RESEARCH PAPER

Ozone-induced stomatal sluggishness changes stomatal parameters of Jarvis-type model in white birch and deciduous oak

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ABSTRACT

- Stomatal ozone flux is closely related to ozone injury to plants. Jarvis-type multiplicative model has been recommended for estimating stomatal ozone flux in forest trees. Ozone can change stomatal conductance by both stomatal closure and less efficient stomatal control (stomatal sluggishness). However, current Jarvis-type models do not account for these ozone effects on stomatal conductance in forest trees.
- We examined seasonal course of stomatal conductance in two common deciduous tree species native to northern Japan (white birch: *Betula platyphylla* var. *japonica*; deciduous oak: *Quercus mongolica* var. *crispula*) grown under free-air ozone exposure. We innovatively considered stomatal sluggishness in the Jarvis-type model using a simple parameter, *s*, relating to cumulative ozone uptake (defined as POD: phytotoxic ozone dose).
- We found that ozone decreased stomatal conductance of white birch leaves after full expansion (−28%). However, such a reduction of stomatal conductance by ozone fell in late summer (−10%). At the same time, ozone reduced stomatal sensitivity of white birch to VPD and increased stomatal conductance under low light conditions. In contrast, in deciduous oak, ozone did not clearly change the model parameters.
- The consideration of both ozone-induced stomatal closure and stomatal sluggishness improved the model performance to estimate stomatal conductance and to explain the dose–response relationship on ozone-induced decline of photosynthesis of white birch. Our results indicate that ozone effects on stomatal conductance (*i.e.* stomatal closure and stomatal sluggishness) are crucial for modelling studies to determine stomatal response in deciduous trees, especially in species sensitive to ozone.

INTRODUCTION

Tropospheric ozone (O_3) is recognised as a widespread phytotoxic air pollutant and its concentration has been increasing in the northern hemisphere since pre-industrial times (Hartmann *et al.* 2013). The phytotoxic nature of O_3 may cause adverse effects to physiological and biochemical processes in forest trees (Matyssek *et al.* 2013). In northern Japan, white birch (*Betula platyphylla* var. *japonica*) and deciduous oak (*Quercus mongolica* var. *crispula*) are widely distributed representative deciduous trees in the cool-temperate region. White birch is a typical pioneer tree species, which has heterophyllous leaves (Clausen & Kozlowski 1965) and rapidly establishes a forest after large disturbances such as wild fire or landslides (Koike & Sakagami 1985; Koike 1995). Deciduous oak, with several succeeding leaf flushes is a late successional species (Koike 1988). These species have been experimentally investigated in view of their O_3 sensitivities (Kohno *et al.* 2005; Yamaguchi *et al.* 2011; Watanabe *et al.* 2013). Kohno *et al.* (2005) reported that the susceptibilities of growth responses to O_3 in white birch and deciduous oak could be classified as intermediate and less

sensitive, respectively. Recent O_3 risk assessments for forest trees have focused on a stomatal flux basis (Matyssek *et al.* 2013; CLRTAP 2015), because stomata are the principal interface for entry of O_3 into a leaf (Omasa *et al.* 2002). Jarvis-type stomatal conductance models are widely used for modelling stomatal components of air quality (Zhang *et al.* 2003) and are recommended for calculating stomatal O_3 fluxes and assessing O_3 risks for forest trees and crops in Europe (CLRTAP 2015) and East Asia (Izuta 2017). Hence, accurate parameterisation of the stomatal conductance model is essential to develop a stomatal flux-based approach for assessment of O_3 impact on forest trees (Hoshika *et al.* 2012b).

Ozone is known to induce stomatal closure, and may therefore limit stomatal O_3 flux (*e.g.* Wittig *et al.* 2007). In addition, several studies show that O_3 may also cause impairment of stomatal control, especially reduced ability to close stomata, referred to as 'O₃-induced stomatal sluggishness' (Paoletti & Grulke 2005; Hoshika *et al.* 2013b). However, comprehensive parameterisations on these two effects of O_3 on stomatal conductance (*i.e.* stomatal closure and stomatal sluggishness) are not achieved with current stomatal models (Damour *et al.*

2010; CLRTAP 2015). It is a matter of discussion whether O_3 could change the Jarvis-type model parameters.

The aim of the present study was to: (i) parameterise the Jarvis-type model for Japanese white birch and deciduous oak grown under free-air O_3 exposure; (ii) test the performance of the Jarvis-type model with or without consideration of O_3 effects on stomatal conductance (both stomatal closure and stomatal sluggishness) to estimate stomatal O_3 flux in these species; and (iii) apply the Jarvis-type model parameters obtained here to establish dose–response relationships for photosynthesis data published in Hoshika *et al.* (2013a) and Watanabe *et al.* (2015).

MATERIAL AND METHODS

Experimental site

The experimental site was located in Sapporo Experimental Forest, Hokkaido University, northern Japan (43°04′ N, 141°20′ E, 15 m a.s.l., annual mean temperature: 9.3 °C, total precipitation: 1279 mm in 2012). The snow-free period is usually from early May to late December. The soil is brown forest soil. Measurements were carried out in a free-air O_3 exposure experiment (for details of the system; see Watanabe *et al.* 2013). We set up two plots, one for ambient O_3 and another for elevated O_3. Size of each plot was 5.5 m × 7.2 m. The distance between the O_3-enhanced plot and the ambient plot was about 20 m.

