Concentration- and flux-based ozone dose–response relationships for five poplar clones grown in North China

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Concentration- and flux-based O3 dose–response relationships were developed for poplars in China. Stomatal conductance (g s) of five poplar clones was measured to parameterize a Jarvis-type multiplicative gs model. The maximum gs and other model parameters varied between clones. The strongest relationship between stomatal O3 flux and total biomass was obtained when phytotoxic ozone dose (POD) was integrated using an uptake rate threshold of 7 nmol m−2 s−1. The R2 value was similar between flux-based and concentration-based dose–response relationships. Ozone concentrations above 28–36 nmol mol−1 contributed to reducing the biomass production of poplar. Critical levels of AOT 40 (accumulated O3 exposure over 40 nmol mol−1) and POD Y in relation to 5% reduction in total biomass for poplar were 12 μmol mol−1 h and 3.8 mmol m−2, respectively.

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1. Introduction

Tropospheric ozone (O3) is an important phytotoxic air pollutant and a significant greenhouse gas formed by photochemical reactions among nitrogen oxides, volatile hydrocarbons and carbon monoxide in the atmosphere (Dumont et al., 2014; Feng et al., 2015). The background O3 level over the mid latitudes of the Northern Hemisphere has increased continuously between 0.5% and 2% per year over the last 30 years (Vingarzan, 2004). It is predicted to rise up to 80 nmol mol−1 in 2100, accompanied by episodes of O3 peaks occasionally exceeding 200 nmol mol−1 (IPCC, 2013; Richet et al., 2012).

Forest ecosystems, which cover 31% of the Earth’s land area and constitute the most important carbon sinks, could be highly vulnerable to O3 damage (FAO, 2010; Fowler et al., 2009; Sitch et al., 2007). Ozone affects tree health through stomatal uptake, causing visible foliar injury, accelerated leaf senescence, reduced photosynthesis, and impaired stomatal function (Feng et al., 2014; Zhang et al., 2011, 2014b). The increasing ozone concentration contributes to a decline in forestry productivity linked to economic losses (Felzer et al., 2007; Percy and Karnosky, 2007; Pye, 1988). A variety of detrimental changes at the biochemical, physiological and molecular levels have been demonstrated in conifers and deciduous trees (Koch et al., 1998; Kopper and Lindroth, 2003; Richet et al., 2011, 2012; Zhang et al., 2014a). Experimental evidence indicates that not only species but also cultivars, genotypes and clones, show different sensitivity to O3 (Calatayud et al., 2011; Castagna et al., 2015; Dumont et al., 2014; Häkiö, 2009; Paolletti and Gruulke, 2010; Zhang et al., 2012, 2014). In the past, the impact of O3 on vegetation was quantified using the dose–response relationship based only on atmospheric O3 concentrations, such as AOT 40 (accumulated hourly O3 concentration over a threshold of 40 nmol mol−1 during daylight hours) (Fuhrer et al., 1997). Nowadays, it has been gradually replaced by the POD Y (phytotoxic ozone dose over a threshold of Y) index defining the amount of O3 entering the leaves through the stomata (Gerosa et al., 2015; LRTAP Convention, 2010; Mills et al., 2011). This latter index, which has been described using different names in the

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Literature, such as $\text{CUO}$ (cumulative uptake of $\text{O}_3$), or $\text{AFsTY}$ (accumulated stomatal flux above a threshold of $Y$) (Azuchi et al., 2014; Uddling et al., 2004), takes into account the $\text{O}_3$ flux by modeling $\text{O}_3$ stomatal conductance ($g_{s\text{O}_3}$) using multiplicative algorithms, such as Jarvis (1976).

It is generally accepted that the damage of $\text{O}_3$ to plants is mainly caused by the amount of $\text{O}_3$ entering into the leaf interior through the open stomata (Kerstiens and Lendzian, 1989). Stomatal conductance of plants is one of the crucial mechanisms of $\text{O}_3$ sensitivity (Dumont et al., 2013; Guidi et al., 2001). Stomatal $\text{O}_3$ uptake considers both biological traits and climatic factors. Therefore, it is better for assessing the adverse effects of $\text{O}_3$ on the plants than external $\text{O}_3$ concentrations (Emberson et al., 2000b; Uddling et al., 2004).

Among different tree species, poplars have received particular attention because of the fast growth rate and relatively high stomatal conductance (Woo and Hinckley, 2005). There are 53 species of poplar distributed in 22 provinces of China (not including crossbreed and imported species), covering a total area of over 10 million ha with a total standing stock of 426 million m$^3$ (Jiang et al., 2006; Xu et al., 2009). Different species or clones of poplar have shown different sensitivity to $\text{O}_3$ with respect to visible leaf injury (Hashika et al., 2012; Novak et al., 2005; Ryan et al., 2005; Strohm et al., 1998), damaged photosystems (Bernacchi et al., 2003; Guidi et al., 2001; Ranieri et al., 2001), and reduced growth (Isbrands et al., 2001; Matyssek et al., 1993; Mooi, 1980). However, the knowledge of $\text{O}_3$ dose–response relationships for poplar, which could be an effective tool for $\text{O}_3$ risk assessment, is still limited. It was hypothesized that the difference in $\text{O}_3$ sensitivity could lead to different parameter values of stomatal conductance model for different poplar species or clones.

