



HOKKAIDO UNIVERSITY

Title	Photosynthetic responses of Monarch birch seedlings to differing timings of free air ozone fumigation
Author(s)	Watanabe, Makoto; Hoshika, Yasutomo; Koike, Takayoshi
Citation	Journal of plant research, 127(2), 339-345 https://doi.org/10.1007/s10265-013-0622-y
Issue Date	2014-03
Doc URL	https://hdl.handle.net/2115/60042
Rights	The final publication is available at Springer via http://dx.doi.org/10.1007/s10265-013-0622-y
Type	journal article
File Information	JPR2014_for_HUSCAP(Watanabe).pdf



Title

Photosynthetic responses of Monarch birch seedlings to differing timings of free air ozone fumigation

Author names and affiliations

Makoto WATANABE^{1,2}, Yasutomo HOSHIKA^{1,3}, Takayoshi KOIKE¹

¹Silviculture and Forest Ecological Studies, Hokkaido University, Sapporo,
Hokkaido 060–8589, Japan

²Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu,
Tokyo 183–8509, Japan

³Institute of Plant Protection, National Research Council of Italy, Via Madonna
del Piano, I-50019 Sesto Fiorentino, Italy

Abstract

To study the effects of different periods of ozone (O₃) fumigation on photosynthesis in leaves of the Monarch birch (*Betula maximowicziana*), we undertook free air O₃ fumigation to Monarch birch seedlings at a concentration of 60 nmol mol⁻¹ during daytime. Plants were exposed to O₃ at early, late or both periods in the growing season. The light-saturated net photosynthetic rate (A_{sat}) in July and August was reduced by O₃ exposure through a reduction in the maximum rate of carboxylation ($V_{\text{c,max}}$). In early September, on the other hand, despite a reduction in $V_{\text{c,max}}$, A_{sat} was not reduced by O₃ due to a counteracting increase in

the stomatal conductance. Through the experiment, there was no difference in sensitivity to O_3 between maturing and matured leaves. We analyzed the relationship between A_{sat} , $V_{c,max}$ and accumulated stomatal O_3 flux (AF_{st}). Whereas $V_{c,max}$ decreased with increasing AF_{st} , the correlation between A_{sat} and AF_{st} was weak because the response of stomatal conductance to O_3 was affected by season. We conclude photosynthetic response of Monarch birch to O_3 exposure changes with season. This is due to the inconstant stomatal response to O_3 but not due to the response of biochemical assimilation capacity in chloroplasts.

Key words:

Monarch birch (*Betula maximowicziana*); Free air ozone exposure;
Photosynthesis; Stomatal ozone uptake

Introduction

Plants respond to various environmental conditions during their life. The health of plants may be influenced by climate conditions and biotic factors and also human activity-induced environmental changes (see e.g. Schulze et al. 2005). Elevated CO₂, nitrogen deposition and elevated ozone (O₃) are considered to be important factors affecting forest health (Paoletti et al. 2010). Ozone in the troposphere is recognized as a widespread phytotoxic air pollutant, and its concentrations have been increasing **in the Northern Hemisphere** (Akimoto 2003; Izuta 1998; Matyssek and Sandermann 2003; Sitch et al. 2007). In Japan, the annual average daytime concentration of O₃ increased from 1985 to 2007 at a rate of 0.25 nmol mol⁻¹ year⁻¹ (Ohara 2011). It is therefore important to determine the responses of forest tree species in Japan to an elevated O₃ environment.

Birch is a representative deciduous broad leaved tree species **in the Northern Hemisphere** (e.g. Kawaguchi et al. 2012). In Japan, the Mountain birch (*Betula ermanii*), Monarch birch (*Betula maximowicziana*) and White birch (*Betula platyphylla* var. *japonica*) are representative species (Kikuzawa 1983; Koike 1988; Mao et al. 2010; Tabata 1966). Of these three species, only the Monarch birch is found only in Japan (e.g. Kurata 1971).

Since the late 1990s, crown diebacks of century-old Monarch birch have been observed in secondary forests after forest fires and landslides in Hokkaido

island, in northern Japan. Ohno et al. (2008, 2009, 2010) determined in field surveys that drought and insect attack are significant in the decline of Monarch birch stands. Matsuki et al. (2004) reported a difference in defense against herbivores between the three birch species. Monarch birch has weaker chemical defense in leaves, but strong physical defense against insect herbivores due to its high trichome density. Ambient levels of O₃ are known to impair growth and physiological functions of forest tree species, including photosynthesis (Izuta 1998; Matyssek and Sandermann 2003; Yamaguchi et al. 2011), so this gas may induce forest decline of the Monarch birch, as has been observed in other tree species in the USA, Europe and Japan (Bytnerowicz et al. 2004; Kume et al. 2009; Matyssek et al. 2012; Sandermann et al. 1997; Takeda and Aihara 2007).

Information on the susceptibility of Monarch birch to O₃ is limited. A single experimental study, by Hoshika et al. (2012b), determined the responses of growth and photosynthesis in the Monarch birch, Mountain birch and White birch to O₃ exposure in late summer. The Monarch birch has relatively low susceptibility to O₃. Differences in susceptibility to O₃ among the three birch species are not settled from a single experimental study conducted in late summer, however. We need further information of O₃ susceptibility of Monarch birch because of its ecological importance and also for reasons of economics, as the timber of this species is valuable (Hokkaido Forest Tree Breeding Association 2008).

Photosynthesis is one of the most important physiological functions that regulate tree growth and health condition. The accumulated exposure over a threshold of 40 nmol mol^{-1} (AOT40) and the accumulative stomatal flux of O_3 (AF_{st}) have been used as measures of the effect of O_3 on growth and physiological parameters that relate to the photosynthetic rate (e.g. Watanabe et al. 2012; 2013; Zhang et al. 2012). It remains unclear whether the extent of O_3 on photosynthesis is the same throughout the life of leaves. Bagard et al. (2008) reported that O_3 did not negatively affect photosynthesis in newly expanding leaves of poplar seedlings, whereas fully grown leaves exhibited significant damage following O_3 exposure.