We employed representative tree species in northern Japan: white birch (*B. platyphylla* var. *japonica*) and deciduous oak (*Q. mongolica* var. *crispula*) as target species. Ten seedlings of 2-year-old deciduous oak were planted in each plot in May 2003, and were grown under ambient conditions from 2003 to 2010. They were therefore 10 years old when the fumigation with O_3 began in 2011. Also, ten seedlings of 3-year-old white birch were planted in each plot in June 2011. Exposure to O_3 is based on the system used at Kranzberg Forest in Germany (Nunn *et al.* 2002; Werner & Fabian 2002; for details of the system see Watanabe *et al.* 2013). The target O_3 concentration was 60 nmol·mol^{-1} during daylight hours. Our previous paper (Watanabe *et al.* 2013) demonstrated the horizontal distributions of O_3 concentration at 2.5 m (canopy height of birches) and 4.0 m (canopy height of oaks) in the free-air ozone facility and concluded that the average O_3 concentrations at the two heights were similar (50.7 nmol·mol^{-1} at 2.5 m and 52.3 nmol·mol^{-1} at 4.0 m, during the assessment in early November 2011). This enhanced daytime O_3 treatment was applied to target trees from August to November 2011, and from May to November 2012. Ozone concentrations at canopy height were recorded continuously with an O_3 monitor (Mod. 202, 2B Technologies, Boulder CO, USA). The daytime hourly mean O_3 concentrations in ambient and elevated O_3 were 25.7 ± 11.4 nmol·mol^{-1} and 56.7 ± 10.5 nmol·mol^{-1} during the experimental period in 2011, and 27.5 ± 11.6 nmol·mol^{-1} and 61.5 ± 13.0 nmol·mol^{-1} during the experimental period in 2012. Soil moisture was measured in the root layer (depth 20 cm) with 10HS sensors equipped with an EM5b data logger (Decagon Devices, Pullman WA, USA). The average soil moisture (volumetric soil water content) was 28.1 ± 2.8% during these measurements. These values were close to field capacity (32%).

Parameterisation of the stomatal conductance model

Diurnal courses of stomatal conductance were measured using a steady-state diffusion porometer (Model LI-1600; Li-Cor, Lincoln, NE, USA) from June to September 2012. All measurements were conducted on fully expanded sun leaves (4th to 8th leaf from the tip of the shoots) at the top of the canopy of five or six trees in each ambient and enhanced O_3 treatment. In white birch, late leaves were selected as target because birch has heterophyllous leaves, *i.e.* a first flush of early leaves, then expansion of late leaves (Clausen & Kozlowski 1965). Late leaves contribute to carbon gain in the summer season (Koike & Sakagami 1985; Koike 1995). Pooled data (430 and 428 measurements in white birch and deciduous oak, respectively) were used to estimate the parameters of the stomatal conductance model.

Our stomatal conductance model was based on the simple multiplicative algorithm, modified from Jarvis (1976) and Emberson *et al.* (2000), as follows:

$$g_{sw} = g_{max} \cdot f_{phen} \cdot f_{O3} \cdot f_{light} \cdot f_{VPD} \cdot f_{SWP} \qquad (1)$$

where g_{max} is the maximum stomatal conductance [mol·H$_2$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. f_{phen} is the variation in stomatal conductance with leaf age. Here f_{O3}, f_{light}, f_{VPD} and f_{SWP} are functions of the O_3 concentration (nmol·mol^{-1}), photosynthetically relevant photon flux density at the leaf surface (PPFD, μmol·photons·m^{-2}·s^{-1}), vapour pressure deficit (VPD, kPa), and volumetric soil water potential (MPa). Here we did not consider the temperature function (denoted as f_{temp} in general) because our measurements did not include freezing temperatures and temperature data are strongly correlated with VPD (Massman & Kaufmann 1991; Misson *et al.* 2004; Oue *et al.* 2008).

The variation in stomatal conductance with leaf age (f_{phen}) is described as follows (Kitajima *et al.* 2002):

$$f_{phen} = 1 - p \cdot [\text{Leaf age}] \qquad (2)$$

where p is the ratio of the reduction of stomatal conductance with leaf age. [Leaf age] is the number of days since full expansion of a leaf.

Elevated O_3 concentration may reduce stomatal conductance (Kitao *et al.* 2009; Hoshika *et al.* 2013b, 2015a,b; Matyssek *et al.* 2015). Therefore f_{O3} can be expressed as:

$$f_{O3} = 1 - q \cdot [O_3] \qquad (3)$$

where q is the parameter reflecting stomatal sensitivity to O_3 concentration, and [O_3] is hourly mean O_3 concentration (nmol·mol^{-1}).

We modified the exponential function of f_{light} suggested by Emberson *et al.* (2000) to consider the minimum stomatal conductance (f_{min}: relative stomatal conductance to g_{max} at PPFD = 0). This is because g_{sw} may not be zero at PPFD = 0 (Matyssek *et al.* 1995; Oue *et al.* 2008; Hoshika *et al.* 2013b). The f_{light} is expressed as:

$$f_{light} = (1 - f_{min}) \cdot \{1 - \exp(a \cdot \text{PPFD})\} + f_{min} \qquad (4)$$

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

Oren *et al.* (1999) suggested a logarithmic function of the response of g_{sw} to VPD:

$$f_{VPD} = \min(1, 1 - m \cdot \ln[VPD]) \qquad (5)$$

where m denotes the sensitivity of g_{sw} to VPD $[\ln(kPa)^{-1}]$. A hydraulic model, which assumed stomatal regulation of leaf water potential, suggested that m is ~0.6 (e.g. Oren *et al.* 1999).

Terms describing modification of stomatal conductance by soil moisture (*i.e.* f_{SWP}) were not used in this study. As mentioned above, the soil moisture approached field capacity, and no reductions in stomatal conductance due to soil water content were recorded (data not shown). Also in the previous studies no effect of soil water content on tree g_{sw} is often reported in Japan (Hiyama *et al.* 2005; Hoshika *et al.* 2012b).