The overall objectives of the present study were: (1) to parameterize the stomatal conductance model of poplar clones widely used in China; (2) to develop exposure concentration and flux-based $\text{O}_3$ dose–response relationships; and (3) to define the critical levels for protecting poplar against $\text{O}_3$ damage.

2. Materials and methods

2.1. Experimental site and plant materials

The experiment was conducted in Changping (40°19′N, 116°13′E), northwest of Beijing, in a warm temperate and semi-humid continental climate. The annual mean temperature in Changping is 11.8 °C and the total annual precipitation is 550 mm.

Rooted cuttings of five hybrid poplar clones were cultivated at the Chinese Academy of Forestry Sciences. The following clones were used: ‘84 K’ (Populus alba × P. glandulosa), ‘107’ (P. × euramericana cv. ‘74/76’), ‘90’ (P. deltoides × P. cathayana cv. Senhai 2), ‘546’ (P. deltoides cv. ‘55/56’ × P. deltoides cv. ‘Imperial’), and ‘156’ (P. deltoides × P. cathayana). The cuttings were transplanted into 20 L circular plastic pots on April 20, 2014 when they were about 65 days old. Pots were filled with local light loamy farmland soil. Seedlings with similar height and stem basal diameter (Table S1 and S2) were selected for this study and pre-adapted to open-top chamber conditions for 10 days before $\text{O}_3$ fumigation. All plants were manually irrigated up to soil field capacity with tap water at 1–2 days interval to avoid drought stress.

When most leaves turned yellow and the growth of height and basal stem diameter had stopped, the plants were harvested and separated into leaves, stems, and roots. All plants were harvested between Sep. 13 and 28, 2014 (see details in Table S3). The plant organs were oven-dried at 80 °C until a constant mass was reached.

2.2. Ozone treatments

The experiment was conducted in six open-top chambers (OTC, octagonal base, 12.5 m$^2$ of growth space and 3.0 m of height, covered with toughened glass) with different treatments: charcoal filtered ambient air (CF), non-filtered ambient air (NF), and NF with targeted $\text{O}_3$ addition of 20 (NF+20), 40 (NF+40), 60 (NF+60), and 80 (NF+80) nmol mol$^{-1}$ for 8-h average $\text{O}_3$ concentration. Ozone was generated from pure oxygen by an $\text{O}_3$ generator (HY003, Chuangcheng Co., Jinan, China) and then mixed with ambient air using a fan (1.1 kW, 1080 Pa, 19 m$^3$ min$^{-1}$, CZF, Fengda, China) to achieve the target $\text{O}_3$ concentration at the top of the canopy in the fumigation treatments. There was no chamber replication for each treatment. In order to eliminate the positional and chamber effects (Potvin and Tardif, 1988), the plant positions within each OTC were changed every 3–5 days, and all seedlings were switched between chambers once a month, according to Feng et al. (2011). Each chamber functioned as one of six treatments randomly at each month during the growing season. The concentration in each chamber was changed every month according to the corresponding treatment.

Six to eight potted plants per poplar clone were exposed to each treatment except there was no ‘156’ in the NF+20 chamber. The daily maximum fumigation period was 9 h (from 08:30 to 17:30) when there was no rain, fog, mist, or dew, according to the protocols in Free Air Ozone Concentration Enrichment System (Feng et al., 2010; Morgan et al., 2006).

2.3. Ozone and climate monitoring

The concentrations of $\text{O}_3$ in the OTCs were continuously monitored using an UV absorption $\text{O}_3$ analyzer (Model 49i, Thermo Scientific, USA), via a Teflon solenoid valve switch system, which collected air from sampling points at approximately 10 cm above the plant canopy. The monitors were calibrated by a 49i-PS calibrator (Thermo Scientific, USA) before the experiment and once a month during the experiment. Fumigation targets and average $\text{O}_3$ concentrations (24 h, 12 h, and 8 h) for all treatments are presented in Table 1.

Air temperature ($T$), relative air humidity ($RH$), and photosynthetic photon flux density (PPFD) under the ambient condition and inside the OTCs were measured every 5 min using a weather station (Campbell Scientific, North Logan, Utah, USA). Water vapor pressure deficit ($VPD$) was estimated using Eq. (1):

$$ VPD = \left(1 - \frac{RH}{100}\right) \times 0.611 \times \exp \left(\frac{17.52}{T + 240.97}\right). $$

Hourly means of environmental variables were employed in $\text{O}_3$ flux calculations. The range of $T$, $VPD$, and PPFD was 6.94–46.48 °C, 0.00–9.15 kPa, and 0–1358 μmol m$^{-2}$ s$^{-1}$, respectively, from May 7 to Sep. 29, 2014. Compared with ambient air, the average air temperature and relative humidity in the OTCs were increased by 1.52 °C and 1.65%, respectively.

