



HOKKAIDO UNIVERSITY

Title	Effect of ambient ozone at the somma of Lake Mashu on growth and leaf gas exchange in <i>Betula ermanii</i> and <i>Betula platyphylla</i> var. <i>japonica</i>
Author(s)	Hoshika, Yasutomo; Tatsuda, Shinpei; Watanabe, Makoto et al.
Citation	Environmental and Experimental Botany, 90, 12-16 https://doi.org/10.1016/j.envexpbot.2012.11.003
Issue Date	2013-06
Doc URL	https://hdl.handle.net/2115/52946
Type	journal article
File Information	Manuscript_Lake Mashu OTC.pdf



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

4
5 ¹Yasutomo HOSHIKA, ¹Shinpei TATSUDA, ¹Makoto WATANABE,
6 ¹Xiao-na WANG, ²Yoko WATANABE, ¹Hideyuki SAITO and
7 ¹Takayoshi KOIKE

8
9
10
11
12
13
14 1. *Silviculture and Forest Ecological Studies, Hokkaido University,*

15 *Sapporo 060-8689, Japan*

16 2. *Forest Research Station, Field Science Center for Northern*

17 *Biosphere, Hokkaido University, Sapporo 060-8689, Japan*

18
19 Corresponding author: Takayoshi Koike. Tel: +81-11-706-3854, Fax:
20 +81-11-706-2517 E-mail: tkoike@for.agr.hokudai.ac.jp

21

22

23 **Abstract**

24 Serious die-back of mountain birch have been observed at the somma
25 of Lake Mashu in northern Japan where ozone concentration has been
26 recently elevated in early growing season. We examined effects of
27 ambient ozone *in situ*, on growth and photosynthetic traits of two
28 common birch species in Japan (mountain birch and white birch).
29 Seedlings of the two birch species were grown in open-top chambers
30 and were exposed to charcoal-filtered ambient air (CF) or non-filtered
31 ambient air (NF) at the somma of Lake Mashu during the growing
32 season in 2009. Ambient ozone increased the ratio of above-ground
33 wood (branch + stem) dry mass to root dry mass (T/R ratio) and
34 decreased the maximum photosynthetic rate ($A_{\max}$) in mountain birch,
35 although no difference in the whole-plant dry mass was observed
36 between the treatments. In white birch, in contrast, ozone exposure at
37 ambient level slightly increased the whole plant dry mass, but had no
38 effect on the T/R ratio and photosynthetic variables. Moreover, an
39 increase in individual leaf size and total leaf area was observed as
40 main O₃ effect in birches. These results suggest that ambient O₃ at the
41 somma of Lake Mashu may have shifted the allocation of biomass to
42 above-ground rather than below-ground in the mountain birch.

43

44

45 **Key words:** tropospheric ozone; birch; forest decline; biomass
46 allocation; photosynthesis

47

48

49

50 1. INTRODUCTION

51 Tropospheric ozone (O_3) is a major secondary air pollutant and causes
52 damage to plants (Bytnerowicz et al. 2007; Serengil et al., 2011;
53 Yamaguchi et al., 2011). In Japan the ground surface O_3 concentration
54 has been increasing since the late 1980s, and this trend is expected to
55 continue (Yamaji et al., 2008). Ohara et al. (2001) reported that this
56 increase in O_3 concentration may be influenced by trans-boundary air
57 pollution. The present O_3 concentration in Japan may have a negative
58 effect on the growth of forest tree species (Watanabe et al., 2010;
59 2012)