In order to assess the impact of O_3 on Monarch birches, we need to determine the effects of O_3 on photosynthesis, taking into account the leaf age. In the present work, we determined the photosynthetic response of individual leaves of Monarch birch seedlings to O_3 fumigation at different timings, and analyzed photosynthetic performance in relation to AF_{st} .

Materials and methods

Plant materials and ozone fumigation

This study was carried out in our free air O_3 fumigation system located at Sapporo Experimental Forest, Hokkaido University, in northern Japan ($43^{\circ}04' \text{ N}$, $141^{\circ}20' \text{ E}$, 15 m a.s.l.). This system is described in full by Watanabe et al. (2013). We

prepared two plots, one a control plot without O₃ fumigation, and the other for elevated O₃ concentrations. The two plots were separated by about 20 m. Each plot comprised a rectangle of sides 5.5 m × 7.2 m and height 5.5 m. We used only part of the system (2.0 m × 1.5 m) for the present study; the rest was used for other studies (e.g. Hoshika et al. 2013a, b; Watanabe et al. 2013). On 15 May 2012, two-year-old seedlings of the Monarch birch were planted in 8.5 l pots filled with a 1:1 (v/v) mixture of Kanuma pumice soil and clay soil. The seedlings were grown in field conditions with no O₃ fumigation until leaved had emerged. To each pot we supplied 500 times diluted liquid fertilizer (N:P:K = 6:10:5, Hyponex Japan, Osaka, Japan) every week, to a total application of 540 mg N per pot.

Fig. 1 shows the time schedule of the present experiment. This is a two-factor factorial experiment comprising two O₃ exposure levels (control and elevated O₃) and two timings of exposure to O₃ (early and late). Twelve seedlings were grown in each of the control and O₃ fumigation plots from 13 June to 3 August (early period). We moved six seedlings in each gas treatment into the other gas treatment on 3 August and let them grow until 26 September (late period). Consequently there were four experimental groups: in the control plot during early and late periods (CC), in the O₃ plot during the early period and later the control plot (OC), in the control plot during the early period and then the O₃ plot (CO) and in the O₃ plot throughout (OO).

The target O₃ concentration in the elevated O₃ plot was 60 nmol mol⁻¹ during daylight hours. The O₃ concentration in the system was monitored continuously by an O₃ monitor (Mod. 202, 2B Technologies, Boulder CO, USA). The observed mean O₃ concentrations $\pm$ standard deviations of the one-hour average O₃ concentrations in the control plot and elevated O₃ plot during daylight hours were 25.0 $\pm$ 10.5 and 63.5 $\pm$ 12.7 nmol mol⁻¹ from 13 June to 3 August, respectively, and 24.1 $\pm$ 10.7 and 62.0 $\pm$ 14.2 nmol mol⁻¹ from 4 August to 26 September, respectively.

Measurement of leaf gas exchange rate

On 7 July, we marked the youngest leaf of each seedling (Fig. 1). The gas exchange rate of the marked leaves was measured on 24 July, 17 August, 4 September and 26 September 2012 using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) with a light-emitting diode (LED) light source (6400-02B, Li-Cor Inc., Lincoln, NE, USA). The measurement was conducted between 8:00 and 15:00 hours. We alternatively measured leaf gas exchange of the seedlings in the four experimental treatments so as to avoid bias caused by diurnal variation of gas exchange traits. The leaf temperature, photosynthetic photon flux and leaf-to-air vapor pressure deficit during measurement were 25.0 $\pm$ 0.5 °C, 1,500 μ mol m⁻² s⁻¹ and 1.2 $\pm$ 0.2 kPa. We determined the net photosynthetic rate (A_{sat}), stomatal conductance to water vapor (g_{sw}), intrinsic water use efficiency ($A_{\text{sat}}/g_{\text{sw}}$),

and intercellular CO₂ concentration (C_i), and calculated the maximum rate of carboxylation ($V_{c,max}$) from A_{sat} and C_i (Farquhar et al. 1980). Values of the Rubisco Michaelis constants for CO₂ (K_c) and O₂ (K_o), and the CO₂ compensation point in the absence of dark respiration (Γ^*) for the calculation of $V_{c,max}$, were estimated from leaf temperature according to Bernacchi et al. (2001). Day respiration was taken as 2 % of $V_{c,max}$ (von Caemmerer 2000). Although several determinations of the net photosynthetic rates, with various values of C_i , are usually used to estimate $V_{c,max}$ (e.g. Long and Bernacchi, 2003), we observed very similar values of $V_{c,max}$ throughout our experiment (data not shown).

Estimation of accumulated stomatal ozone flux

Diurnal courses of the g_{sw} of the leaf, which was the same leaf as in the gas exchange measurement described above, were determined from 11 July to 14 September 2012 using a steady-state diffusion porometer (Model LI-1600, Li-Cor instruments, Lincoln, NE, USA); a total of seven measurements was made. Pooled data from all 567 measurements were used to estimate the parameters of the stomatal conductance model (Jarvis 1976, Emberson et al. 2000a, Mills et al. 2010). Details of the model and estimates for the parameters are given in the supplementary information (Table S1, Fig. S1). Although a slightly smaller slope and larger intercept were observed in the regression line for the measured g_{sw} than the

model-estimated g_s (Fig. S1), the accuracy of estimation was comparable with other studies (e.g. Emberson et al. 2000b, Hoshika et al. 2012a).

