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

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ARTICLE INFO

Article history:

Received 25 June 2015

Received in revised form

14 August 2015

Accepted 20 August 2015

Available online 1 September 2015

Keywords:

Stomatal conductance

Ozone flux

Dose–response relationship

Critical level

Poplar

ABSTRACT

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 g_s model. The maximum g_s 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 AOT₄₀ (accumulated O₃ exposure over 40 nmol mol^{−1}) and POD₇ 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; Häikiö, 2009; Paoletti and Grulke, 2010; Zhang et al., 2012, 2014c).

In the past, the impact of O₃ on vegetation was quantified using the dose–response relationship based only on atmospheric O₃ concentrations, such as AOT₄₀ (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 *CUO* (cumulative uptake of O_3), or *AFstY* (accumulated stomatal flux above a threshold of Y) (Azuchi et al., 2014; Uddling et al., 2004), takes into account the O_3 flux by modeling O_3 stomatal conductance (g_{sto}) using multiplicative algorithms, such as Jarvis (1976).

It is generally accepted that the damage of O_3 to plants is mainly caused by the amount of O_3 entering into the leaf interior through the open stomata (Kerstiens and Lenzian, 1989). Stomatal conductance of plants is one of the crucial mechanisms of O_3 sensitivity (Dumont et al., 2013; Guidi et al., 2001). Stomatal O_3 uptake considers both biological traits and climatic factors. Therefore, it is better for assessing the adverse effects of O_3 on the plants than external 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 (Liang et al., 2006; Xu et al., 2009). Different species or clones of poplar have shown different sensitivity to O_3 with respect to visible leaf injury (Hoshika et al., 2012; Novak et al., 2005; Ryan et al., 2009; Strohm et al., 1998), damaged photosystems (Bernacchi et al., 2003; Guidi et al., 2001; Ranieri et al., 2001), and reduced growth (Isebrands et al., 2001; Matyssek et al., 1993; Mooi, 1980). However, the knowledge of O_3 dose–response relationships for poplar, which could be an effective tool for O_3 risk assessment, is still limited. It was hypothesized that the difference in 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 O_3 dose–response relationships; and (3) to define the critical levels for protecting poplar against 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 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 O_3 addition of 20 (NF+20), 40 (NF+40), 60 (NF+60), and 80 (NF+80) $nmol\ mol^{-1}$ for 8-h average O_3 concentration. Ozone was generated from pure oxygen by an O_3 generator (HY003, Chuangcheng Co., Jinan, China) and then mixed with ambient air using a fan (1.1 kW, 1080 Pa, 19 $m^3\ min^{-1}$, CZR, Fengda, China) to achieve the target 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 O_3 in the OTCs were continuously monitored using an UV absorption 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 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):

$$VPD = \left(1 - \frac{RH}{100}\right) \times 0.611 \times \exp\left(\frac{17.502 \times T}{T + 240.97}\right). \quad (1)$$

Hourly means of environmental variables were employed in 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 $\mu mol\ m^{-2}\ s^{-1}$, 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
 O_3 concentrations ($nmol\ mol^{-1}$, mean $\pm$ SE) during the experiments from May 7 to Sep. 29, 2014.

Target	24-h average	12-h average, 08:00–20:00	8-h average, 09:00–17:00
CF	25.7 $\pm$ 0.9	28.1 $\pm$ 1.1	28.0 $\pm$ 1.3
NF	35.7 $\pm$ 1.2	48.4 $\pm$ 1.6	53.2 $\pm$ 1.8
NF+20	41.4 $\pm$ 1.3	59.4 $\pm$ 1.7	67.1 $\pm$ 1.9
NF+40	46.3 $\pm$ 1.4	69.4 $\pm$ 2.0	79.5 $\pm$ 2.3
NF+60	52.8 $\pm$ 1.6	81.9 $\pm$ 2.5	95.4 $\pm$ 2.9
NF+80	58.1 $\pm$ 1.8	92.4 $\pm$ 2.8	108.7 $\pm$ 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 $\pm$ 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 (Embersson 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 (f_{O_3}).

$$g_{sto} = g_{max} \times \min(f_{phen}, f_{O_3}) \times f_{PPFD} \times \max[f_{min}, (f_{temp} \times f_{VPD} \times f_{SWP})], \quad (2)$$

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 $A_{start} \leq y_d < (A_{start} + f_{phen_c})$,

$$f_{phen} = (1 - f_{phen_a}) \times [(y_d - A_{start}) / f_{phen_c}] + f_{phen_a}, \quad (3)$$

when $(A_{start} + f_{phen_c}) \leq y_d < (A_{end} - f_{phen_d})$,

$$f_{phen} = 1, \quad (4)$$

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

$$f_{phen} = (1 - f_{phen_b}) \times [(A_{end} - y_d) / f_{phen_d}] + f_{phen_b}, \quad (5)$$