Ozone may induce slower or less efficient stomatal control to environmental stimuli, *i.e.* stomatal sluggishness. Each parameter of stomatal responses might be modified by stomatal sluggishness (*e.g.* q for f_{O3}, a for f_{light}). Hoshika *et al.* (2012a) suggested that the degree of sluggishness of stomatal light response increased with increasing cumulative O_3 uptake (defined as phytotoxic O_3 dose (POD); CLRTAP 2015). We therefore employed the modification factor by stomatal sluggishness (s), which is expressed as:

$$s = r \cdot POD \qquad (6)$$

where r is an empirical factor, POD is a phytotoxic O_3 dose (mmol·m^{-2}, see equation 10). Ozone may reduce the sensitivity parameters, q, a and m in equations (3)–(5) and may decrease the closing response of stomata under low light conditions (denoted as $(1-f_{min})$) in equation (4). Those parameters therefore can be modified as:

$$
\begin{aligned}
q &= (1-s) \cdot q' \\
a &= (1-s) \cdot a' \\
m &= (1-s) \cdot m' \\
1-f_{min} &= (1-s) \cdot (1-f'_{min})
\end{aligned} \qquad (7)
$$

where q', a', m' and f_{min} are the parameters without stomatal sluggishness for stomatal responses to O_3, PPFD, VPD and minimum conductance, respectively (obtained from measurements under ambient conditions in June). The parameter s can range from 0 to 1. Here we propose the new equations for the Jarvis-type model as the combination of equations (1) and (7) to consider the O_3-induced stomatal sluggishness.

The parameterisation was made using the following procedure: (i) presumed non-limiting g_{sw} data (under PPFD > 500μmol·m^{-2}·s^{-1}, VPD < 1.2 kPa) were extracted; (ii) a potential g_{max} value without O_3 (=0 nmol·mol^{-1}) was estimated by extrapolation from data in ambient and elevated O_3 (Figure S1), and this potential g_{max} can be interpreted as the g_{max} without O_3 effects; (iii) the extrapolation line between the g_{sw} data and hourly mean O_3 concentration indicates the O_3-induced stomatal closure response (f_{O3}) and the parameter

q in equation (3) can be calculated (Figure S1); (iv) a trend of the estimated potential g_{max} values with leaf age indicates the variation in stomatal conductance with leaf age without O_3 effects (f_{phen}) and p in equation (2) can be calculated (Figure S2, dotted line); (v) the f_{VPD} was estimated by fitting the functional form $(1 - m \cdot \ln[VPD])$ to presumably non-light-limited data (PPFD > 500 μmol·m^{-2}·s^{-1}); (vi) The determination of f_{min} was achieved as the average of g_{sw} measurements at sunset (~19:00 h, PPFD < 10 μmol·m^{-2}·s^{-1}); (vii) we fitted the f_{light} function to g_{sw} data measured under VPD < 1.2 kPa using the determined f_{min}.

We assumed that the sluggishness factor (s) might be related to POD and the empirical parameter r equations (6, 7). We used the iterative method (Kercher & Chambers 2001; Martin *et al.* 2001) to estimate the empirical parameter r. Here r is described as r_i for explanation (i is the number of iteration). The computation starts from $r_0 = 0$ (no sluggishness) using the parameters without stomatal sluggishness (q', a', m' and f'_{min}, obtained from measurements under ambient conditions in June). By calculating POD, the parameter r_1 was obtained to minimise the error (RMSE, Root Mean Square Error) of the model estimation of g_{sw} (using Solver in Microsoft Excel). Then, using the value of r_1, we again calculated POD to obtain values of s_2. On average, less than five iterations were needed to achieve a deviation of 5% in the successive value of r.

To estimate stomatal O_3 flux in white birch and deciduous oak, we compared the performance of the Jarvis-type models: (i) with consideration of O_3 effects on stomatal conductance ($+f_{O3}$ and $+s$), and (ii) without consideration of O_3 effects on stomatal conductance (no f_{O3} and no s, *i.e.* $f_{O3} = 1$ and $s = 0$).

Table 1. Summary of model parameters in stomatal conductance for white birch (*B. platyphylla* var. *japonica*) and deciduous oak (*Q. mongolica* var. *crispula*) obtained from measured data in 2012. g_{max}, maximum stomatal conductance; f_{phen}, variation of stomatal conductance with leaf age; f_{O3}, f_{light} and f_{VPD} depend on daily mean O_3 concentration (nmol·mol^{-1}), photosynthetically relevant photon flux density at the leaf surface (PPFD, μmol·m^{-2}·s^{-1}) and VPD (kPa), respectively; p', fraction of decline of stomatal conductance with leaf age; q', fraction of decline of stomatal conductance with O_3 concentration; f'_{min}, relative stomatal conductance to g_{max} at PPFD = 0 (measured at sunset ~19:00 h); a' is the parameter determining an exponential curve of stomatal response to light, and m' is a sensitivity parameter of stomatal response to VPD; p', q', f'_{min} and m' are parameters without ozone-induced stomatal sluggishness (obtained from the measurements under ambient conditions in June); s is the modification factor of stomatal responses to environmental stimuli due to stomatal sluggishness; r is an empirical parameter to determine s.