60 Lake Mashu is located in Akan National Park in northern Japan.
61 This lake was formed in the caldera of a potentially active volcano,
62 and has been called the clearest lake in the world. Mountain birch
63 (*Betula ermanii*) forest covers the somma at Lake Mashu. Birches are
64 typical light demanding species (Koike, 1988; Mao et al., 2010), and
65 these species comprise 12% of the total forest stock in northern Japan
66 (Kawaguchi et al., 2012). Birch forests are natural resources that also
67 constitute tourist attractions as a result of the beautiful scenery of
68 northern Japan. A serious decline or dieback of mountain birch has
69 been recently observed in the somma of Lake Mashu (Yamaguchi and
70 Noguchi, 2011). Ozone might be one of the factors involved in this
71 decline, as a relatively high O_3 concentration of at least 60 nmol mol^{-1}
72 (monthly average) is recorded in spring in northern Japan, including
73 the Lake Mashu area (Watanabe, 2011; Yamaguchi and Noguchi, 2011),
74 while O_3 concentration in summer is relatively low (about 20 nmol
75 mol^{-1}) (Yamaguchi and Noguchi, 2011). This implies that relatively
76 high O_3 concentrations are recorded at the beginning of growing

77 season of birches.

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

93

94 **2. MATERIALS AND METHODS**

95 **2.1 Plant materials**

96 We studied 2-year-old seedlings of mountain birch (*B. ermanii*) and
 97 white birch (*B. platyphylla* var. *japonica*) obtained from the nursery in
 98 the town of Naganuma in central part of Hokkaido, i.e. the main area
 99 of distribution for these birch species, in order to adjust the
 100 acclimation of day length for these birches (e.g. Evans, 1963; Larcher,
 101 2004). The seedlings were grown outdoors in the nursery. The seeds
 102 were collected around central Hokkaido, northern Japan. In May 2009,
 103 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 O₃ 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 O₃ concentration has been continuously monitored by commercial O₃ analyzer (Dylec, model 1150, Japan) at the measurement site. Fig. 1 shows a seasonal variation of ambient O₃ concentration in measurement site. Monthly mean concentration of nitrogen dioxide (NO₂) and sulfur dioxide (SO₂) 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, O₃=14.1 nmol mol⁻² as monthly average value during the experimental period) and non-filtered ambient air (NF, O₃=18.5 nmol mol⁻² as monthly average value during the same period). We measured monthly mean O₃ concentrations in OTCs by passive sampler (Ogawa Co., Kobe, Japan) and estimated daytime mean O₃ concentration in both treatments as (a ratio between monthly mean O₃ 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 (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 CO₂ concentration in the leaf chamber (C_a, 50-1700 μmol mol⁻¹). We determined A at growth CO₂ 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 CO₂ 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 CO₂ (K_c) and O₂ (K_o), and the CO₂ 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 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_{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.

239

240 **4. Discussion**

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

259 Leaf gas-exchange measurements revealed that ambient O₃ at the
 260 somma of Lake Mashu significantly reduced the value of A_{max} and J_{max}
 261 for the mountain birch, although the treatment had no effect on the
 262 A_{growth} (Table 4). This result suggests that O₃ may inhibit the
 263 Ribulose-1,5-bisphosphate (RuBP) regeneration in the Calvin cycle for
 264 the mountain birch, depending on the electron transport rate in the
 265 thylakoid membrane and/or the supply or utilization of inorganic

266 phosphate (e.g., Farquhar et al., 1980; Sharkey, 1985).

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

282 The white birch had lower G_s than the mountain birch, which may
283 lead to lower stomatal O₃ uptake and stress. Similar findings were
284 reported in a previous OTC experiment for the 3 kinds of Japanese
285 birch species by Hoshika et al. (2012). They reported that O₃ exposure
286 of 60 nmol mol⁻¹ caused a reduction in branch biomass and net
287 photosynthetic rate and an increase in the number of defoliated leaves
288 in the mountain birch, while no adverse effect caused by O₃ was
289 recorded in the white birch. Although G_s might have been altered by
290 irrigation and the OTC climate (Ashenden et al., 1992), a previous
291 study also reported that the white birch had lower G_s than the mountain
292 birch in chamberless conditions (Watanabe et al., 2011b).