Stomatal O_3 uptake (F_{st} ; $\text{nmol } O_3 \text{ m}^{-2} \text{ s}^{-1}$) was calculated as:

$$F_{st} = [O_3] \cdot g_{sO_3} \cdot \frac{r_c}{r_b + r_c} \quad (1)$$

where $[O_3]$ is the atmospheric O_3 concentration, r_b is the leaf boundary layer resistance (s m^{-1}), and g_{sO_3} is the stomatal conductance for O_3 ($g_{sO_3} = g_{sw}/1.65$; $\text{mmol } O_3 \text{ m}^{-2} \text{ s}^{-1}$); the factor 1.65 accounts for the difference in diffusivity of water in air and in O_3 . Also, r_c is the leaf surface resistance ($= 1 / (g_{sO_3} + g_{ext})$; s m^{-1}), and g_{ext} is the external leaf or cuticular conductance (m s^{-1}), chosen as 0.0004 m s^{-1} (Mills et al. 2010). We calculated r_b from the wind speed, u (m s^{-1}), and the cross-wind leaf dimension, L_d (0.07 m for the Monarch birch, obtained as the mean value of 12 leaves $\times$ 6 trees in each O_3 treatment) (Mills et al. 2010):

$$r_b = 1.3 \cdot 150 \cdot (L_d/u)^{0.5} \quad (2)$$

where the factor 1.3 accounts for differences in diffusivity between heat and O_3 .

We calculated the accumulative stomatal flux (AF_{st}) of O_3 with a threshold value of 1

$\text{nmol m}^{-2} \text{ s}^{-1}$ (designated $\text{AF}_{\text{st}1}$) according to Mills et al. (2010).

Statistical analysis

Statistical analyses were performed using PASW Statistics v.18 (IBM, NY, USA). We undertook a two-way analysis of variance to test the effects of early O_3 exposure, late O_3 exposure and their interaction. The relationship between parameters was analysed by Pearson's correlation test.

Results and discussion

The values of A_{sat} of the Monarch birch on 24 July and 17 August were significantly reduced by early and late O_3 exposures, respectively (Table 1). At the same time, a significant or marginally significant reduction was found in g_{sw} and $V_{\text{c,max}}$, but there was no change in C_i . Late O_3 exposure induced a marginally significant increase in $A_{\text{sat}}/g_{\text{sw}}$ on 17 August only. Stomatal conductance is in general regulated so as to maintain the ratio of C_i to ambient CO_2 concentration (Lambers et al. 2008). We therefore believe that the lower value of g_{sw} was not the direct reason for the O_3 -induced reduction in A_{sat} , but rather the balance to lower $V_{\text{c,max}}$. This hypothesis is supported by the increase in $A_{\text{sat}}/g_{\text{sw}}$ on 17 August. Several reports have suggested a decline of photosynthetic activity in chloroplasts rather than stomatal closure is the main cause of the reduction in A_{sat} induced by O_3 (Watanabe et al.

2007; 2013; Yamaguchi et al. 2007), while Kitao et al. (2009) found a decrease of A_{sat} in mature European beech induced by stomatal closure (*Fagus sylvatica*).

No significant effect of O_3 fumigation on A_{sat} was observed on 4 September. On the other hand, g_{sw} and C_i underwent significant increases following late O_3 exposure, and a significant and marginally significant decrease were found for $A_{\text{sat}}/g_{\text{sw}}$ and $V_{\text{c,max}}$, respectively. The unaffected A_{sat} of the seedlings exposed to O_3 in the late period was due to the increase in C_i as a result of high g_{sw} , which counteracted the decrease in A_{sat}/g_s and $V_{\text{c,max}}$. An increase in g_{sw} due to O_3 has been observed by several researchers (Mills et al. 2009; Yamaguchi et al. 2007).

A large reduction in g_{sw} was observed from 17 August to 4 September. Since $V_{\text{c,max}}$ did not fall and $A_{\text{sat}}/g_{\text{sw}}$ became large during this period, photosynthetic activity in chloroplast cannot be the main factor responsible for the large reduction in g_{sw} . There were frequent clear sky days and higher temperature and vapor pressure deficit between 17 August and 4 September (data not shown). It is possible that the leaves experienced drought stress, whereas we maintained damp soil and adequate environmental conditions for photosynthesis and stomatal conductance during the leaf gas exchange measurements, as described in **Materials and methods**.

Fig. 2 shows A_{sat} , $V_{\text{c,max}}$ and g_{sw} plotted against $\text{AF}_{\text{st}1}$. We found a decreasing trend in A_{sat} with increasing $\text{AF}_{\text{st}1}$, but the correlation coefficients were not high (Fig. 2a). One reason of scatter plot is considered as recover of A_{sat} of the OC seedlings

from 24 July to 17 August (see Table 1 and the plot indicated by a solid arrow in Fig. 2a). In addition, unaffected A_{sat} with increasing C_i , especially in OO on 4 September (the plot indicated by dashed arrow in Fig. 2a), also contributed to the weakness of the correlation. In contrast, a relatively high coefficient of correlation was found between $V_{\text{c,max}}$ and $AF_{\text{st}1}$. In particular, if we remove the plot of OC on 17 August (indicated by a dashed arrow in Fig. 2b), the coefficient of correlation would be -0.79 ($P = 0.002$). In regard to the recovery of photosynthetic activity in OC on 17 August, leaf biochemical, physiological and/or structural changes induced by early O_3 exposure (e.g. Günthardt-Goerg et al. 1993; Hoshika et al. 2013a, b; Watanabe et al. 2005) might continue after O_3 exposure ceased on 4 August and increase photosynthesis, although the recovery did not persist to the next measurement in September. There was no significant correlation between g_{sw} and $AF_{\text{st}1}$ (Fig. 2c).

Bagard et al. (2008) found that mature leaves of hybrid poplar seedlings were more sensitive to O_3 than newly expanded leaves of poplar seedlings. Similar findings were reported by Pääkkönen et al. (1995b) and Strohm et al. (1999). The greater resistance of maturing leaves to O_3 could involve anatomical and ultrastructural features (Pääkkönen et al. 1995a), a higher capacity for detoxification (Polle 1997), and compensatory processes that mobilize resources from senescent leaves to expanding ones (Brendley and Pell 1998). Our results did not match these previous studies, however. Comparison of photosynthetic responses for OC on 24

July and CO on 17 August should suffice for analysis of the differing response of maturing and matured leaves (Table 1). The temperature and relative humidity for three weeks before these measurements were similar (data not shown). The rates of reduction of A_{sat} and V_{cmax} as compared to CC were similar in OC on 24 July and CO on 17 August. It follows that the sensitivity of Monarch birch leaves to O_3 does not change with leaf development.