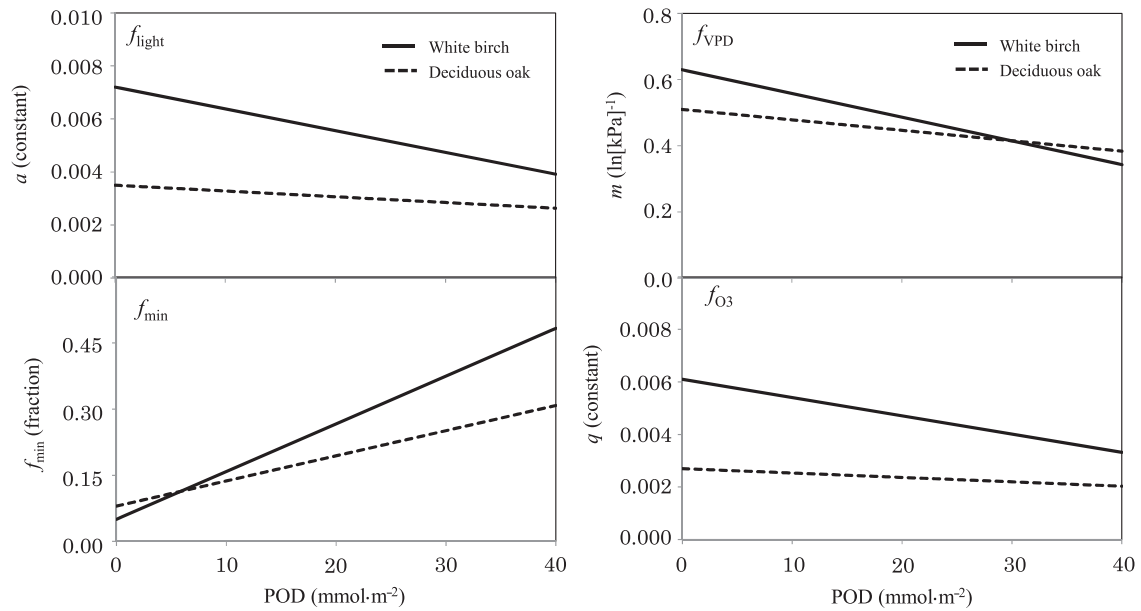| parameter			white birch (*Betula platyphylla* var. *japonica*)	deciduous oak (*Quercus mongolica* var. *crispula*)
g_{max} (mol·H$_2$O·m^{-2}·PLA·s^{-1})			0.84	0.60
f_{phen}	p'	(constant)	0.0061	0.0028
f_{O3}	q'	(constant)	0.0061	0.0027
f_{light}	f'_{min}	(fraction)	0.05	0.08
	a'	(constant)	0.0072	0.0035
f_{VPD}	m'	$[\ln(kPa)^{-1}]$	0.63	0.51
s	r	(constant)	0.0114	0.0062

Fig. 1. Changes of parameters of the Jarvis-type stomatal conductance model with POD (phytotoxic O_3 dose) due to O_3-induced stomatal sluggishness in white birch and deciduous oak. a is the parameter determining an exponential curve of stomatal response to light; f_{min}, relative stomatal conductance to g_{max} at PPFD = 0; m is a sensitivity parameter of stomatal response to VPD; q, fraction of decline of stomatal conductance with O_3 concentration. a of f_{light}: black line: y = 0.0072*(1−0.0114x), dotted line: y = 0.0035*(1−0.0062x); f_{min}: black line: y = 0.05 + (0.0114*x)*(1−0.05), dotted line: y = 0.08 + (0.0062*x)* (1−0.08); m of f_{VPD}: black line: y = 0.63*(1−0.0114x), dotted line: y = 0.51*(1−0.0062x); q of f_{O3}: black line: y = 0.0061*(1−0.0114x), dotted line: y = 0.0027*(1−0.0062x).
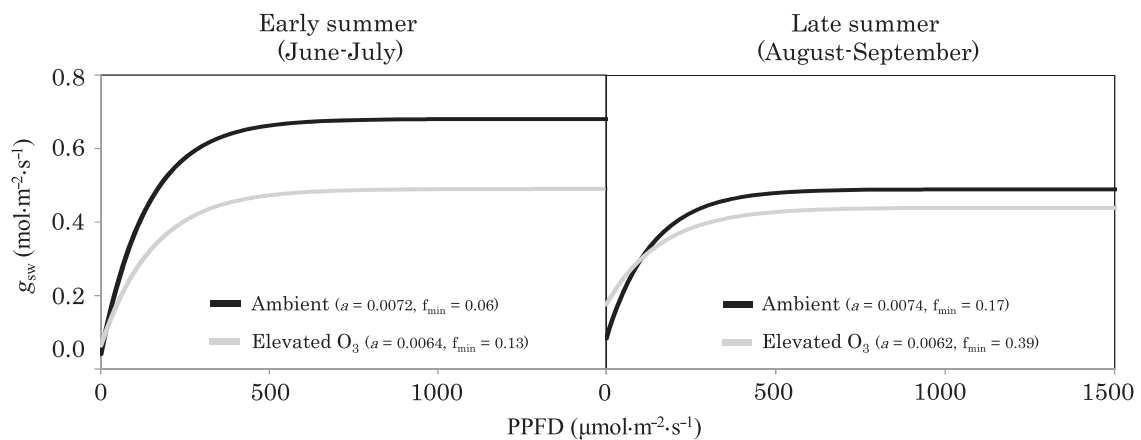

Fig. 2. Stomatal conductance response to PPFD of white birch grown under ambient (black line) and elevated (grey line) O_3. Measurements were carried out in early summer (June–July) and in late summer (August–September). a is an empirical parameter to determine an exponential curve of stomatal response to light, and f_{min} denotes the ratio of stomatal conductance to g_{max} at PPFD = 0.

Estimation of stomatal ozone flux

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

$$F_{st} = [O_3] \cdot g_{sO3} \cdot \frac{r_c}{r_b + r_c} \qquad (8)$$

where $[O_3]$ is hourly mean O_3 concentration (nmol·mol^{-1}), r_b is leaf boundary layer resistance (s·m^{-1}) and g_{sO3} is stomatal conductance for O_3 ($g_{sO3} = g_{sw} \cdot 0.663$; mol·$O_3 \cdot m^{-2} \cdot PLA \cdot s^{-1}$); the factor 0.663 is the ratio of diffusivities between O_3 and water vapour (CLRTAP 2015). Here, r_c is the leaf surface

resistance ($=1/(g_{sO3}+g_{ext})$; s·m^{-1}) and g_{ext} is the external leaf or cuticular conductance (s·m^{-1}), chosen as 0.0004 m·s^{-1} (CLRTAP 2015).

