Effect of ambient ozone at the somma of Lake Mashu on growth and leaf gas exchange in *Betula ermanii* and *B. platyphylla* var. *japonica*

[1]Yasutomo HOSHIKA, [1]Shinpei TATSUDA, [1]Makoto WATANABE, [1]Xiao-na WANG, [2]Yoko WATANABE, [1]Hideyuki SAITO and [1]Takayoshi KOIKE

1. *Silviculture and Forest Ecological Studies, Hokkaido University, Sapporo 060-8689, Japan*

2. *Forest Research Station, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-8689, Japan*

Corresponding author: Takayoshi Koike. Tel: +81-11-706-3854, Fax: +81-11-706-2517 E-mail: tkoike@for.agr.hokudai.ac.jp

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 *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_{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 O_3-induced decline of whole-plant carbon gain, although subsequent O_3 exposure may cause reductions in leaf and stem biomass (Pääkkönen et al., 1996). Also, O_3 may limit photosynthate allocation to roots (Anderson, 2003). We hypothesized that compensatory leaf growth and limitation of root growth may occur simultaneously under O_3 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 O_3 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 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 O_3 concentration has been continuously monitored by commercial O_3 analyzer (Dylec, model 1150, Japan) at the measurement site. Fig. 1 shows a seasonal variation of ambient 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, O_3=14.1 nmol mol^{-2} as monthly average value during the experimental period) and non-filtered ambient air (NF, O_3=18.5 nmol mol^{-2} as monthly average value during the same period). We measured monthly mean O_3 concentrations in OTCs by passive sampler (Ogawa Co., Kobe, Japan) and estimated daytime mean O_3 concentration in both treatments as (a ratio between monthly mean O_3 concentration in OTCs and those in the ambient condition) × (daytime hourly mean O_3 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 m^3 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 O_3 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 CO_2 concentration (C_i) response curve of the net photosynthetic rate (A), i.e., the A/C_i curve, was obtained by measurements over 12 steps of CO_2 concentration in the leaf chamber (C_a, 50-1700 μmol mol^{-1}). We determined A at growth CO_2 concentration (i.e., 380 μmol mol^{-1} for CF and NF treatment, A_{growth}) and at 1700 μmol mol^{-1} (A_{max}), and determined the stomatal conductance at growth CO_2 concentrations (G_s). The maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were calculated from the A/C_i curve (Farquhar et al., 1980; Long and Bernacchi, 2003). The Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) Michaelis constants for CO_2 (K_c) and O_2 (K_o), and the CO_2 compensation point in the absence of dark respiration (Γ^*) for the analysis of the A/C_i 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 O_2 using a NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Osaka, Japan).

2.6 Statistics

The main and interactive effects of O_3 and species on leaf traits, gas exchange, growth and plant dry mass were tested via two-way analysis of variance (ANOVA). The effects of O_3 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 O_3 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 O_3 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_{max} for the mountain birch was found in NF relative to CF (Table 4). It is noted that J_{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 O_3 at the somma of Lake Mashu changed an allocation to leaves and roots of birch seedlings; there was an O_3-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 O_3 induced compensatory leaf development (Karlsson et al., 2003; Wittman et al., 2007). Pääkkönen et al. (1996) reported that O_3 exposure initially stimulated an increase in leaf area of *B. pendula*, although subsequent exposure to O_3 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 O_3 stress. Such stimulation of leaf growth by O_3 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 O_3 levels at the somma of Lake Mashu.

Leaf gas-exchange measurements revealed that ambient O_3 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 O_3 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 O_3 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 O_3 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, O_3 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 O_3 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 O_3 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 G_s than the mountain birch, which may lead to lower stomatal O_3 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 O_3 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 O_3 was recorded in the white birch. Although G_s 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 G_s 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

We thank the Environment Research & Technology Development Fund of Japan (B-1105) and a Grant-in-aid from the Japanese Society for Promotion of Science (Type B 23380078, and Young Scientists B

24710027 and ditto B 24780239) for financial support. We are grateful to the permission of Ministry of Environement, Japan for our study and all staff of Teshikaga Town Office, Hokkaido, and Dr. Izumi Noguchi and Dr. Takashi Yamaguchi in Hokkaido Research Organization for their support during this research. We also thank Dr. Anthony Garrett of SCITEXT CAMBRIDGE for proof-reading.