293 Koike (1995) reported a difference in growth pattern between the
294 mountain birch and the white birch. The mountain birch exhibited a
295 determinate-like growth pattern, i.e., shoot development was ceased at
296 the early summer. On the other hand, the white birch showed an
297 indeterminate growth pattern, i.e., shoot development was continued
298 until autumn. Manninen et al. (2009a, b) pointed out that rapid
299 phenological development and determinate growth pattern may limit
300 the ability to produce new leaves to compensate for the O₃ damage.
301 Compared with the white birch, the mountain birch with determinate
302 growth pattern may thus be more susceptible to cumulative effects of
303 O₃ on photosynthetic capacity.

304 In conclusion, our hypothesis was supported by responses we found
305 in the mountain birch. Ambient O₃ at the somma of Lake Mashu may
306 shift the allocation of biomass to above-ground rather than
307 below-ground in the mountain birch. At the somma of Lake Mashu,
308 poor root system for the mountain birch was observed and nutrients
309 such as phosphorus are usually deficient in the volcanic ash soils that
310 comprise the surface soil layer (Sakuma et al., 2012). A long-term
311 monitoring on tree growth including above- and below-ground
312 processes in the field is needed, in addition to continuous
313 measurements of O₃ concentrations and other environmental factors, to
314 clarify a cause of the decline of trees.

315

316 **Acknowledgements**

317 We thank the Environment Research & Technology Development Fund
318 of Japan (B-1105) and a Grant-in-aid from the Japanese Society for
319 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 Environment, 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 CO₂. *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

- 347 model of photosynthetic CO₂ assimilation in leaves of C3 species.
348 Planta 149, 78–90.
- 349 Hoshika, Y., Watanabe, M., Inada, N., Koike, T., 2012. Growth and
350 leaf gas exchange in three birch species exposed to elevated ozone
351 and CO₂ in summer. Water Air Soil Pollut. DOI:
352 10.1007/s11270-012-1253-y.
- 353 Karlsson, P.E., Uddling, J., Skärby, L., Wallin, G., Selldén, G., 2003.
354 Impact of ozone on the growth of birch (*Betula pendula*) saplings.
355 Environ. Pollut. 124, 485–495.
- 356 Kawaguchi, K., Hoshika, Y., Watanabe, M., Koike, T., 2012.
357 Ecophysiological responses of northern birch forests to changing
358 atmospheric environment. Asian J. Atmos. Environ. 6, 192-205.
- 359 Koike, T., 1988. Leaf structure and photosynthetic performance as
360 related to the forest succession of deciduous broad-leaved trees.
361 Plant Species Biol. 3, 77–87.
- 362 Koike, T., 1995. Physiological ecology of the growth characteristics of
363 Japanese mountain birch in northern Japan: a comparison with
364 Japanese mountain white birch, In: E.O. Box et al. (Eds.) Vegetation
365 Science in Forestry: Global Perspective based on Forest Ecosystems
366 of East & Southeast Asia. Kluwer Academic Publishers, The
367 Netherlands, 409-422.
- 368 Larcher, W. (2004). Physiological plant ecology 4 ed. Springer Verlag,
369 Berlin, Heidelberg.
- 370 Long, S.P., Bernacchi, C.J., 2003. Gas exchange measurements, what
371 can they tell us about the underlying limitations to photosynthesis?
372 Procedures and sources of error. J. Exp. Bot. 54, 2393-2401.
- 373 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 O₃ and/or CO₂ 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