Leaf boundary layer resistance (r_b) was calculated from the wind speed, u (m·s^{-1}) and the cross-wind leaf dimension, L_d (0.06 and 0.08 m for white birch and deciduous oak, respectively, obtained as mean value of 3–5 leaves × six trees in each O_3 treatment; CLRTAP 2015):

$$r_b = 1.3 \cdot 150 \cdot (L_d/u)^{0.5} \qquad (9)$$

where the factor 1.3 accounts for differences in diffusivity between heat and O_3.
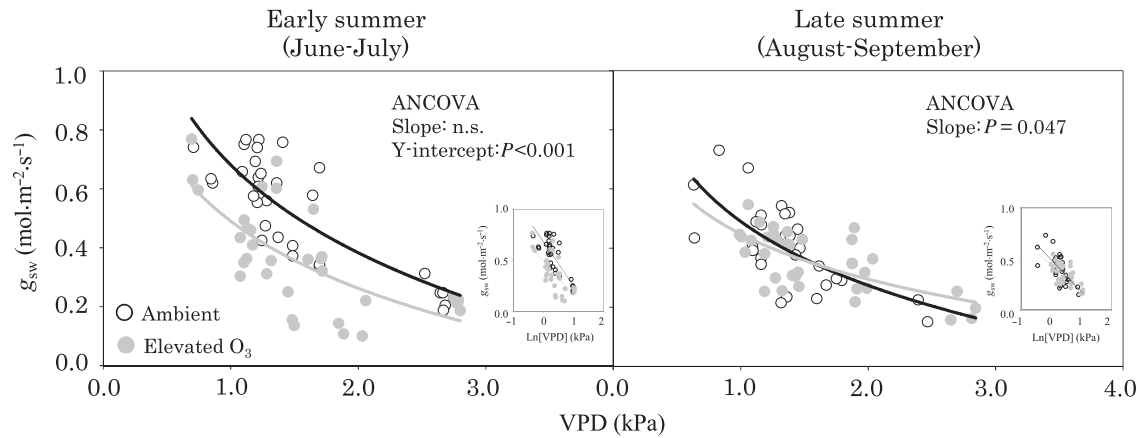
Fig. 3. Relationship between stomatal conductance and leaf-to-air VPD for white birch grown under ambient (open circle) and elevated O_3 (grey circle). Data were obtained under a PPFD > 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The fitting line denotes the model function $g_{sw} = g_{max} \cdot [l - m \cdot ln(VPD)]$ in ambient (black line) and elevated (grey line) O_3. The stomatal sensitivity parameter (m) in ambient and elevated O_3 was 0.63 and 0.67 in early summer, and 0.64 and 0.47 in late summer. The determination coefficient (R^2) in ambient and elevated O_3 was 0.67 and 0.50 in early summer, and 0.47 and 0.42 in late summer.

Table 2. Results of the correlation analyses between measured and estimated stomatal conductance values using the models (case 1: no f_{O3}, no s; case 2: +f_{O3}, no s; case 3: +f_{O3}, +s) in white birch (*B. platyphylla* var. *japonica*) and deciduous oak (*Q. mongolica* var. *crispula*) in 2012. RMSE = Root mean square error (mol·m^{-2}·s^{-1}).

species	R^2	RMSE
White birch (n = 430)		
no f_{O3}, no s	0.62	0.130
+f_{O3}, no s	0.66	0.121
+f_{O3}, +s	0.67	0.105
Deciduous oak (n = 428)		
no f_{O3}, no s	0.65	0.091
+f_{O3}, no s	0.65	0.090
+f_{O3}, +s	0.66	0.087

Phytotoxic O_3 dose (POD) was recommended to assess O_3 injury to physiological parameters for forest tree species (Lombardozzi *et al.* 2012). It is given by:

$$POD = \sum F_{st} \quad (10)$$

Here we also tried to investigate a relationship between ozone-induced decline of photosynthesis and POD using the published photosynthesis data in the same free-air O_3 exposure experiment (white birch: Hoshika *et al.* 2013a; deciduous oak: Watanabe *et al.* 2015).

Data analysis

The effects of O_3 on the relationship between g_{sw} and log-transformed VPD were tested using analysis of covariance (ANCOVA). Simple correlation analysis was used to test the relationship between measured and estimated stomatal conductance. The relationship between light-saturated net photosynthetic rate and POD was assessed with a linear regression. Results were considered significant at $P < 0.05$. All analyses were performed using SPSS software (SPSS, Chicago, IL, USA).

RESULTS

Parameterisations of Jarvis-type stomatal conductance model

The g_{max} values were set to 0.84 and 0.60 mol $H_2O \cdot m^{-2} \cdot PLA \cdot s^{-1}$ in white birch and deciduous oak, respectively (Table 1). The reduction in rate of g_{sw} with leaf aging was greater in white birch than in deciduous oak (parameter p of f_{phen}: 0.0061 in white birch, 0.0028 in deciduous oak). The parameter q' of f_{O3} was higher in white birch than in deciduous oak, reflecting a larger reduction of g_{sw} by O_3 in white birch (Figure S1). Typical response functions were obtained in stomatal response to PPFD (f_{light}) and VPD (f_{vpd}) in both tree species. However, these parameters of the stomatal conductance model may have been changed by O_3-induced stomatal sluggishness (Fig. 1). The parameter r of the sluggishness factor s was higher in white birch than in deciduous oak, suggesting that O_3 may have caused significant stomatal sluggishness in white birch. In fact, although O_3 greatly decreased g_{sw} of white birch leaves after full expansion (Figs 2 and S2: −26% in leaves with leaf age of 5 days), such a reduction of g_{sw} by O_3 fell in late summer (Figs 2 and S2: −10% with leaf age of 85 days). Ozone also reduced stomatal sensitivity to VPD for white birch in late summer (Fig. 3: $m = 0.64$ and 0.47 in ambient and elevated O_3, respectively. ANCOVA: $P = 0.047$). At the same time, a loss of closing response of stomata under low light conditions in white birch was observed under elevated O_3 (Fig. 2: increased f_{min}, +116% in late summer). In contrast, such lower sensitivity of stomata to VPD and light intensity under elevated O_3 was not found in deciduous oak (data not shown).