Table 1

| Target | 24-h average | 12-h average, 08:00–20:00 | 8-h average, 09:00–17:00 |
|--------|--------------|---------------------------|-------------------------|
| CF     | 25.7 ± 0.9   | 28.1 ± 1.1                | 28.0 ± 1.3              |
| NF     | 35.7 ± 1.2   | 48.4 ± 1.6                | 53.2 ± 1.8              |
| NF+20  | 41.4 ± 1.3   | 59.4 ± 1.7                | 67.1 ± 1.9              |
| NF+40  | 46.3 ± 1.4   | 69.4 ± 2.0                | 79.5 ± 2.3              |
| NF+60  | 52.8 ± 1.6   | 81.9 ± 2.5                | 95.4 ± 2.9              |
| NF+80  | 58.1 ± 1.8   | 92.4 ± 2.8                | 108.7 ± 3.3             |
2.4. Stomatal conductance measurements and modeling

The stomatal conductance of five poplar clones from NF and NF-40 treatments were measured in situ with a Li-6400 photosynthesis system (LiCor, Lincoln, Nebraska, USA) during the experiment in 2014. A red/blue LED light source chamber was used to avoid shading part on the leaf. The PPFD was adjusted every 30 min to get close to the sun light PPFD. The temperature and humidity was not controlled during the measurements. The $g_{sto}$ value was stabilized for at least 1.5 min before it was recorded. The diurnal variation in $g_{sto}$ was measured every 1–2 h, i.e. every measurement cycle, from 9:00 AM to 6:00 PM on fully developed leaves in the middle layer of leaves of 4–8 replicated trees of each treatment. After discarding the obvious outliers (i.e. some data that were outside the mean ± 2SD of all measurements at each measurement cycle for each clone, around 10% of the total measurements), all valid $g_{sto}$ and their linked meteorological data were averaged for each clone at each treatment in one measurement cycle, and were used to calibrate the stomatal conductance model. The Jarvis-type multiplicative stomatal conductance model was applied (Emerson et al., 2000a; Jarvis, 1976; LRTAP Convention, 2010). It includes mathematical functions for phenology ($f_{phen}$), photosynthetically photon flux density ($f_{PPFD}$), water vapor pressure deficit ($f_{VPD}$), temperature ($f_{temp}$), soil water potential ($f_{SWP}$) and O$_3$ ($g_{sto}$):

$$g_{sto} = g_{max} \times \min\left(f_{phen} \times f_{O_3} \times f_{PPFD} \times \max\left(f_{min}, \left(f_{temp} \times f_{VPD} \times f_{SWP}\right)\right)\right),$$

(2)

where $g_{sto}$ denotes the actual stomatal conductance of O$_3$ (mmol O$_3$ m$^{-2}$ projected leaf area (PLA) s$^{-1}$), which is converted from the stomatal conductance of water vapor using a conversion factor of 0.663 to account for the difference in the molecular diffusivity of water vapor to that of O$_3$; $g_{max}$ refers to the highest value of stomatal conductance of O$_3$ (mmol O$_3$ m$^{-2}$ PLA s$^{-1}$); and $f_{min}$ is the relative minimum stomatal conductance (fraction of $g_{max}$).

$f_{phen}$ is calculated based on a fixed time interval:

when $A_{start} \leq y_d < (A_{start} + f_{phen, c})$,

$$f_{phen} = \left(1 - f_{phen, a}\right) \times \left[\left(y_d - A_{start}\right) / f_{phen, c}\right] + f_{phen, a},$$

(3)

when $(A_{start} + f_{phen, c}) \leq y_d < (A_{end} - f_{phen, a})$,

$$f_{phen} = 1,$$

(4)

when $(A_{end} - f_{phen, a}) \leq y_d < A_{end}$,

$$f_{phen} = \left(1 - f_{phen, b}\right) \times \left[\left(A_{end} - y_d\right) / f_{phen, d}\right] + f_{phen, b},$$

(5)

where $y_d$ represents the year day; $A_{start}$ and $A_{end}$ are the year days for the start and end of O$_3$ fumigation period, which was May 7, 2014 and the corresponding harvest date in Table S3, respectively; $f_{phen, a}$ and $f_{phen, b}$ denote the maximum fraction of $g_{max}$ that $g_{sto}$ takes at the start and the end of the fumigation period for O$_3$ flux, respectively. $f_{phen, c}$ and $f_{phen, d}$ are model parameters to describe the shape of the function within the fumigation period.