- 401 ultrastructural changes of the photosynthetic apparatus in birch
402 (*Betula pendula*) saplings exposed to low concentrations of ozone.
403 Tree physiol. 16, 597-605.
- 404 Reich, P.B., 1987. Quantifying plant response to ozone: a unifying
405 theory. Tree physiol. 3, 63-91.
- 406 Serengil, Y., Augustaitis, A., Bytnerowicz, A., Grulke, N., Kozovitz,
407 A.R., Matyssek, R., Müller-Starck, G., Schaub, M., Wieser, G.,
408 Coskun, A.A., Paoletti, E., 2011. Adaptation of forest ecosystems to
409 air pollution and climate change: a global assessment on research
410 priorities. iForest – Biogeosci. Forestry 4, 44-48.
- 411 Sakuma, A., Watanabe, M., Yamaguchi, T., Noguchi, I., Watanabe, T.,
412 Wakamatsu, A., Saito, H., Shibuya, M., Koike, T., 2012. Effects of
413 soil conditions and/or terrain conditions on the forest decline of
414 *Betula ermanii* at Lake Mashu somma. Boreal For. Res. 60, 43-44.
415 (in Japanese)
- 416 Sharkey, T.D., 1985. Photosynthesis in intact leaves of C3 plants:
417 physics, physiology and rate limitations. Bot. Rev. 51, 53–105.
- 418 Watanabe, M., 2011. Changes in atmosphere caused by human
419 activities, In: Society of Boreal Forests (eds.) Forests in Hokkaido,
420 Hokkaido News Paper Co. Ltd., Sapporo, Japan, 18-22. (in
421 Japanese)
- 422 Watanabe, M., Matsuo, N., Yamaguchi, M., Matsumura, H., Kohno, Y.,
423 Izuta, T., 2010. Risk assessment of ozone impact on the carbon
424 absorption of Japanese representative conifers. Europ. J. For. Res.
425 129, 421-430.
- 426 Watanabe, M., Watanabe, Y., Kitaoka, S., Utsugi, H., Kita, K., Koike,
427 T., 2011a. Growth and photosynthetic traits of hybrid larch F1

- 428 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) under elevated CO₂
 429 concentration with low nutrient availability. *Tree physiol.* 31,
 430 965-975.
- 431 Watanabe, M., Mao, Q., Novriyanti, E., Ito, H., Ueda, T., Takagi, K.,
 432 Sasa, K., Koike, T. 2011b. Photosynthetic activity of 3 birches
 433 grown under free air CO₂ enrichment system. *Bor. For. Res.*, 59,
 434 39-40 (in Japanese).
- 435 Watanabe, M., Yamaguchi, M., Matsumura, H., Kohno, Y., Izuta, T.,
 436 2012. Risk assessment of ozone impact on *Fagus crenata* in Japan:
 437 consideration of atmospheric nitrogen deposition. *Europ. J. For. Res.*
 438 131, 475-484.
- 439 Wittman, C., Matyssek, R., Pfanz, H., Humar, M., 2007. Effects of
 440 ozone impact on the gas exchange and chlorophyll fluorescence of
 441 juvenile birch stems (*Betula pendula* Roth.). *Environ. Pollut.* 150,
 442 258–266.
- 443 Yamaguchi, M., Watanabe, M., Matsumura, H., Kohno, Y., Izuta, T.,
 444 2011. Experimental studies on the effects of ozone on growth and
 445 photosynthetic activity of Japanese forest species. *Asian J. Atmos.*
 446 *Environ.* 5, 65-78.
- 447 Yamaguchi, T., Noguchi, I., 2011. Atmospheric environment around
 448 lake Mashu – Fog and ozone-.*Northern Forestry.* 63, 30-32. (in
 449 Japanese)
- 450 Yamaji, K., Ohara, T., Uno, I., Kurokawa, J., Pochanart, P., Akimoto,
 451 H., 2008. Future prediction of surface ozone over east Asia using
 452 models-3 community multiscale air quality modeling system and
 453 regional emission inventory in Asia. *J. Geophys. Res.* 113, D08306,
 454 doi: 10.1029/2007JD008663.