Predicted stomatal conductance by the model fitted well with the measured values under both O_3 treatments in the two deciduous species (Table 2). The estimated values from the model explained 60–70% of the variation of measured stomatal conductance. The consideration of effects of O_3 on stomatal conductance (f_{O3} and sluggishness factor s) improved performance of the model to estimate g_{sw} in white birch (increased R^2 values from 0.62 to 0.67, and reduced RMSE from 0.130 to 0.105 mol·m^{-2}·s^{-1}).
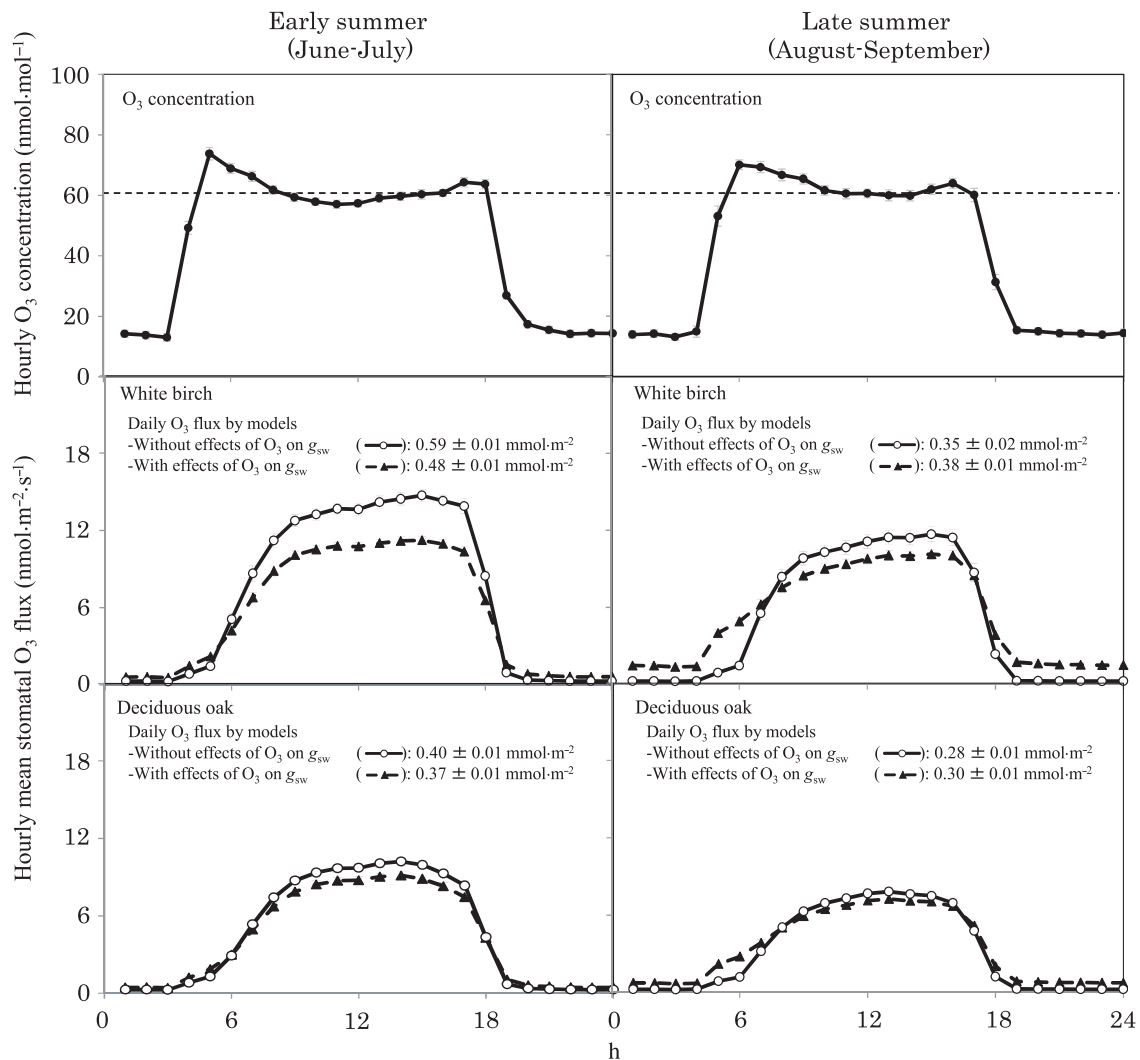
Fig. 4. Daily course of O_3 concentration (mean ± SE) and stomatal O_3 flux values (mean ± SE) under elevated O_3 treatment. Stomatal O_3 fluxes are estimated by the model with the effects of O_3 on stomatal conductance (open circle and solid black line) or without the effects of O_3 on stomatal conductance (closed triangle and dotted black line) in white birch and deciduous oak. 60 nmol·mol^{-1} during daylight hours was set as a target value for the elevated O_3 treatment.

Diurnal course of stomatal O_3 flux

Figure 4 shows the daily course of O_3 concentrations and estimated stomatal O_3 fluxes under elevated O_3 in white birch and deciduous oak. In both early and late summer, stomatal O_3 fluxes were higher in white birch than in deciduous oak. The estimates of stomatal O_3 flux of white birch had 19% lower values in early summer when we applied the model with consideration of O_3 effects on stomatal conductance. In late summer, daily stomatal O_3 fluxes of white birch did not differ between the two simulations by the model with or without O_3 effects on stomatal conductance. However, the estimates of stomatal O_3 flux were higher during the night (+288%) considering the O_3 effects on stomatal conductance. In contrast, stomatal O_3 fluxes of deciduous oak were similar in the two the simulations regardless of season.