Concentrations of low-molecular-weight secondary metabolites are frequently highest in immature leaves (Herms and Mattson 1992). Immature leaves need stronger chemical defense against herbivores, because of their softness. The concentrated secondary metabolites in immature leaves may affect leaf sensitivity to O_3 , because some secondary metabolites are important for defense against O_3 stress (e.g. Peltonen et al. 2005). However, the concentrations of total phenolics and condensed tannin in Monarch birch leaves were lower than in Mountain birch and White birch (Matsuki et al. 2004; Matsuki and Koike 2004). Accumulation of secondary metabolites might therefore be low even in immature leaves of the Monarch birch, and this might be at least partly why we found no difference in O_3 sensitivity was observed between maturing and matured leaves of Monarch birch seedlings.

We found a significant reduction in photosynthesis in Monarch birch exposed to elevated O_3 , although there was no significant effect due to O_3 in a

previous study (i.e. Hoshika et al. 2012b). The duration of O₃ fumigation was different in the two studies: during daytime (twelve hours average) in the present study, and seven hours per day in Hoshika et al. (2012b). The higher O₃ uptake in the present study might also result in higher susceptibility. The value of g_{st} in young leaves in the present study ranged from 0.47 to 0.65 mol m⁻² s⁻¹, whereas in Hoshika et al. (2012b) it ranged from 0.20 to 0.23 mol m⁻² s⁻¹, so that O₃ uptake in the present study was potentially 2–3 times higher. The soil materials were the same, but the amount of fertilizer was different in the two studies, at 540 mg per pot in the present study and 192 mg pot⁻¹ in Hoshika et al. (2012b). Higher photosynthetic activity supported by good nutrient status would lead to wider stomatal opening and greater O₃ uptake (e.g. Yamaguchi et al. 2007). Several studies have demonstrated a change in O₃ susceptibility of woody species as a result of soil N loading (Häikiö et al. 2007; Pääkkönen and Holopainen 1995; Pell et al. 1995; Watanabe et al. 2006; Yamaguchi et al. 2007).

Based on the results of the present study, we conclude photosynthetic response of Monarch birch to O₃ exposure changes with season. This is due to the inconstant stomatal response to O₃ but due not to the response of biochemical assimilation capacity in chloroplasts. When we assess the impact of O₃ on photosynthesis in the Monarch birch, it is necessary to consider separately the effects of O₃ on stomatal CO₂ uptake and biochemical assimilation capacity in chloroplasts.

Furthermore, we found recovery from the O₃-induced reduction in A_{sat} after O₃ fumigation ceased. A study of this recovery would be valuable.

Acknowledgments

This study was supported partly by the Environment Research and Technology Development Fund (5B-1105) of the Ministry of the Environment of Japan (grant to T. Koike), and by a Grant-in-Aid from the Japan Society for the Promotion of Science through its Type B program (to T. Koike, grant 23380078) and Young Scientists B (to M. Watanabe, grant 24710027 and to Y. Hoshika, grant 24780239). Thanks are also due to Dr. Anthony Garret of SCITEXT-Cambridge for English proofread.

References

- Akimoto H (2003) Global air quality and pollution. *Science* 302:1716–1719
- Bagard M, Le Thiec D, Delacote E, Hasenfratz-Sauder M-P, Banvoy J, Gerard J, Dizengremel P, Jolivet Y (2008) Ozone-induced changes in photosynthesis and photorespiration of hybrid poplar in relation to the developmental stage of the leaves. *Physiol Plant* 134:559–574
- Bernacchi CJ, Singaas EL, Pimentel C, Portis AR, Long SP (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis.

Plant Cell Environ 24:253–259

Brendley BW, Pell EJ (1998) Ozone-induced changes in biosynthesis of Rubisco and associated compensation to stress in foliage of hybrid poplar. Tree Physiol 18:81–90

Bytnerowicz A, Godzik B, Grodzińska K, Frączek W, Musselman R, Manning W, Badea O, Popescu F, Fleischer P (2004) Ambient ozone in forests of the Central and Eastern European mountains. Environ Pollut 130:5–16

Emberson LD, Ashmore MR, Cambridge HM, Simpson D, Tuovinen JP (2000a) Modelling stomatal ozone flux across Europe. Environ Pollut 109:403–413

Emberson LD, Wieser G, Ashmore MR (2000b) Modelling stomatal conductance and ozone flux of Norway spruce: comparison with field data. Environ Pollut 109:393–402

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. Planta 149:78–90

Günthardt-Goerg MS, Matyssek R, Scheidegger C, Keller T (1993) Differentiation and structural decline in the leaves and bark of birch (*Betula pendula*) under low ozone concentrations. Trees 7:104–114

Häikiö E, Freiwald V, Silfver T, Beuker E, Holopainen T, Oksanen E (2007) Impacts of elevated ozone and nitrogen on growth and photosynthesis of European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* × *Populus tremuloides*)

- clones. Can J For Res 37:2326–2336
- Hermes DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Quart Rev Biol 67:283–335
- Hokkaido Forest Tree Breeding Association (2008) Forest tree breeding and forest genetic resources in Hokkaido. Hokkaido Forest Tree Breeding Association. Ebetsu, Hokkaido, Japan (in Japanese)
- Hoshika Y, Watanabe M, Inada N, Koike T (2012a) Modeling of stomatal conductance for estimating ozone uptake of *Fagus crenata* under experimentally enhanced free-air ozone exposure. Water Air Soil Pollut 223:3893–3901
- Hoshika Y, Watanabe M, Inada N, Koike T (2012b) Growth and leaf gas exchange in three birch species exposed to elevated ozone and CO₂ in summer. Water Air Soil Pollut 223:5017–5025
- Hoshika Y, Watanabe M, Inada N, Koike T. (2013a) Model-based analysis of avoidance of ozone stress by stomatal closure in Siebold's beech (*Fagus crenata*). Ann Bot 112:1149–1158
- Hoshika Y, Watanabe M, Inada N, Mao Q, Koike T. (2013b) Photosynthetic response of early and late leaves of white birch (*Betula platyphylla* var. *japonica*) grown under free-air ozone exposure. Environ Pollut 182:242–247
- Izuta T (1998) Ecophysiological response of Japanese forest tree species to ozone,