REFERENCES

Anderson, C.P., 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol. 157, 213–228.

Ashenden, T.W., Baxter, R., Rafarel, C.R., 1992. An inexpensive system for exposing plants in the field to elevated concentrations of CO_2. Plant, Cell and Environ. 15, 365-372.

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis Jr., A.R., Long, S.P., 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant, Cell and Environ. 24, 253-259.

Bytnerowicz, A., Omasa, K., Paoletti, E., 2007. Integrated effects of air pollution and climate change on forests: A northern hemisphere perspective. Environ. Pollut. 147, 438–445.

Calabrese, E.J., 2005. Paradigm lost, paradigm found: The re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. Environ. Pollut. 138, 378–411.

Evans. L.T. (1963) Environmental control of plant growth, Academic press.

Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149, 78–90.

Hoshika, Y., Watanabe, M., Inada, N., Koike, T., 2012. Growth and leaf gas exchange in three birch species exposed to elevated ozone and CO_2 in summer. Water Air Soil Pollut. DOI: 10.1007/s11270-012-1253-y.

Karlsson, P.E., Uddling, J., Skärby, L., Wallin, G., Selldén, G., 2003. Impact of ozone on the growth of birch (*Betula pendula*) saplings. Environ. Pollut. 124, 485–495.

Kawaguchi, K., Hoshika, Y., Watanabe, M., Koike, T., 2012. Ecophysiological responses of northern birch forests to changing atmospheric environment. Asian J. Atmos. Environ. 6, 192-205.

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.

Koike, T., 1995. Physiological ecology of the growth characteristics of Japanese mountain birch in northern Japan: a comparison with Japanese mountain white birch, In: E.O. Box et al. (Eds.) Vegetation Science in Forestry: Global Perspective based on Forest Ecosystems of East & Southeast Asia. Kluwer Academic Publishers, The Netherlands, 409-422.

Larcher, W. (2004). Physiological plant ecology 4 ed. Springer Verlag, Berlin, Heidelberg.

Long, S.P., Bernacchi, C.J., 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. Exp. Bot. 54, 2393-2401.

Manninen, S., Huttunen, S., Tømmervik, H., Hole, L.R., Solberg, S., 2009a. Northern plants and ozone. AMBIO 38, 406-413.

Manninen, S., Huttunen, S., Vanhatalo, M., Pakonen, T., Hämäläinen, A., 2009b. Inter- and intra-specific response to elevated ozone and chamber climate in northern birches. Environ. Pollut. 157, 1679-1688.

Mao, Q.Z,, Watanabe, M., Koike, T., 2010. Growth characteristics of two promising tree species for afforestation, birch and larch in the northeastern part of Asia. Eurasian J. For. Res. 13, 69-76.

Matsumura, H., Mikami, C., Sakai, Y., Murayama, K., Izuta, T., Yonekura, T., Miwa, M., Kohno, Y., 2005. Impacts of elevated O_3 and/or CO_2 on growth.of *Betula platyphylla*, *Betula ermanii*, *Fagus crenata*, *Pinus densiflora* and *Cryptomeria japonica* seedlings. J. Agric. Meteorol. 60, 1121-1124.

Matyssek, R., Keller, T., Koike, T., 1993. Branch growth and leaf gas exchange of *Populus tremula* exposed to low ozone concentrations throughout two growing seasons. Environ. Pollut. 79, 1-7.

Matyssek, R., Sandermann, H., 2003. Impact of ozone on trees: an ecophysiological perspective. In: Esser, K., Lüttge, U., Beyschlag, W., Hellwig, F. (Eds.), An Ecophysiological Perspective. Progress in Botany vol.64. Springer-Verlag, Berlin Heidelberg, 349-404.

Oksanen, E., 2003. Responses of selected birch (*Betula pendula* Roth) clones to ozone change over time. Plant Cell Environ. 26, 875-886.

