 (0.34)a</td>
<td>1.32 (0.13)a</td>
<td>2.23 (0.16)b</td>
</tr>
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</table>

Each value is the mean (±SD) of three replications. Different letters show significant differences among values within each row (Tukey HSD test, p<0.05).
Table 3 Total leaf area and dry mass of plant organs, and above-ground wood (branch + stem) to root dry mass ratio (T/R) of mountain birch and white birch seedlings grown in charcoal filtered air (CF) and non-filtered air (NF) open-top chambers at somma of Lake Mashu measured on September 2nd, 2009

<table>
<thead>
<tr>
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<th>Mountain birch</th>
<th>White birch</th>
<th>p value for two-way ANOVA</th>
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<tr>
<td></td>
<td>CF</td>
<td>NF</td>
<td>O₃</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>154.5 (11.5)a</td>
<td>187.7 (24.6)a</td>
<td>345.2 (41.9)b</td>
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<tr>
<td>Dry mass (g)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leaf</td>
<td>0.88 (0.03)a</td>
<td>1.01 (0.07)a</td>
<td>1.97 (0.20)b</td>
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<tr>
<td>Branch</td>
<td>0.54 (0.01)a</td>
<td>0.52 (0.10)a</td>
<td>0.97 (0.14)b</td>
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<tr>
<td>Stem</td>
<td>0.78 (0.10)a</td>
<td>0.76 (0.03)a</td>
<td>2.12 (0.15)b</td>
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<td>Root</td>
<td>3.33 (0.14)a</td>
<td>2.97 (0.20)a</td>
<td>6.27 (0.22)b</td>
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<td>Whole plant</td>
<td>5.42 (0.12)a</td>
<td>5.21 (0.33)a</td>
<td>11.33 (0.58)b</td>
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<td>T/R ratio</td>
<td>0.66 (0.04)a</td>
<td>0.76 (0.02)b</td>
<td>0.82 (0.04)b</td>
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Each value is the mean (±SD) of three replications.
Different letters show significant differences among values within each row (Tukey HSD test, p<0.05).
Table 4 Gas exchange traits of leaves of mountain birch and white birch seedlings grown in charcoal filtered air (CF) and non-filtered air (NF) open-top chambers at somma of Lake Mashu measured on September 1st, 2009

<table>
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<tbody>
<tr>
<td></td>
<td>CF</td>
<td>NF</td>
<td>CF</td>
</tr>
<tr>
<td>A_{growth} (μmol m⁻² s⁻¹)</td>
<td>9.3 (0.1)a</td>
<td>9.1 (0.4)ab</td>
<td>7.5 (0.8)b</td>
</tr>
<tr>
<td>Gₛ (mmol m⁻² s⁻¹)</td>
<td>245.7 (26.2)a</td>
<td>210.9 (36.2)ab</td>
<td>132.7 (2.3)c</td>
</tr>
<tr>
<td>V_{cmax} (μmol m⁻² s⁻¹)</td>
<td>45.4 (2.7)a</td>
<td>46.9 (2.4)a</td>
<td>47.5 (5.4)a</td>
</tr>
<tr>
<td>J_{max} (μmol m⁻² s⁻¹)</td>
<td>107.8 (3.8)a</td>
<td>99.5 (3.1)a</td>
<td>85.5 (4.0)b</td>
</tr>
<tr>
<td>A_{max} (μmol m⁻² s⁻¹)</td>
<td>21.2 (0.6)a</td>
<td>19.2 (0.2)b</td>
<td>14.9 (0.9)c</td>
</tr>
</tbody>
</table>

Each value is the mean (±SD) of three replications.

A_{growth}, net photosynthetic rate at growing CO₂ concentration (380 μmol mol⁻¹); Gₛ, stomatal conductance to water vapour; V_{cmax}, maximum rate of carboxylation; J_{max}, maximum rate of electron transport; A_{max}, net photosynthetic rate at 1700 μmol mol⁻¹ CO₂.

Different letters show significant differences among values within each row (Tukey HSD test, p<0.05).
Table 5 Leaf traits of mountain birch and white birch seedlings grown in charcoal filtered air (CF) and non-filtered air (NF) open-top chambers at somma of Lake Mashu measured on September 2nd, 2009

<table>
<thead>
<tr>
<th></th>
<th>Mountain birch</th>
<th></th>
<th>White birch</th>
<th></th>
<th>p value for two-way ANOVA</th>
<th>O₃</th>
<th>Species</th>
<th>O₃ x Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF</td>
<td>NF</td>
<td>CF</td>
<td>NF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf size (cm²/leaf)</td>
<td>6.7 (0.4)a</td>
<td>7.9 (1.5)a</td>
<td>9.1 (0.9)ab</td>
<td>11.4 (1.2)b</td>
<td>0.025</td>
<td></td>
<td>0.002</td>
<td>0.442</td>
</tr>
<tr>
<td>LMA (g m⁻²)</td>
<td>54.2 (2.4)a</td>
<td>54.3 (0.5)a</td>
<td>56.9 (1.9)a</td>
<td>55.0 (0.2)a</td>
<td>0.105</td>
<td></td>
<td>0.323</td>
<td>0.297</td>
</tr>
<tr>
<td>N (g m⁻²)</td>
<td>0.78 (0.13)a</td>
<td>0.80 (0.12)a</td>
<td>0.61 (0.10)a</td>
<td>0.70 (0.09)a</td>
<td>0.427</td>
<td></td>
<td>0.060</td>
<td>0.622</td>
</tr>
</tbody>
</table>

Each value is the mean (±SD) of three replications. LMA, leaf mass per area. N, leaf nitrogen content. Different letters show significant differences among values within each row (Tukey HSD test, p<0.05).