- simulated acid rain and soil acidification. *J Plant Res* 111:471–480
- Jarvis PG (1976) Interpretation of variations in leaf water potential and stomatal conductance found in canopies in field. *Philos Trans R Soc B* 273:593–610
- Kawaguchi K, Hoshika Y, Watanabe M, Koike T (2012) Ecophysiological responses of northern birch forests to the changing atmospheric CO₂ and O₃ concentration. *Asian J Atmos Environ* 6:192–205
- Kikuzawa K (1983) Leaf survival of woody plants in deciduous broad-leaved forests. 1. Tall trees. *Can J Bot* 61:2133–2139
- Kitao M, Löw M, Heerdt C, Grams TEE, Häberle K.-H, Matyssek R (2009) Effects of chronic elevated ozone exposure on gas exchange responses of adult beech trees (*Fagus sylvatica*) as related to the within-canopy light gradient. *Environ Pollut* 157:537–544
- 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
- Kurata S (1971) Illustrated important forest trees of Japan, vol. 1. Chikyu Shuppan, Tokyo
- Kume A, Numata S, Watanabe K, Honoki H, Nakajima H, Ishida M (2009) Influence of air pollution on the mountain forests along the Tateyama–Kurobe Alpine route. *Ecol Res* 24:821–830
- Lambers H, Chapin III FS, Pons TL (2008) *Plant physiological ecology*, second

edition. Springer, New York

Long SP, Bernacchi CJ (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

Mao Q, 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

Matsuki S., Sano Y., Koike T. (2004) Chemical and physical defence in early and late leaves in three heterophyllous birch species native to northern Japan. *Ann Bot* 93:141–147

Matsuki S., Koike T. (2004) Comparison of leaf life span, photosynthesis and defensive traits across seven species of deciduous broad-leaf tree seedlings. *Ann Bot* 97:813–817

Matyssek R, Sandermann H (2003) Impact of ozone on trees: an ecophysiological perspective. *Progress in Botany* 64:349–404

Matyssek R, Schnyder H, Osswald W, Ernst D, Munch J C, Pretzsch H (2012) *Growth and Defence in Plants*, Springer Ecol St 220

Mills G, Hayes F, Wilkinson S, Davies WJ (2009) Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. *Glob Chang Biol* 15:1522–1533

- Mills G, Pleijel H, Büker P, Braun S, Emberson LD, Harmens H, Hayes F, Simpson D, Grünhage L, Karlsson PE, Danielsson H, Bermejo V, Gonzalez-Fernandez I (2010) Mapping critical levels for vegetation. Revision undertaken in summer 2010 to include new flux-based critical levels and response functions for ozone, in: Mapping manual 2004. International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops
- Ohara T (2011) Why is the increase of tropospheric ozone concentration in mountain and island regions in Japan? Japanese Journal of Ecology 60:77–81 (In Japanese)
- Ohno Y, Umeki K, Watanabe I, Takiya M, Terazawa K, Yasaka M, Matsuki S (2009) Basal area growth and mortality of *Betula maximowicziana* affected by crown dieback in a secondary forest in Hokkaido, northern Japan. J For Res 14:37–43
- Ohno Y, Umeki K, Watanabe I, Takiya M, Terazawa K, Hara H, Matsuki S (2008) Variation in shoot mortality within crowns of severely defoliated *Betula maximowicziana* trees in Hokkaido, northern Japan. Ecol Res 23:355–362
- Ohno Y, Umeki K, Terazawa K, Yasaka M, Watanabe I, Takiya M (2010) Competition as a predisposing factor of crown dieback in a secondary forest of *Betula maximowicziana* in Hokkaido, northern Japan. J For Res 14:37–43
- Pääkkönen E, Holopainen T (1995) Influence of nitrogen supply on the response of

- clones of birch (*Betula pendula* Roth.) to ozone. *New Phytol* 129:595–603
- Pääkkönen E, Holopainen T, Kärenlampi L (1995a) Ageing-related anatomical and ultrastructure changes in leaves of birch (*Betula pendula* Roth.) clones as affected by low ozone exposure. *Ann Bot* 75:285–294
- Pääkkönen E, Metsärinne S, Holopainen T, Kärenlampi L (1995b) The ozone sensitivity of birch (*Betula pendula*) in relation to the developmental stage of leaves. *New Phytol* 132:145–154
- Paoletti E, Schaub M, Matyssek R, Wieser G, Augustaitis A, Bastrup-Birk AM, Bytnerowicz A, Günthardt-Goerg MS, Müller-Starck G, Serengil Y (2010) Advances of air pollution science: from forest decline to multiple-stress effects on forest ecosystem services. *Environ Pollut* 158:1986–1989
- Pell EJ, Sinn JP, Johansen CV (1995) Nitrogen supply as a limiting factor determining the sensitivity of *Populus tremuloides* Michx. to ozone stress. *New Phytol* 130:437–446
- Peltonen PA, Vapaavuori E, Julkunen-Tiitto R (2005) Accumulation of phenolic compounds in birch leaves is changed by elevated carbon dioxide and ozone. *Glob Change Biol* 11:1305–1324
- Polle A (1997) Defense against photooxidative damage in plants. In: Scandalios J (ed) *Oxidative Stress and the Molecular Biology of Antioxidants Defences*. Cold Spring Harbor Laboratory Press, New York, pp 623–666