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

$$f_{temp} = \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right) \times \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right)^{b_t}, \quad b_t = \frac{T_{max} - T_{opt}}{T_{opt} - T_{min}} \quad (6)$$

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

$$f_{temp} = f_{min}, \quad (7)$$

where T denotes the leaf temperature ($^{\circ}C$), 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:

$$f_{PPFD} = 1 - \exp(-light_a \times PPFD), \quad (8)$$

where $light_a$ is model parameter; the unit of PPFD is $\mu mol m^{-2} s^{-1}$;

$$f_{VPD} = \min \left\{ 1, \max \left\{ f_{min}, \frac{(1 - f_{min}) \times (VPD_{min} - VPD)}{VPD_{min} - VPD_{max}} + f_{min} \right\} \right\}, \quad (9)$$

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; Vizcayno-Soto and Côté, 2004). It was based on the principle that biological materials have an upper response limit to a given variable (Imhoff et al., 2010). 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 ($g_{\max}$) was measured under optimal conditions where there is no limiting effect of VPD, PPFD, and O_3 . However, such optimal conditions are difficult to achieve during measurements. In the present study, clone-specific $g_{\max}$ was estimated by fitting observed g_{sto} into the bell-shaped Eq. (10) using a boundary analysis technique.

$$g_{\text{sto}} = g_{\max} \times f_{\text{temp}} \quad (10)$$

For forest trees, the response of g_{sto} to O_3 (f_{O_3}) 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_{sto} was not considered in the calculations ($f_{\text{SWP}} = 1$). The ability of this model to predict g_{sto} was validated by comparing the observations and simulations.

2.5. Stomatal ozone flux

Stomatal flux of O_3 at the leaf level (F_{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).

$$F_{\text{st}} = \frac{[O_3]_{\text{can}} - [O_3]_{\text{int}}}{r_b + r_s + r_b \cdot r_s / r_{\text{ns}}}, \quad (11)$$

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; $r_b = 1/g_b$, $r_s = 1/g_{\text{sto}}$, and $r_{\text{ns}} = 1/g_{\text{ns}}$ are the resistances for O_3 flux through the leaf-boundary layer, stomatal and non-stomatal surface deposition, respectively; g_b , g_{sto} and g_{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 Lenzian, 1989). Therefore, Eq. (11) can be written as:

$$F_{\text{st}} = \frac{[O_3]_{\text{can}} - [O_3]_{\text{int}}}{r_b + r_s}. \quad (12)$$

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:

$$F_{\text{st}} = \frac{[O_3]_{\text{can}}}{r_b + r_s}. \quad (13)$$

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:

$$r_b = k\sqrt{d/u}, \quad (14)$$

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 $Y \text{ nmol } O_3 \text{ m}^{-2} \text{ PLA s}^{-1}$), 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).

$$POD_Y = \int \max(F_{\text{st}} - Y, 0) dt. \quad (15)$$

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_3 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 RB s 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 $120 \text{ nmol mol}^{-1}$, and as POD with the stomatal O_3 flux threshold (Y) ranging from 0 to $20 \text{ nmol } O_3 \text{ m}^{-2} \text{ PLA s}^{-1}$. AOT_X is the accumulated O_3 exposure over a concentration of $X \text{ nmol mol}^{-1}$ 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_{40} - and POD_1 -based dose–response relationships were analyzed, since they have been widely used and have been accepted by the United Nations Economic Commission for Europe (UNECE) 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_{40} - or POD_1 -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

Fig. 1. Average total biomass productions (g dry weight per plant, mean $\pm$ SE) of five poplar clones in different O_3 treatments.

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_{sto} functions are provided in Table 2. The slope of the linear regression analyses for the relationship between simulated g_{sto} and observed g_{sto} 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 100%, 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_{40} is presented in Fig. 4A with a R^2 value of 0.88. The best performing concentration-based index is AOT_{60} ($R^2 = 0.91$, Fig. 4B). The regression relationships of RB for POD_1 and POD_7 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_{40} , AOT_{60} , POD_1 , and POD_7 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 $mmol\ m^{-2}$, and 3.8 $mmol\ m^{-2}$, 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_7 varies between 28 and 36 $nmol\ mol^{-1}$ for five poplar clones according to their respective value for g_{sto} . It is clear that the ambient 8-h (09:00–17:00) average O_3 concentration ($68.3 \pm 2.3\ nmol\ mol^{-1}$, mean $\pm$ 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_{max}	$mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$	299.8	275.2	240.3	343.9	322.4
f_{min}	Fraction	0.01	0.01	0.01	0.01	0.01
f_{phen_a}	Fraction	—	−0.09	—	—	—
f_{phen_b}	Fraction	0.006	0.798	0.512	0.665	0.327
f_{phen_c}	day	—	80	—	—	—
f_{phen_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
$light_a$	Constant	3.3×10^{-3}	4.6×10^{-3}	5.9×10^{-3}	3.8×10^{-3}	4.0×10^{-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