Net photosynthetic rate *versus* POD

Figure 5 shows the relationship between light-saturated net photosynthetic rate (A_{sat}) and POD, estimated by the model

with or without the effects of O_3 on stomatal conductance in white birch and deciduous oak. A_{sat} declined with increasing POD in both species. The slope of the regression line was higher in white birch than in deciduous oak, suggesting a larger O_3-induced decline of A_{sat} per unit O_3 uptake in white birch. The inclusion of parameters f_{O_3} and s improved the predictive power of the dose–response relationship in white birch (increased R^2 values from 0.62 to 0.70).

DISCUSSION

Ozone-induced reduction in stomatal conductance occurred in white birch (Figs 2, 3, S1, S2). The mechanism of stomatal closure may have been a reaction to an O_3-induced decline of photosynthetic capacity in the mesophyll (Reich 1987; Farage & Long 1995; Heath & Taylor 1997), because stomatal conductance is generally correlated with photosynthesis (Lambers *et al.* 2008). Previous studies also suggested that O_3 induces stomatal closure due to direct modulation of K$^+$ channels (Torsethaugen *et al.* 1999; Vahisalu *et al.* 2010), alteration in
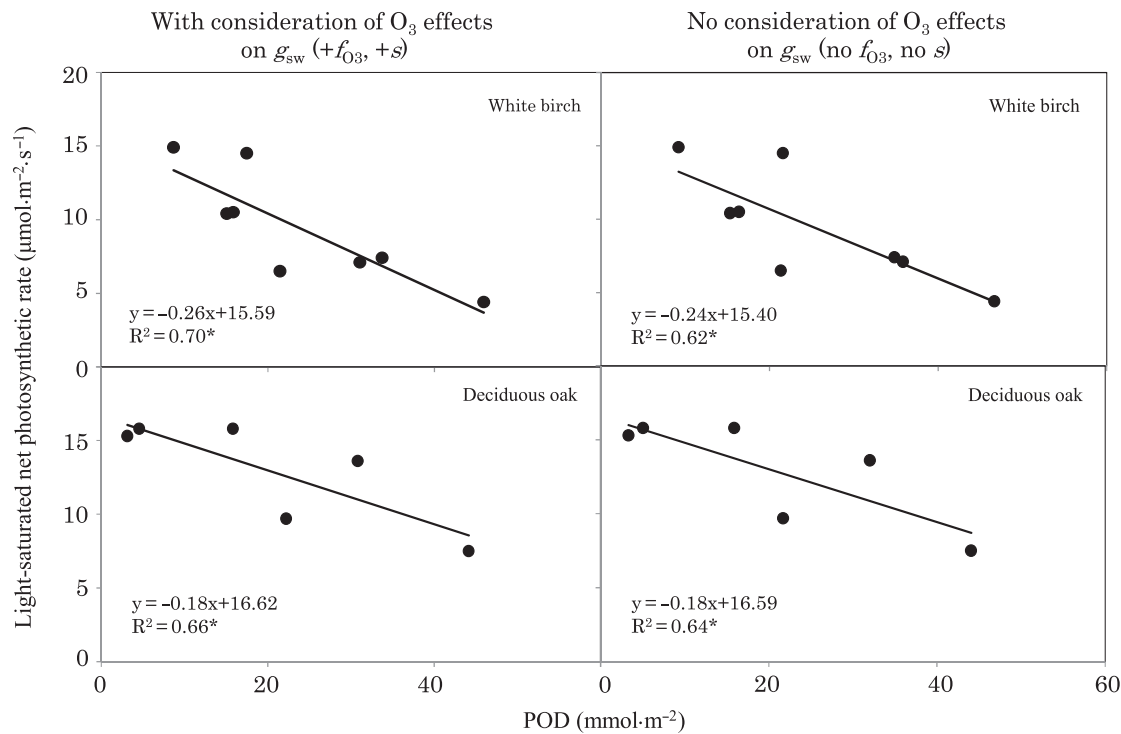
Fig. 5. Relationships between light-saturated net photosynthetic rate and POD (phytotoxic O_3 dose), which is estimated by the model with consideration of the effects of O_3 on stomatal conductance (left figures) and without consideration of the effects of O_3 on stomatal conductance (right figures) in white birch and deciduous oak. Linear regression analysis: *$P < 0.05$.

Ca^{2+} homeostasis of guard cells (McAinsh *et al.* 2002; Dumont *et al.* 2014) and phytohormone production (Overmyer *et al.* 2008). In white birch, such stomatal closure fell in late summer (Figs 2 and S2). Previous studies similarly reported a gradual loss of O_3-induced stomatal closure in European birch (*B. pendula*: Oksanen 2003), European beech (*Fagus sylvatica*: Löw *et al.* 2007; Matyssek *et al.* 2015) and Siebold's beech (*F. crenata*: Hoshika *et al.* 2015b). This response could translate into a weaker ability to close stomata after O_3 exposure, *i.e.* increasing loss of stomatal regulation, which is here called 'O_3-induced stomatal sluggishness' (Paoletti 2005; Mills *et al.* 2009; Hayes *et al.* 2012; Hoshika *et al.* 2012a, 2013b, 2015b; Dumont *et al.* 2013). In late summer, O_3 caused lower sensitivity of stomata to VPD (Fig. 3) and loss of closing response of stomata under low light conditions in white birch (Fig. 2). Omasa *et al.* (2002) reported that such stomatal responses might be caused by O_3-induced alteration to the osmotic pressure modulating a balance in turgor between guard and subsidiary cells. Mills *et al.* (2009) reported that O_3 might reduce the sensitivity of stomata to abscisic acid (ABA). This loss of stomatal response to ABA may be related to O_3-induced ethylene emissions (Wilkinson & Davies 2010). However, the mechanisms are still under investigation.