- Sandermann H, Wellburn A R, Heath RL (1997) Forest decline and ozone. Springer-Verlag, Berlin
- Schulze E-D, Beck E, Müller-Hohenstein K (2005) Plant Ecology, Berlin/Heidelberg: Springer.
- Sitch S, Cox PM, Collins WJ, Huntingford C (2007) Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. *Nature* 448:791–794
- Strohm M, Eiblmeier M, Langebartels C, Jouanin L, Polle A, Sandermann H, Rennenberg H (1999) Responses of transgenic poplar (*Populus tremula* x *P. alba*) overexpressing glutathione synthetase or glutathione reductase to acute ozone stress: visible injury and leaf gas exchange. *J Exp Bot* 50:365–374
- Tabata H (1966) A contribution to the biology of Japanese birches. *Mem Coll Sci Univ Kyoto Ser B* 17:239–271
- Takeda M, Aihara K (2007) Effects of ambient ozone concentrations on Beech (*Fagus crenata*) seedlings in the Tanzawa Mountains, Kanagawa Prefecture, Japan. *J Jpn Soc Atmos Environ* 42:107–117 (In Japanese with English summary)
- von Caemmerer S (2000) Biochemical models of leaf photosynthesis. CSIRO publishing, Collingwood
- Watanabe M, Yonekura T, Honda Y, Yoshidome M, Nakaji T, Izuta T (2005) Effects

- of ozone and soil water stress, singly and in combination, on leaf antioxidative systems of *Fagus crenata* seedlings. J Agric Meteorol 60:1105–1108
- Watanabe M, Yamaguchi M, Iwasaki M, Matsuo N, Naba J, Tabe C, Matsumura H, Kohno Y, Izuta T (2006) Effects of ozone and/or nitrogen load on the growth of *Larix kaempferi*, *Pinus densiflora* and *Cryptomeria japonica* seedlings. J Jpn Soc Atmos Environ 41:320–334
- Watanabe M, Yamaguchi M, Tabe C, Iwasaki M, Yamashita R, Funada R, Fukami M, Matsumura H, Kohno Y, Izuta T (2007) Influences of nitrogen load on the growth and photosynthetic responses of *Quercus serrata* seedlings to O₃. Trees 21:421–432
- Watanabe M, Yamaguchi M, Matsumura H, Kohno Y Koike, T Izuta, T (2012) Risk assessment of ozone impact on *Fagus crenata* in Japan: Consideration of atmospheric nitrogen deposition. Eur J For Res 131:475–484
- Watanabe M, Hoshika Y, Inada N, Wang X, Mao Q, Koike T (2013) Photosynthetic traits of Siebold's beech and oak saplings grown under free air ozone exposure in northern Japan. Environ Pollut 174:50–56
- Yamaguchi M, Watanabe M, Iwasaki M, Tabe C, Matsumura H, Kohno Y, Izuta T (2007) Growth and photosynthetic responses of *Fagus crenata* seedlings to O₃ under different nitrogen loads. Trees 21:707–718

- 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 tree species. *Asian J Atmos Environ* 5:65–87
- Zhang WW, Feng ZZ, Wang XK, Niu JF (2012) Responses of native broadleaved woody species to elevated ozone in subtropical China. *Environ Pollut* 163:149–157

Table 1 Light-saturated net photosynthetic rate (A_{sat}), stomatal conductance for water vapor (g_{sw}), intrinsic water use efficiency ($A_{\text{sat}}/g_{\text{sw}}$), intercellular CO_2 concentration (C_i) and maximum rate of carboxylation ($V_{\text{c,max}}$) of Monarch birch seedlings exposed to differing timing of free air ozone fumigation. See the legend of Fig. 1 for abbreviations relating to the experimental treatment.

		24 Jul.		17 Aug.		4 Sep.		26 Sep.	
A_{sat}	CC	14.9	(0.5)	15.3	(0.5)	10.7	(1.0)	6.5	(0.6)
	OC	12.6	(0.9)	16.2	(0.5)	9.7	(0.2)	5.3	(0.6)
	CO	15.0	(0.3)	13.4	(0.7)	10.1	(0.4)	5.0	(0.6)
	OO	12.6	(1.1)	13.1	(0.2)	10.2	(0.9)	4.0	(1.4)
ANOVA	EE	**		n.s.		n.s.		n.s.	
	LE	n.s.		***		n.s.		n.s.	
g_{sw}	CC	0.65	(0.03)	0.63	(0.08)	0.20	(0.04)	0.23	(0.02)
	OC	0.47	(0.02)	0.66	(0.04)	0.19	(0.02)	0.19	(0.02)
	CO	0.62	(0.06)	0.44	(0.06)	0.24	(0.00)	0.22	(0.05)
	OO	0.52	(0.06)	0.46	(0.04)	0.26	(0.04)	0.17	(0.07)
ANOVA	EE	**		n.s.		n.s.		n.s.	
	LE	n.s.		**		*		n.s.	
$A_{\text{sat}}/g_{\text{sw}}$	CC	23.0	(1.3)	26.6	(3.6)	55.7	(3.9)	31.0	(5.7)
	OC	27.1	(2.5)	24.9	(1.3)	53.9	(4.2)	27.5	(2.3)
	CO	25.5	(2.6)	31.8	(2.9)	42.2	(2.0)	27.0	(4.5)
	OO	25.8	(3.3)	29.5	(2.5)	41.6	(4.8)	34.0	(15.4)
ANOVA	EE	n.s.		n.s.		n.s.		n.s.	
	LE	n.s.		0.085		**		n.s.	
C_i	CC	300.2	(4.2)	299.2	(7.9)	252.7	(5.2)	307.4	(10.9)
	OC	301.2	(5.5)	298.0	(4.6)	259.0	(6.7)	312.3	(4.7)
	CO	296.8	(6.0)	291.3	(6.0)	276.2	(4.0)	318.4	(6.6)
	OO	299.8	(8.2)	295.5	(5.8)	276.8	(7.3)	307.0	(24.4)
ANOVA	EE	n.s.		n.s.		n.s.		n.s.	
	LE	n.s.		n.s.		**		n.s.	
$V_{\text{c,max}}$	CC	70.0	(4.3)	67.0	(3.5)	83.2	(7.2)	27.7	(6.1)
	OC	58.5	(6.8)	68.8	(2.6)	74.8	(4.8)	22.6	(3.2)
	CO	69.1	(4.0)	59.9	(3.2)	67.3	(4.6)	20.7	(2.1)
	OO	59.5	(7.3)	57.2	(2.2)	69.0	(7.3)	17.9	(5.5)
ANOVA	EE	0.083		n.s.		n.s.		n.s.	
	LE	n.s.		**		0.092		n.s.	