The response of $g_{sto}$ to leaf temperature ($f_{temp}$) was expressed as Eq. (6).

when $T_{min} < T < T_{max}$,

$$f_{temp} = \left(\frac{T - T_{min}}{T_{opt} - T_{min}}\right) \times \left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)^{b_1}, \quad b_1 = \frac{T_{max} - T_{opt}}{T_{opt} - T_{min}}$$

(6)

when $T < T_{min}$ or $T > T_{max}$,

$$f_{temp} = f_{min},$$

(7)

where $T$ denotes the leaf temperature ($^{°}C$), $T_{min}$ and $T_{max}$ are the minimum and maximum leaf temperatures at which stomatal closure occurs to $f_{min}$. $T_{opt}$ corresponds to the optimum leaf temperature.

Response functions for radiation and VPD were described as follows:

$$f_{PPFD} = 1 - \exp(-light_a \times PPFD),$$

(8)

$$f_{VPD} = \min\left\{1, \max\left\{f_{min}, \frac{1 - f_{min} \times (VPD_{min} - VPD) + f_{min}}{VPD_{max} - VPD_{min}}\right\}\right\},$$

(9)

where the unit of VPD is kPa. It was assumed that there is no strong correlation between $f_{VPD}$ and $f_{temp}$ even though VPD was estimated based on temperature (Pleijel et al., 2002).

These mathematical functions were parameterized using a boundary line technique, which was first introduced by Webb (1972), and has been widely used in plant and soil science models (Bhat and Sujatha, 2013; Blanco-Macías et al., 2010; Lewandowski and Schmidt, 2006; Topp et al., 2013; Vizcaíno-Soto and Côté, 2004). It was based on the principle that biological materials have an upper response limit to a given variable. The construction and fitting of boundary lines took the following four steps (Schmidt et al., 2000).

1. Scattergrams were plotted between relative stomatal conductance (fraction of $g_{max}$) as dependent variable and each of the independent variables, such as $y_d$, $T$, PPFD, and VPD, for each poplar clone.
2. The x-axis of each scattergram was divided into 8 sections of the same length. The data set of the scattergram, therefore, was split into 8 equidistant segments to cover all the points.
3. For each segment, 99% percentile was computed and used as the boundary point. It was assumed to be affiliated to the arithmetic mean of the x-values of the corresponding section.
4. On each entire scattered data set, the boundary points were plotted against the arithmetic centers of the segments from which they were derived. The curves were then fitted to Eqs. (3)–(9), respectively.
In previous studies, the maximum stomatal conductance ($g_{\text{max}}$) was measured under optimal conditions where there is no limiting effect of VPD, PPDF, and O$_3$. However, such optimal conditions are difficult to achieve during measurements. In the present study, clone-specific $g_{\text{max}}$ was estimated by fitting observed $g_{\text{sto}}$ into the bell-shaped Eq. (10) using a boundary analysis technique.

$$g_{\text{sto}} = g_{\text{max}} \times f_{\text{rmp}}$$

(10)

For forest trees, the response of $g_{\text{sto}}$ to O$_3$ $(f_{\text{sto}})$ was set to 1 in Eq. (2) according to LRTAP Convention (2010). Moreover, measurements of soil water potential were unavailable and the water availability was hypothesized to be sufficient since all plants were kept well watered with manual irrigation every 1–2 days. The limitation of soil water potential on $g_{\text{sto}}$ was not considered in the calculations ($f_{\text{VPD}} = 1$). The ability of this model to predict $g_{\text{sto}}$ was validated by comparing the observations and simulations.

### 2.5. Stomatal ozone flux

Stomatal flux of O$_3$ at the leaf level ($F_{\text{st}}$, expressed on a one-sided leaf area basis) is determined by the O$_3$ concentration at plant height and the resistance analogue principle derived from Ohm’s law (Campbell and Norman, 1998), considering the leaf-boundary layer as a resistance to O$_3$ flux connected in series with the parallel resistances of stomatal and non-stomatal leaf surfaces (Uddling et al., 2010).

$$F_{\text{st}} = \frac{|O_3|_{\text{can}} - |O_3|_{\text{int}}}{r_b + r_s + r_{b-s}/r_{ns}}.$$  

(11)

where $|O_3|_{\text{can}}$ and $|O_3|_{\text{int}}$ are the O$_3$ concentrations at the canopy top and in the intercellular air space of the leaves, respectively; $r_b = 1/g_{\text{sto}}$, $r_s = 1/g_{\text{sto}}$, and $r_{b-s} = 1/g_{\text{sto}}$ are the resistances for O$_3$ flux through the leaf-boundary layer, stomatal and non-stomatal surface deposition, respectively; $g_{\text{sto}}$, $g_{\text{sto}}$, and $g_{\text{ns}}$ represent the leaf boundary layer conductance, stomatal conductance, and non-stomatal conductance, respectively.