Current Jarvis-type models do not take into account O_3-induced stomatal sluggishness (Damour *et al.* 2010; CLRTAP 2015), although several studies have pointed out that stomatal sluggishness could affect the calculation of transpiration and stomatal O_3 flux (Paoletti & Grulke 2005; Dumont *et al.* 2013; Hoshika *et al.* 2015c). Here we developed the Jarvis-type model with consideration of O_3 effects on stomatal conductance by employing the simple parameters f_{O3} and s. The new model

provided a better fit of estimated stomatal conductance to the measured value, when both O_3-induced stomatal closure (f_{O3}) and stomatal sluggishness (s) were considered (Table 2). A simplified model is preferred because the increased number of parameters requires more calibration of these parameters and needs more observed data (Alexandrov *et al.* 2002). Here the simple parameters, f_{O3} and s, provide a realistic framework for stomatal conductance modelling under O_3 pollution in forest trees.

Ozone-induced stomatal closure might have limited stomatal O_3 flux in early summer for white birch (Fig. 4: −19%). Similarly, Matyssek *et al.* (2015) reported that stomatal closure reduced seasonal O_3 influx in European beech by ~20% under elevated O_3. It is still under discussion whether such stomatal closure could have a protective value to avoid damage to physiological and biochemical processes in forest trees (Robinson *et al.* 1998). However, an optimal stomatal conductance model developed by Hoshika *et al.* (2013b), which involves water, CO_2 and O_3 influx, suggests the O_3-induced stomatal closure during early summer to reduce O_3 influx is an avoidance mechanism for O_3 injury, allowing maximum photosynthetic capacity to be reached in Siebold's beech. On the other hand, in late summer, daily stomatal O_3 flux was not limited due to an increase in evening or night-time stomatal O_3 flux through stomatal sluggishness (Fig. 4). Current modelling studies generally ignore the night-time values of stomatal conductance. However, nocturnal stomatal O_3 flux may enhance the deleterious effects of O_3. Matyssek *et al.* (1995) found that a 24-h exposure to O_3, including exposure at night, caused a stronger decrease in growth relative to daytime exposure only for European birch and concluded that night-time O_3 exposure should be considered in the assessment of O_3 impacts. In fact, our

previous study found that O_3 increased the night-time stomatal conductance in Siebold's beech (Hoshika *et al.* 2013b). Ozone concentration can remain elevated at night, particularly in mountainous areas (Musselman & Minnick 2000), and plants can be more susceptible to O_3 at night than during daytime, because the capacity of plant defence mechanisms is lower at night (Musselman & Minnick 2000).

As a result, the innovative parameterisation of the Jarvis-type model for elevated O_3 condition improved the dose–response relationship for the O_3-induced decline of photosynthesis of white birch (Fig. 5). Our result indicates that the O_3-induced decline of photosynthesis per unit O_3 uptake was higher in white birch than in deciduous oak (1.7% $mmol^{-1}$ POD *versus* 1.1% $mmol^{-1}$ POD), suggesting that deciduous oak might have higher resistance to O_3 than white birch. These findings are supported by previous studies (growth response: Kohno *et al.* 2005; photosynthetic traits: Watanabe *et al.* 2013). Ozone-induced decline in photosynthesis per unit O_3 uptake was reported to be 1.2% $mmol^{-1}$ POD in *F. sylvatica* (Löw *et al.* 2007) and 1.5–5.0% $mmol^{-1}$ POD in *Populus* spp. (Bagard *et al.* 2008; Dumont *et al.* 2013), which is the same order of magnitude as our value. The smaller value of the parameters of O_3 effects of g_{sw} (f_{O3} and s) in deciduous oak implies that O_3 had a much smaller effect on the model parameters for deciduous oak, which is less sensitive to O_3 (Figs 3, S1, S2).

Based on the results of the present study, we conclude that our hypothesis is supported. Ozone-induced stomatal sluggishness changed the parameters of the Jarvis-type stomatal conductance model. When both O_3-induced stomatal closure and stomatal sluggishness were taken into account in a stomatal conductance model, the model provided good agreement with the measurements. The present results innovatively propose a simple parameter (s) to consider stomatal sluggishness. This new parameter will provide new insights to stomatal O_3 response in the model assumptions and contribute to validation of results under field conditions. Ozone-induced stomatal sluggishness can affect stomatal O_3 flux especially in the evening or during the night, as stomata may close slowly or show impairment of the closing response. We also note that deciduous oak, which is classified as a less sensitive species to O_3, did not show clear O_3 effects on stomatal conductance. The examined level of O_3 concentration used here might not have been sufficient to modify stomatal conductance of deciduous oak. We conclude that the O_3 effects on stomatal conductance (*i.e.* f_{O3} and s) could be crucial for modelling studies to determine stomatal response in O_3-sensitive deciduous trees.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Relationships between stomatal conductance (g_{sw}) and hourly mean O_3 concentration in white birch and deciduous oak.

Figure S2. Relationships between stomatal conductance (g_{sw}) and leaf age in white birch and deciduous oak. effect. O_3 treatment.

Figure S3. Relationships between measured and estimated stomatal conductance (g_{sw}) for white birch and deciduous oak in 2012. Models with or without ozone-induced stomatal closure (f_{O3}) and stomatal sluggishness (s) were tested.

Figure S4. Parameters of the Jarvis-type stomatal conductance model for white birch.

Figure S5. Parameters of the Jarvis-type stomatal conductance model for deciduous oak.

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