Ohara, T., Uno, I., Wakamatsu, S., Murano, K., 2001. Numerical simulation of the springtime trans-boundary air pollution in East Asia. Water, Air, Soil Pollut. 130, 295-300.

Pääkkönen, E., Vahala, J., Holopainen, T., Karjalainen, R., Kärenlampi, L., 1996. Growth responses and related biochemical and ultrastructural changes of the photosynthetic apparatus in birch (*Betula pendula*) saplings exposed to low concentrations of ozone. Tree physiol. 16, 597-605.

Reich, P.B., 1987. Quantifying plant response to ozone: a unifying theory. Tree physiol. 3, 63-91.

Serengil, Y., Augustaitis, A., Bytnerowicz, A., Grulke, N., Kozovitz, A.R., Matyssek, R., Müller-Starck, G., Schaub, M., Wieser, G., Coskun, A.A., Paoletti, E., 2011. Adaptation of forest ecosystems to air pollution and climate change: a global assessment on research priorities. iForest – Biogeosci. Forestry 4, 44-48.

Sakuma, A., Watanabe, M., Yamaguchi, T., Noguchi, I., Watanabe, T., Wakamatsu, A., Saito, H., Shibuya, M., Koike, T., 2012. Effects of soil conditions and/or terrain conditions on the forest decline of *Betula ermanii* at Lake Mashu somma. Boreal For. Res. 60, 43-44. (in Japanese)

Sharkey, T.D., 1985. Photosynthesis in intact leaves of C3 plants: physics, physiology and rate limitations. Bot. Rev. 51, 53–105.

Watanabe, M., 2011. Changes in atmosphere caused by human activities, In: Society of Boreal Forests (eds.) Forests in Hokkaido, Hokkaido News Paper Co. Ltd., Sapporo, Japan, 18-22. (in Japanese)

Watanabe, M., Matsuo, N., Yamaguchi, M., Matsumura, H., Kohno, Y., Izuta, T., 2010. Risk assessment of ozone impact on the carbon absorption of Japanese representative conifers. Europ. J. For. Res. 129, 421-430.

Watanabe, M., Watanabe, Y., Kitaoka, S., Utsugi, H., Kita, K., Koike, T., 2011a. Growth and photosynthetic traits of hybrid larch F1

(*Larix gmelinii* var. *japonica* × *L. kaempferi*) under elevated CO_2 concentration with low nutrient availability. Tree physiol. 31, 965-975.

Watanabe, M., Mao, Q., Novriyanti, E., Ito, H., Ueda, T., Takagi, K., Sasa, K., Koike, T. 2011b. Photosynthetic activity of 3 birches grown under free air CO_2 enrichment system. Bor. For. Res., 59, 39-40 (in Japanese).

Watanabe, M., Yamaguchi, M., Matsumura, H., Kohno, Y., Izuta, T., 2012. Risk assessment of ozone impact on *Fagus crenata* in Japan: consideration of atmospheric nitrogen deposition. Europ. J. For. Res. 131, 475-484.

Wittman, C., Matyssek, R., Pfanz, H., Humar, M., 2007. Effects of ozone impact on the gas exchange and chlorophyll fluorescence of juvenile birch stems (*Betula pendula* Roth.). Environ. Pollut. 150, 258–266.

Yamaguchi, M., Watanabe, M., Matsumura, H., Kohno, Y., Izuta, T., 2011. Experimental studies on the effects of ozone on growth and photosynthetic activity of Japanese forest species. Asian J. Atmos. Environ. 5, 65-78.

Yamaguchi, T., Noguchi, I., 2011. Atmospheric environment around lake Mashu – Fog and ozone-.Northern Forestry. 63, 30-32. (in Japanese)

Yamaji, K., Ohara, T., Uno, I., Kurokawa, J., Pochanart, P., Akimoto, H., 2008. Future prediction of surface ozone over east Asia using models-3 community multiscale air quality modeling system and regional emission inventory in Asia. J. Geophys. Res. 113, D08306, doi: 10.1029/2007JD008663.