The non-stomatal O$_3$ deposition to the exterior leaf surface is negligible because the cuticle is considered to be highly impermeable to O$_3$ compared to open stomata (Kerstiens and Lendzian, 1989). Therefore, Eq. (11) can be written as:

$$F_{\text{st}} = \frac{|O_3|_{\text{can}} - |O_3|_{\text{int}}}{r_b + r_s}.$$  

(12)

It is empirically assumed that the O$_3$ concentration inside the intracellular spaces of the leaf was approximately zero (Laisk et al., 1989). Hence, Eq. (12) can be simplified as:

$$F_{\text{st}} = \frac{|O_3|_{\text{can}}}{r_b + r_s}.$$  

(13)

The boundary layer resistance ($r_b$) depends on wind speed and the shape of the leaf. It can be measured with a heat transfer method (Jones, 1983) or calculated using the Unsworth et al. (1984) equation:

$$r_b = k \sqrt{d/u},$$

(14)

where $k$ is the empirical coefficient, $d$ refers to the leaf mean width, and $u$ denotes the wind mean velocity at the top canopy level. In this study, the value for $r_b$ was considered negligible because of the high velocity of the airflow through the chambers (Ryan et al., 2009).

A flux-based index ($POD_Y$, phytotoxic O$_3$ dose above a flux threshold of Y nmol O$_3$ m$^{-2}$ PLAs$^{-1}$), was calculated by integrating positive values of $(F_{\text{st}} - Y)$ during the fumigation period (start from May 7, 2014 to the harvest date displayed in Table S3).

$$POD_Y = \int \max(F_{\text{st}} - Y, 0) \, dt.$$  

(15)

For the calculation of $POD_Y$, the leaf temperature was replaced by air temperature in Eq. (6) since air temperature is easily obtained from the meteorology station and this simplification does not have a large influence on the calculated $POD_Y$ (Pleijel et al., 2002).

### 2.6. Relationships between O$_2$ exposure and biomass loss

The dose–response relationship for the relative loss of total biomass was analyzed, based on the principles introduced by Fuhrer (1994). Firstly, a linear regression for each poplar clone was made between the actual biomass and accumulated O$_3$ exposure or stomatal uptake to obtain the $y$-intercept as hypothetically maximum biomass at zero O$_3$ exposure or uptake. The relative biomass (RB) was obtained as the biomass at each O$_3$ treatment divided by the $y$-intercept of each clone regression. The RB of each O$_3$ treatment and clone thus becomes comparable on a common, relative scale. The RBs for all five poplar clones and treatments were then regressed against the O$_3$ dose metrics.

Biomass was plotted against O$_3$ exposure expressed as AOT with an O$_3$ concentration threshold (X) from 0 to 120 nmol mol$^{-1}$, and as $POD$ with the stomatal O$_3$ flux threshold (Y) ranging from 0 to 20 nmol O$_3$ m$^{-2}$ PLAs$^{-1}$. AOT$_X$ is the accumulated O$_3$ exposure over a concentration of X nmol mol$^{-1}$ based on hourly averages during daylight hours. Both AOT$_X$ and POD$_Y$ were calculated over the specific period for each clone in each treatment (start from May 7, 2014 to the harvest date displayed in Table S3). The test of a wide range of thresholds X and Y was made in order to find the relationship with the highest $R^2$ for the relationship between RB and O$_3$ exposure. Moreover, the AOT$_{20}$–$POD_Y$–based dose–response relationships were analyzed, since they have been widely used and have been accepted by the United Nations Economic Commission for Europe (UNEEC) Convention on Long-range Transboundary Air Pollution (LRTAP) in assessing the O$_3$ effects on forests (LRTAP Convention, 2010). The O$_3$ sensitivity of poplar could be evaluated by comparing the slope of AOT$_{20}$–$POD_Y$–based dose–response relationship for poplar with the one for other forest tree species, like beech/birch and spruce, included in LRTAP Convention (2010).

### 2.7. Mathematical analysis

All calculations of stomatal conductance and fluxes, as well as the statistical analysis were performed in MATLAB, version R2013a (The MathWorks, Inc., Natick, MA, USA). The effects of O$_3$, clone, and their interactions on total biomass were tested using ANOVA. The differences among five clones and six O$_3$ treatments were analyzed using a multiple comparison test.

### 3. Results

#### 3.1. Effects of O$_3$ treatments on the total biomass of poplar clones

Elevated O$_3$ had significant effects ($p < 0.001$) on the biomass production of all clones (Fig. 1). Generally, the total biomass decreased with increasing O$_3$ concentration. Significant O$_3 \times$ clone interactions ($p < 0.001$) were also observed. The corresponding results of multiple comparison are shown in Table S4 with different letters representing significant differences ($p < 0.05$). Compared with the CF treatment, the NF+80 treatment reduced total biomass
by 29% for ‘107’, 33% for ‘84 K’ and ‘156’, and 41% for ‘90’ and ‘546’, respectively. However, there was no significant difference between the biomass production in the NF treatment compared to that in CF treatment across all poplar clones.