Fig. 2. Simulated versus observed stomatal conductance (g_{sto}) of five poplar clones from NF and NF+40 treatments. Blue solid line represents regression, and red dashed line denotes theoretical 1:1 relationship. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

To develop the flux-based O_3 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 O_3 . 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 $mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$ 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 O_3 flux. The averaged g_{max} ($296.3\ mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$) for poplar is higher than for beech ($205\ mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$, Azuchi et al. (2014)) and oak ($255\text{--}285\ mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$, Hoshika et al. (2011)) in East Asia, as well as birch in Continental Central Europe ($150\ mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$, LRTAP Convention (2010)) and in North America ($230\ mmol\ O_3\ m^{-2}\ PLA\ s^{-1}$, Uddling et al. (2010)). Even though VPD was estimated using air temperature and relative humidity in the present study, the correlation coefficient between f_{VPD} 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.

Fig. 3. Coefficient of determination (R^2), slope and intercept (%) of regressions between relative biomass (RB, %, y-axis) and accumulated O_3 exposure over an hourly cut-off threshold concentration of $X\ nmol\ mol^{-1}$ during daylight hours (AOT_x , x-axis) (A) or between relative biomass (RB, %, y-axis) and estimated accumulated stomatal O_3 flux over a threshold of $Y\ nmol\ O_3\ m^{-2}\ PLA\ s^{-1}$ (POD_Y , x-axis) (B).

The dose–response relationships between O_3 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 AOT_{60} is the highest (0.91). For AOT_{40} , 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 O_3 during daylight hours and have no sensitivity at all to the factors which influence O_3 uptake by plants. The stomatal flux-response index POD_7 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 O_3 indices presented in Fig. 4. However, the intercepts on y-axis for the regression between RB and POD 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 O_3 exposure over a threshold of 40 nmol mol^{-1} during daylight hours (AOT_{40}) (A) and AOT_{60} (B), as well as the accumulated O_3 flux over an uptake threshold of $1 \text{ nmol m}^{-2} \text{ PLA s}^{-1}$ (POD_1) (C) and POD_7 (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 R^2 value than concentration-based exposure indices in assessing O_3 -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 pratense*, 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 O_3 uptake by plants. Its performance may highly depend on the experimental conditions, such as temperature and soil water content.

AOT_{40} has been regarded and retained as the basis for the O_3 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 O_3 uptake. POD_Y is more biologically realistic than AOT_{40} , since the impact of O_3 on plants depends on the amount of O_3 entering the stomata and its detoxification by antioxidants (Emberson 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 AOT_{40} -response relationship with regard to 5% reduction in total biomass for poplar was $12 \text{ μmol mol}^{-1} \text{ h}$, which is 2.4 times the critical value ($5 \text{ μmol mol}^{-1} \text{ h}$) reported for birch and beech species in Europe

(LRTAP Convention, 2010). The AOT_{40} value accumulated using ambient O_3 concentration over the same duration in the experimental site was $40.5 \text{ μmol mol}^{-1} \text{ h}$, which corresponded to 16.7% loss of total poplar biomass. Expert judgment was used to set the flux-based threshold Y to $1 \text{ nmol O}_3 \text{ m}^{-2} \text{ PLA s}^{-1}$ to represent the detoxification capacity for forest trees (LRTAP Convention, 2010; Pleijel et al., 2004). The critical levels of POD_1 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 POD_1 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 POD_1 -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 O_3 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 O_3 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 AOT_{40} -based critical level cannot be applied for the tree species even with the similar sensitivity to O_3 .

POD_7 will start to accumulate at a threshold O_3 concentration of approximately $28\text{--}36 \text{ nmol mol}^{-1}$ due to high g_{sto} in poplar clones.

Fig. 5. Hourly POD_7 values ($\mu\text{mol m}^{-2}$) in relation to hourly O_3 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 O_3 flux (when $g_{sto} = g_{max}$) expressed as hourly POD_7 at any O_3 concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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 POD_7 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 the greatest (36 nmol mol^{-1}) and the slope is lowest (0.71). Moreover, at concentrations slightly above this threshold value, the contribution to POD_7 is small even if g_{sto} is close to g_{max} . At higher O_3 concentrations, the contribution to POD_7 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 O_3 damage due to current ground-level O_3 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 O_3 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 O_3 dose–response relationship for poplar risk assessment.

Acknowledgements

This study has been funded by the Hundred Talents Program, Chinese Academy of Sciences, State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, and the National Natural Science Foundation of China (No. 41401225). We thank Dr. Harry Harmens for English improvement throughout the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.08.034>.

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