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

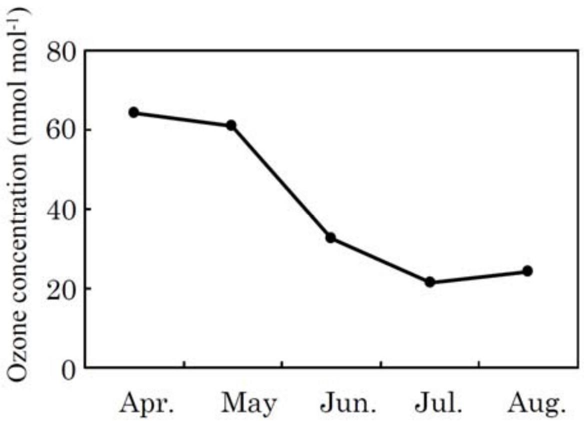
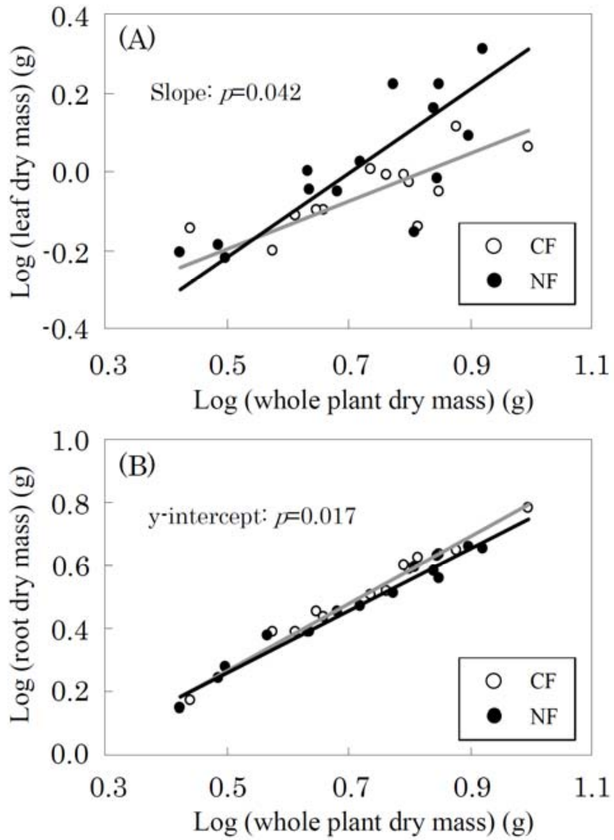

Ozone concentration (nmol mol-1)
80
60
40
20
0
Apr.
May
Jun.
Jul.
Aug.

Fig.2


(A)
Slope: p=0.042
CF
NF
Log (leaf dry mass) (g)
Log (whole plant dry mass) (g)
0.4
0.2
0.0
-0.2
-0.4
0.3
0.5
0.7
0.9
1.1
(B)
y-intercept: p=0.017
CF
NF
Log (root dry mass) (g)
Log (whole plant dry mass) (g)
1.0
0.8
0.6
0.4
0.2
0.0
0.3
0.5
0.7
0.9
1.1

Tables

Table 1 Daytime hourly mean O_3 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

Month	NF	CF
June	27.4 (8.8)	18.4 (5.9)
July	14.5 (5.4)	10.1 (3.7)
August	17.0 (5.5)	12.6 (4.1)

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

	Mountain birch		White birch		*p* value for two-way ANOVA		
	CF	NF	CF	NF	O_3	Species	O_3 x Species
Height increment (cm)							
July 14th	7.6 (0.5)ab	6.1 (3.4)a	12.1 (1.2)bc	13.8 (1.3)c	0.951	**0.001**	0.198
August 6th	11.0 (0.9)ab	9.4 (3.7)a	16.5 (2.0)bc	19.3 (1.3)c	0.642	**<0.001**	0.129
September 2nd	11.4 (0.6)ab	11.0 (5.5)a	18.9 (1.2)bc	23.3 (1.3)c	0.274	**<0.001**	0.187
Diameter increment (mm)							
July 14th	0.67 (0.19)a	0.37 (0.10)a	1.30 (0.08)b	1.31 (0.26)b	0.188	**<0.001**	0.168
August 6th	1.37 (0.31)ab	1.05 (0.13)a	2.00 (0.13)c	1.94 (0.30)bc	0.192	**<0.001**	0.374
September 2nd	1.53 (0.34)a	1.32 (0.13)a	2.23 (0.16)b	2.30 (0.29)b	0.650	**<0.001**	0.362