### 3.2. Stomatal conductance model test

The clone-specific parameters of the $g_{s0}$ functions are provided in Table 2. The slope of the linear regression analyses for the relationship between simulated $g_{s0}$ and observed $g_{s0}$ was 0.94, and the coefficient of determination ($R^2$) was 0.72 ($p < 0.001$, Fig. 2). The regression line was not significantly different from the 1:1 line. The small intercept (20.4, 95% confidence interval, 9.5 to 31.4) on the y-axis and the slope of 0.942 (95% confidence interval, 0.879 to 1.006) showed that the parameterized model performed very well.

### 3.3. Ozone uptake and biomass response

Fig. 3 displays the different determination coefficients, slopes and intercepts of the regression of $RB$ for $AOT_x$ and $POD_y$ indices across five poplar clones with different thresholds. The $R^2$ value in the regression of $RB$ and $AOT_x$ increased with $O_3$ concentration threshold up to 60 nmol mol$^{-1}$ and then decreased (Fig. 3A). An $O_3$ flux threshold of 7 nmol $O_3$ m$^{-2}$ PLA s$^{-1}$ provided the strongest correlation between $RB$ and $POD_y$, using a step of 1 nmol $O_3$ m$^{-2}$ PLA s$^{-1}$ (Fig. 3B). For each clone, the $R^2$ value showed a similar pattern, but the maximum values of $R^2$ occurred at different $O_3$ concentration or flux thresholds (Fig. S1). The y-intercept of the regression of $RB$ for $AOT_x$ indices increased with the rising of $O_3$ concentration threshold. However, the $POD_y$-based $y$-intercept values showed a peak value at an $O_3$ flux threshold of 2 nmol $O_3$ m$^{-2}$ PLA s$^{-1}$. The intercepts of all regressions deviated very little from 0.85, which is desirable since zero exposure should be associated with no effect on biomass production. The $AOT_x$-based intercepts were relatively higher than the $POD_y$-based ones. Generally, slopes decreased with the increase of threshold value, and the greater the threshold value is, the faster the slope decreased (Fig. 3A and B). The response function based on $AOT_y$ is presented in Fig. 4A with a $R^2$ value of 0.88. The best performing concentration-based index is $AOT_{0.05}$ ($R^2 = 0.91$, Fig. 4B). The regression relationships of $RB$ for $POD_1$ and $POD_2$ are illustrated in Fig. 4C and D, with determination coefficients of 0.85 and 0.91, respectively. Based on the relationships illustrated in Fig. 4, the critical levels of $AOT_{50}$, $AOT_{10}$, $POD_1$, and $POD_2$ for a 5% reduction of total biomass for poplar were 12.0 $\mu$mol mol$^{-1}$ h, 9.4 $\mu$mol mol$^{-1}$ h, 6.1 nmol m$^{-2}$, and 3.8 nmol m$^{-2}$, respectively.

Fig. 5 illustrates the relationships between the estimated $O_3$ flux above the threshold 7 nmol $O_3$ m$^{-2}$ PLA s$^{-1}$ and the $O_3$ concentration. The minimum $O_3$ concentration, above which there could be a theoretical contribution to $POD_y$ varies between 28 and 36 nmol mol$^{-1}$ for five poplar clones according to their respective value for $g_{s0}$. It is clear that the ambient 8-h (09:00–17:00) average $O_3$ concentration (68.3 ± 2.3 nmol mol$^{-1}$, mean ± SE) during the experiment period was already affecting poplar growth. As shown in Fig. 5, poplar ‘546’ had the lowest intercept on the x-axis and the highest slope among all five clones and the opposite was true for poplar ‘90’. The smaller the x-axis intercept and the higher the slope are, the greater is the potential for $O_3$ damage.

### Table 2

Parameterization for the stomatal conductance model for five poplar clones.

| Parameter | Units | 84 K | 107 | 90 | 546 | 156 |
|-----------|-------|------|-----|----|-----|-----|
| $g_{s0}$ | mmol $O_3$ m$^{-2}$ PLA s$^{-1}$ | 299.8 | 275.2 | 240.3 | 343.9 | 322.4 |
| $f_{min}$ | Fraction | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| $f_{shen,s}$ | Fraction | – | –0.09 | – | – | – |
| $f_{shen,b}$ | Fraction | 0.006 | 0.798 | 0.512 | 0.665 | 0.327 |
| $f_{shen,c}$ | day | – | 80 | – | – | – |
| $f_{shen,d}$ | day | 83 | 15 | 32 | 16 | 22 |
| $T_{min}$ | $^\circ$C | 23.2 | 13.7 | 17.0 | 16.5 | 23.2 |
| $T_{opt}$ | $^\circ$C | 33.5 | 30.7 | 30.8 | 29.7 | 32.5 |
| $T_{max}$ | $^\circ$C | 46.9 | 44.2 | 43.7 | 39.1 | 38.6 |
| light$_e$ | Constant | $3.3 \times 10^{-3}$ | $4.6 \times 10^{-3}$ | $5.9 \times 10^{-3}$ | $3.8 \times 10^{-3}$ | $4.0 \times 10^{-3}$ |
| VPD$_{min}$ | kPa | 8.4 | 8.2 | 7.2 | 5.2 | 6.4 |
| VPD$_{max}$ | kPa | 2.5 | 2.5 | 3.0 | 2.2 | 1.8 |
4. Discussion