455

456 Figure captions

457 Fig.1 Monthly mean ambient O₃ concentration (nmol mol⁻¹) at somma
458 of Lake Mashu in 2009.

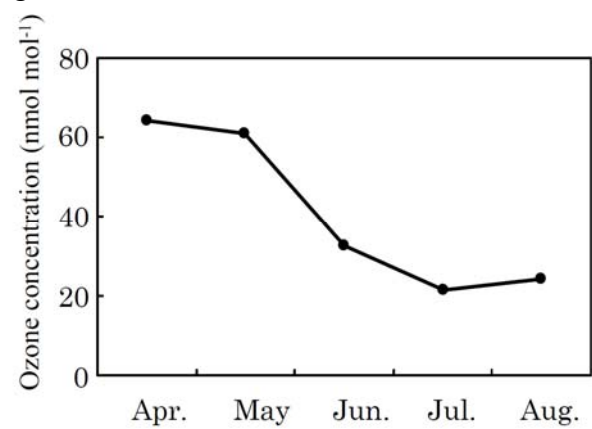
459

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

464

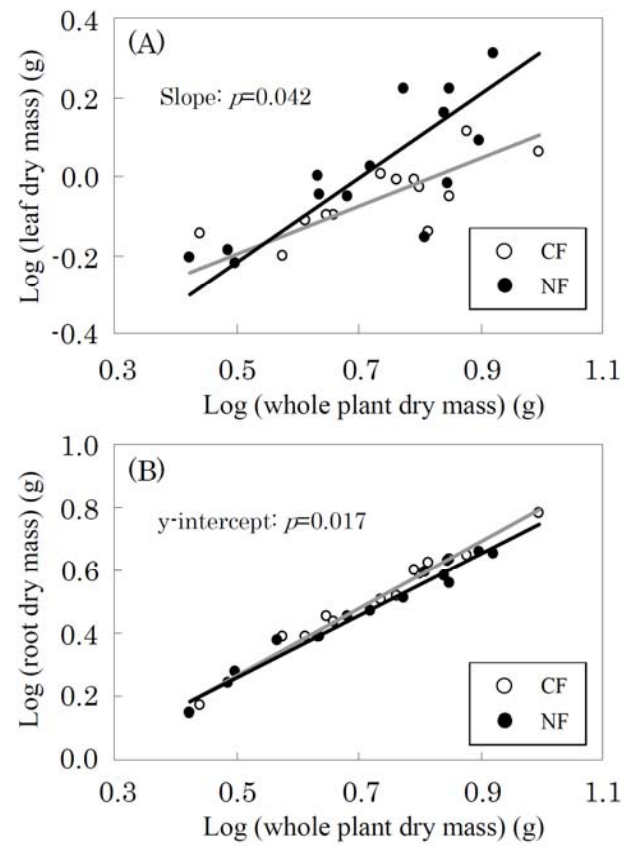
465

466 Fig.1



467
468

469 Fig.2



470

471

472 Tables

473 Table 1 Daytime hourly mean O₃ concentrations (nmol mol⁻¹) in charcoal filtered air (CF) and non-filtered air (NF)
474 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)

475

476

477

478 Table 2 Height and diameter increment of mountain birch and white birch seedlings grown in charcoal filtered
 479 air (CF) and non-filtered air (NF) open-top chambers at somma of Lake Mashu

	Mountain birch		White birch		<i>p</i> 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 ($\pm$ SD) of three replications.

Different letters show significant differences among values within each row (Tukey HSD test, $p < 0.05$).

480

481

482

483 Table 3 Total leaf area and dry mass of plant organs, and above-ground wood (branch + stem) to root dry mass
 484 ratio (T/R) of mountain birch and white birch seedlings grown in charcoal filtered air (CF) and non-filtered air
 485 (NF) open-top chambers at somma of Lake Mashu measured on September 2nd, 2009

	Mountain birch		White birch		<i>p</i> 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 ($\pm$ SD) of three replications.

Different letters show significant differences among values within each row (Tukey HSD test, $p < 0.05$).

486
 487
 488
 489

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		<i>p</i> 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 CO₂ concentration (380 μmol mol⁻¹);

G_s, 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).

494 Table 5 Leaf traits of mountain birch and white birch seedlings grown in charcoal filtered air (CF) and
 495 non-filtered air (NF) open-top chambers at somma of Lake Mashu measured on September 2nd, 2009

	Mountain birch		White birch		<i>p</i> 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).