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

	Mountain birch		White birch		*p* value for two-way ANOVA		
	CF	NF	CF	NF	O_3	Species	O_3 x Species
Leaf area (cm^2)	154.5 (11.5)a	187.7 (24.6)a	345.2 (41.9)b	421.0 (46.5)b	**0.024**	**<0.001**	0.311
Dry mass (g)							
Leaf	0.88 (0.03)a	1.01 (0.07)a	1.97 (0.20)b	2.31 (0.26)b	**0.044**	**<0.001**	0.308
Branch	0.54 (0.01)a	0.52 (0.10)a	0.97 (0.14)b	1.26 (0.11)c	0.053	**<0.001**	**0.027**
Stem	0.78 (0.10)a	0.76 (0.03)a	2.12 (0.15)b	2.37 (0.34)b	0.323	**<0.001**	0.267
Root	3.33 (0.14)a	2.97 (0.20)a	6.27 (0.22)b	7.43 (0.63)c	0.087	**<0.001**	**0.006**
Whole plant	5.42 (0.12)a	5.21 (0.33)a	11.33 (0.58)b	13.38 (1.17)c	**0.046**	**<0.001**	**0.021**
T/R ratio	0.66 (0.04)a	0.76 (0.02)b	0.82 (0.04)b	0.80 (0.02)b	0.069	**<0.001**	**0.012**

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

	Mountain birch		White birch		*p* value for two-way ANOVA		
	CF	NF	CF	NF	O_3	Species	O_3 × Species
A_{growth} (μmol m^{-2} s^{-1})	9.3 (0.1)a	9.1 (0.4)ab	7.5 (0.8)b	8.4 (1.0)ab	0.485	**0.014**	0.124
G_s (mmol m^{-2} s^{-1})	245.7 (26.2)a	210.9 (36.2)ab	132.7 (2.3)c	154.0 (37.0)bc	0.687	**0.009**	0.192
V_{cmax} (μmol m^{-2} s^{-1})	45.4 (2.7)a	46.9 (2.4)a	47.5 (5.4)a	53.3 (4.7)a	0.287	**0.039**	0.195
J_{max} (μmol m^{-2} s^{-1})	107.8 (3.8)a	99.5 (3.1)a	85.5 (4.0)b	88.4 (5.5)b	0.151	**0.006**	0.145
A_{max} (μmol m^{-2} s^{-1})	21.2 (0.6)a	19.2 (0.2)b	14.9 (0.9)c	14.9 (0.5)c	**0.043**	**<0.001**	**0.043**

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

A_{growth}, net photosynthetic rate at growing CO_2 concentration (380 μmol mol^{-1});

G_s, stomatal conductance to water vapour; V_{cmax}, maximum rate of carboxylation;

J_{max}, maximum rate of electron trasport; A_{max}, net photosynthetic rate at 1700 μmol mol^{-1} CO_2.

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

	Mountain birch		White birch		*p* value for two-way ANOVA		
	CF	NF	CF	NF	O_3	Species	O_3 x Species
Leaf size (cm^2/leaf)	6.7 (0.4)a	7.9 (1.5)a	9.1 (0.9)ab	11.4 (1.2)b	**0.025**	**0.002**	0.442
LMA ($g\ m^{-2}$)	54.2 (2.4)a	54.3 (0.5)a	56.9 (1.9)a	55.0 (0.2)a	0.105	0.323	0.297
N ($g\ m^{-2}$)	0.78 (0.13)a	0.80 (0.12)a	0.61 (0.10)a	0.70 (0.09)a	0.427	0.060	0.622

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, $p<0.05$).