To develop the flux-based O₃ dose–response relationship for poplar in China, a factorial Jarvis-type stomatal conductance model was parameterized using the measurements of $g_{sto}$ and corresponding meteorological and phenological data. In previous studies, the maximum stomatal conductance ($g_{max}$) was measured under optimal conditions where there is no limiting effect of VPD, PPFD, and O₃. However, such optimal conditions are hardly achieved during measurements. On the contrary, fitting the observed $g_{sto}$ into a bell-shaped equation (Eq. (10)) using a boundary analysis technique can extrapolate the $g_{max}$ from the optimum of the curve. The maximum $g_{sto}$ measured in this experiment was 295.7, 272.5, 240.7, 309.3 and 311.2 mmol O₃ m⁻² PLA s⁻¹ for ‘84 K’, ‘107’, ‘90’, ‘546’ and ‘156’, respectively. The estimated $g_{max}$ from the bell-shaped curve by an improved temperature-effect method in this study were slightly higher than the maximum measurements except for the clone ‘90’, suggesting that using the maximum of random measurements may underestimate stomatal O₃ flux. The averaged $g_{max}$ (296.3 mmol O₃ m⁻² PLA s⁻¹) for poplar is higher than for beech (205 mmol O₃ m⁻² PLA s⁻¹, Azuchi et al. (2014)) and oak (255–285 mmol O₃ m⁻² PLA s⁻¹, Hoshika et al. (2011)) in East Asia, as well as birch in Continental Central Europe (150 mmol O₃ m⁻² PLA s⁻¹, LRTAP Convention (2010)) and in North America (230 mmol O₃ m⁻² PLA s⁻¹, Uddling et al. (2010)). Even though VPD was estimated using air temperature and relative humidity in the present study, the correlation coefficient between $f_{PPFD}$ and $f_{temp}$ was very small (0.094), which is in agreement with the results reported by Pleijel et al. (2002). The Jarvis-type Eq. (2) is a mathematical model multiplying $g_{max}$ by the limiting functions of phenology and environmental factors. The ability of this model to predict $g_{sto}$ needs to be validated by comparing the observations and simulations, even though each limiting function was calibrated based on the experimental data using a boundary line technique (Azuchi et al., 2014). The determination coefficient of the regression between calculated and observed $g_{sto}$ across all five poplar clones in the present study was 0.72.

The dose–response relationships between O₃ metrics and relative biomass for poplar were developed. The regressions based on both concentration-based AOT indices and flux-based POD indices are all highly significant ($p < 0.001$). Among all AOT indices for the same accumulation period, the determination coefficient of the regression between RB and AOT₁₀ is the highest (0.91). For AOT₄₀, the regression resulted in a slightly lower, but still acceptable $R^2$ value of 0.88. The AOT indices are based only on the concentration of O₃ during daylight hours and have no sensitivity to all to the factors which influence O₃ uptake by plants. The stomatal flux-response index PODₙ led to the highest $R^2$ value (0.91) in the regression between RB and POD. The intercepts did not statistically differ from 100% for any of the O₃ indices presented in Fig. 4. However, the intercepts on y-axis for the regression between RB and AOT indices were slightly lower than that for the regression between RB and AOT indices.

Both AOT-based and POD-based dose–response relationships performed similar with respect to biomass reduction for poplars in China, as indicated by similar $R^2$ value. Similar results were also found for winter wheat (Triticum aestivum L.) grown in subtropical China (Feng et al., 2012) and UK (Gonzalez–Fernandez et al., 2010). Nevertheless, most European studies found that flux-based...
Fig. 4. Relative biomasses (RB, %) of five poplar clones in relation to the accumulated O₃ exposure over a threshold of 40 nmol mol⁻¹ during daylight hours (AOT₄₀) (A) and AOT₆₀ (B), as well as the accumulated O₃ flux over an uptake threshold of 1 nmol m⁻² PL s⁻¹ (POD₁) (C) and POD₁ (D) based on the model parameterization of this study. Blue solid lines represent linear regressions. Red and green dashed lines denote 95% confidence intervals and prediction intervals of the regression, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dose–response relationship provided a higher R² value than concentration-based exposure indices in assessing O₃-induced biomass loss of wheat (T. aestivum L., Danielsson et al., 2003; Pleijel et al., 2004), potato (Solanum tuberosum L., Pleijel et al., 2002, 2004), timothy (Phleum pretense, Danielsson et al., 2013), juvenile silver birch (Betula pendula Roth, Uddling et al., 2004), and subterranean clover (Trifolium subterraneum L., cv. Geraldton, Karlsson et al., 2004). The POD-based approach is more analytic to explain the stomatal limitation on O₃ uptake by plants. Its performance may highly depend on the experimental conditions, such as temperature and soil water content.