Each value is the mean of six measurements; the standard error is shown in parentheses.

ANOVA: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant. The actual P value is shown if $0.05 < P < 0.10$. EE: early O_3 exposure, LE: late O_3 exposure. There was no significant interaction between EE and LE for any parameters and day combination.

Figure legends

Fig. 1 Time schedule of the present study. Seedlings of the Monarch birch were sorted into four experimental groups according to early and late O₃ exposure. Seedlings were grown in the control plot during both early and late periods (CC); in the O₃ plot in the early period and then changed to the control plot (OC); in the control plot in the early period and then the O₃ plot (CO); or in O₃ plot throughout (OO).

Fig. 2 Relationship between accumulative stomatal flux of O₃ with threshold value 1 nmol m⁻² s⁻¹. (AF_{st}1) and net photosynthetic rate (A_{sat} , a), the maximum rate of carboxylation ($V_{\text{c,max}}$, b) and stomatal conductance to water vapor (g_{sw} , c) in leaves of Monarch birch seedlings exposed to differing timing of free air ozone fumigation. The values on the y-axis are relative to the CC treatment. See the legend of Fig. 1 for abbreviation of experimental treatment. The plots indicated by a solid arrow indicate the OC treatment on 17 August. The dashed arrow indicates the OO treatment on 4 September.

Fig. 1

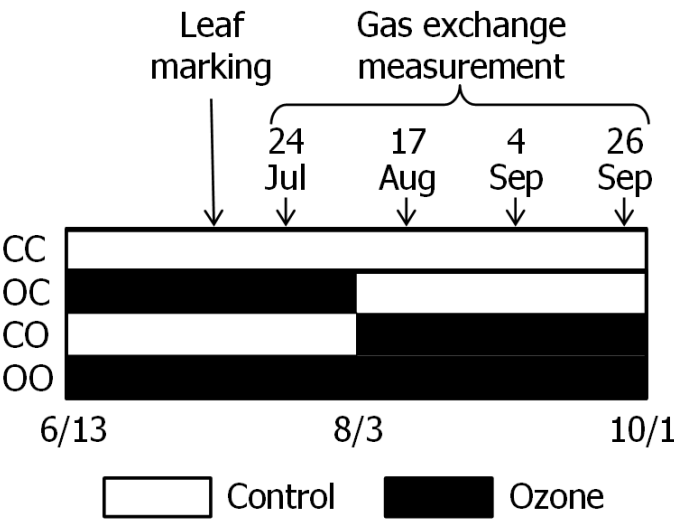
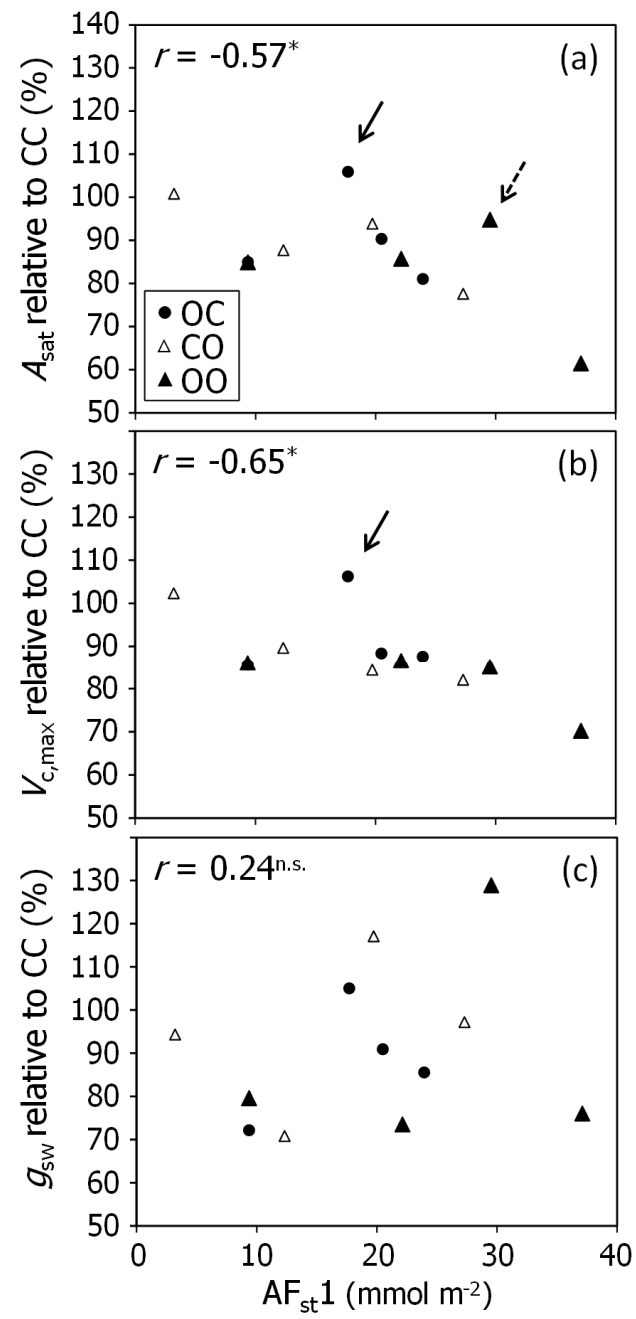


Fig. 2



Supplementary Information

Parameterization of the stomatal conductance model

The stomatal conductance model in the present study was based on the multiplicative algorithm described by Jarvis (1976) and modified by Emberson et al. (2000), as follows:

$$g_{sw} = g_{max} \cdot f_{phen} \cdot f_{light} \cdot \max\{f_{min}, (f_{temp} \cdot f_{VPD} \cdot f_{SWP})\} \quad (1)$$

where g_{max} is the maximum stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}$ Projected Leaf Area (PLA) s^{-1}). The other functions are limiting factors of g_{max} and are scaled from 0 to 1. Here, f_{min} is the minimum stomatal conductance, f_{phen} is the variation in stomatal conductance with leaf age, and f_{light} , f_{temp} , f_{VPD} , and f_{SWP} depend respectively on the photosynthetic photon flux at the leaf surface (PPF, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the temperature (T , $^{\circ}\text{C}$), the vapor pressure deficit (VPD, kPa), and the volumetric soil water potential (MPa).