AOT₄₀ has been regarded and retained as the basis for the O₃ critical levels for trees because it has a relatively simple definition to make calculations easy (LRTAP Convention, 2010). However, it is not sufficiently robust to apply to sensitive plants growing under a diverse range of climates (Karlsson et al., 2004). The POD approach is more complicated and requires more data. But it could take into account the stomatal limitation on O₃ uptake. POD₁ is more biologically realistic than AOT₄₀, since the impact of O₃ on plants depends on the amount of O₃ entering the stomata and its detoxification by antioxidants (Emerson et al., 2000a; Matyssek et al., 2007; Zona et al., 2014). Thus, from a mechanistic point of view it seems to be a step forward to promote this flux-based approach (Danielsson et al., 2003; Mills et al., 2011; Paoletti and Manning, 2007; Pleijel et al., 2004; Uddling et al., 2004).

The critical level based on the AOT₄₀–response relationship with regard to 5% reduction in total biomass for poplar was 12 μmol mol⁻¹ h, which is 2.4 times the critical value (5 μmol mol⁻¹ h) reported for birch and beech species in Europe (LRTAP Convention, 2010). The AOT₄₀ value accumulated using ambient O₃ concentration over the same duration in the experimental site was 40.5 μmol mol⁻¹ h, which corresponded to 16.7% loss of total poplar biomass. Expert judgment was used to set the flux-based threshold Y to 1 nmol O₃ m⁻² PL s⁻¹ to represent the detoxification capacity for forest trees (LRTAP Convention, 2010; Pleijel et al., 2004). The critical levels of POD₁ for 4% biomass loss of beech/birch and 2% biomass loss of Norway spruce were defined as 4 mmol m⁻² and 8 mmol m⁻², respectively (LRTAP Convention, 2010). Using the POD₁ dose–response relationship for poplar resulted in a 2% biomass loss at 2.1 mmol m⁻² and a 4% loss at 4.8 mmol m⁻². The slope of the POD₁-response relationship in the poplars suggests that ozone effects per unit ozone uptake of poplar was similar to that of European beech/birch, and was steeper than that of spruce. This means the capacity of tolerance such as biochemical detoxification or repair may be similar between poplar and European beech/birch. It is somewhat inconsistent with the previous observations reported that fast-growing tree species, such as most poplar clones and hybrids, are more sensitive and responsive to tropospheric O₃ than slower-growing species, such as beech (Bortier et al., 2000; Novak et al., 2003, 2005), because poplar has a relatively high stomatal conductance and thus high O₃ uptake (Pye, 1988; Zona et al., 2014). The comparisons of flux- and concentration-based critical levels between poplar examined in the present study and beech/birch in Europe may also suggest that the same AOT₄₀-based critical level cannot be applied for the tree species even with the similar sensitivity to O₃.

POD₁ will start to accumulate at a threshold O₃ concentration of approximately 28–36 nmol mol⁻¹ due to high gₛₛₜₛ in poplar clones.
Ozone uptake at concentrations above this threshold value has the potential to cause biomass loss in poplar. Unlike AOT indices, such concentration threshold and the size of the contribution to POD7 depend strongly on $g_{sto}$ of different poplar clones. For ‘546’ with the maximum $g_{max}$, the threshold is the smallest (28 nmol mol$^{-1}$) and the slope is the highest (0.94). In contrast, for ‘90’ with the minimum $g_{max}$, the threshold is greatest (36 nmol mol$^{-1}$) and the slope is lowest (0.71). Moreover, at concentrations slightly above this threshold value, the contribution to POD7 is small even if $g_{sto}$ is close to $g_{max}$. At higher O3 concentrations, the contribution to POD7 would be much greater, whereas was limited by $g_{sto}$ to the extent of $g_{max}$.

There are over 7 million ha of poplar plantation in China, constituting 19% of the total forest plantation area (Xu et al., 2009). As one of the representative deciduous broadleaved tree species of plantations, poplar is under the risk of O3 damage due to current ground-level O3 pollution in China (Feng et al., 2015; Zhang et al., 2012). The five poplar clones used in the present study are widely planted in China as plantations, urban landscaping, farmland shelterbelts, as well as industrial timber (Jia et al., 2013). The O3 dose–response relationships based on these five poplar clones could be used to evaluate the worst-case ozone risk on poplars in China (i.e. without water supply limiting stomatal conductance). Admittedly, a more comprehensive experiment with more poplar clones at different climate conditions, and with the observation of soil water potential could improve the stomatal conductance model and thus O3 dose–response relationship for poplar risk assessment.

Fig. 5. Hourly POD7 values (µmol m$^{-2}$) in relation to hourly O3 concentrations (nmol mol$^{-1}$) for poplar ‘84 K’ (A), ‘107’ (B), ‘90’ (C), ‘546’ (D) and ‘156’ (E). The blue solid lines represent the highest possible O3 flux (when $g_{sto} = g_{max}$) expressed as hourly POD7 at any O3 concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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Appendix A. Supplementary data

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