The response of g_{sw} to phenology (f_{phen}) during the experiments is described as follows:

for $LE \leq DOY \leq (LE + f_{phen_b})$,

$$f_{phen} = (1 - f_{phen_a}) \cdot ((DOY - EE) / f_{phen_b}) + f_{phen_a},$$

for $(LE + f_{phen_b}) \leq DOY \leq (EE - f_{phen_d})$,

$$f_{phen} = 1;$$

for $(EE - f_{phen_d}) \leq DOY \leq EE$,

$$f_{\text{phen}} = (1 - f_{\text{phen_c}}) \cdot ((\text{EE} - \text{DOY}) / f_{\text{phen_d}}) + f_{\text{phen_c}}, \quad (2)$$

where DOY is the day of the year. Here LE is the date of leaf emergence (i.e. July 7th) and EE is the date of end of the experiment (i.e. September 30th). The parameters $f_{\text{phen_a}}$ and $f_{\text{phen_c}}$ denote the maximum fraction of g_{max} at LE and EE, respectively. The parameters $f_{\text{phen_b}}$ and $f_{\text{phen_d}}$ represent the number of days for f_{phen} to reach its maximum and the number of days during the decline of f_{phen} to the minimum value.

The response of g_{sw} to PPF, i.e., f_{light} , is specified as:

$$f_{\text{light}} = 1 - \exp(-a \cdot \text{PPF}) \quad (3)$$

where a is a species-specific parameter defining the shape of the exponential relationship.

The parameter for of air temperature (T , °C) is expressed as:

$$f_{\text{temp}} = \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right) \left\{ \left(\frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} \right)^{\left(\frac{T_{\max} - T_{\text{opt}}}{T_{\text{opt}} - T_{\min}} \right)} \right\} \quad (4)$$

where T_{opt} , $T_{\min}$, and $T_{\max}$ respectively denote the optimum, minimum, and maximum temperature (°C) for stomatal conductance.

The response of g_{sw} to the vapor pressure deficit (VPD, kPa) is given by:

$$f_{\text{VPD}} = \frac{(1 - f_{\text{min}}) \cdot (\text{VPD}_{\text{min}} - \text{VPD})}{\text{VPD}_{\text{min}} - \text{VPD}_{\text{max}}} + f_{\text{min}} \quad (5)$$

where VPD_{min} and VPD_{max} denote the threshold of VPD (kPa) for attaining minimum and full stomatal opening, respectively. If VPD exceeds VPD_{min} then f_{VPD} is set to f_{min} . If VPD is lower than VPD_{max} then f_{VPD} is 1.

Terms describing modification of g_{sw} by the soil moisture (i.e. f_{SWP}) were not used in this study because we keep the soil moisture by irrigation to avoid drought stress.

Parameter estimation was carried out using a boundary line analysis. Firstly, the g_{sw} data were divided into classes with the following step-wise increases for each variable: 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for PPF (when PPF values were less than 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, PPF classes at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ steps were adopted), 2 °C for T and 0.2 kPa for VPD. A function was fitted against each model variable based on 95th percentile values per each class of environmental factors. The g_{max} value corresponds to a maximum value recorded throughout the experiment. We took f_{min} as the 5th percentile values during the daytime period of conductance. The results of parameterization of stomatal conductance model and the relationship between measured g_{sw} and model estimated g_{sw} for Monarch birch seedlings are shown in Table S1, and Fig. S1, respectively.

References

- Emberson LD, Ashmore MR, Cambridge HM, Simpson D, Tuovinen JP (2000) Modelling stomatal ozone flux across Europe. *Environ Pollut* 109: 403–413
- Jarvis PG (1976) Interpretation of variations in leaf water potential and stomatal conductance found in canopies in field. *Philos Trans R Soc B* 273: 593–610

Table S1

Summary of model parameters in stomatal conductance for Monarch birch

$g_{\max}$ (mmol H ₂ O m ⁻² PLA s ⁻¹)	710
$f_{\min}$ (fraction)	0.12
f_{phen}	
$f_{\text{phen_a}}$ (fraction)	0.5
$f_{\text{phen_b}}$ (days)	20
$f_{\text{phen_c}}$ (fraction)	0.3
$f_{\text{phen_d}}$ (days)	36
f_{light}	
a (constant)	0.0075
f_{temp}	
T_{opt} (°C)	22
$T_{\min}$ (°C)	7
$T_{\max}$ (°C)	48
f_{VPD}	
VPD _{max} (kPa)	1
VPD _{min} (kPa)	3.3

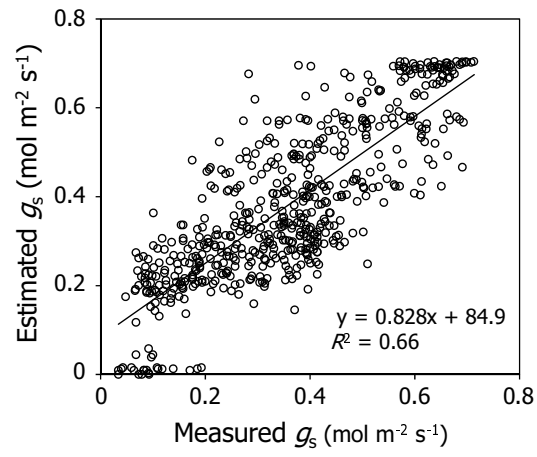


Fig. S1. Relationship between measured stomatal conductance to water vapour (g_{sw}) and estimated g_{sw} by model. Solid line and dashed line indicate regression line and 1:1 line, respectively.